VOL. II.

ADDITIONAL PUBLISHED PAPERS.

1. "Blood Tumours" or Hameangioendotheliomata in the Fowl. (With F. Blakemore). (1931). 2nd. Rep. I.A.P. Cambs.

2. The Comparative Pathology of Tumours. (1932). Proc.Roy.Soc.Med.

3. The Chemical Identification of Vitamin C. (With L.J. Harris & I. Mills). (1932). Lancet.

4. The Pathogenesis of Avitaminosis A. (With L.J. Harris & S. Griffith). (1932). Lancet.

5. Beitrag zur Wirkung des Cyanids auf die Schilddrüsen der Ratte. (1933). Endokrinologie.

6. "Über Nebennierenveränderungen bei experimentellen Skorbut. (1934). "

7. Vergleichende Untersuchung der sog. Umgebungsreaktion der Tumoren und ihrer Metastases. (1934). Z.f.Krebsforschung

8. The Pathological Diagnosis of Swine Fever. (1935). Vet. Rec.

9. A Case of Suppurative Epididymitis in the Dog caused by B. Abortus. (With T.J. Bosworth & T.L. Whitby. (1935). Vet. Rec.

10. The Effect of P. Deficient Diets in Metabolism Blood Bones of Sheep by J. Stewart, with addendum. The Anatomical Changes in the Bones and their relationship to Rickets. (1936). 4th. Rep. I.A.P. Cambs.

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14.	The Assimilation of the Stenboch Diet in Normal and Vitamin D. deficient rats with and without Caecum. (With I. Nicolaysen).	(1937).	Biochem J.
15.	Studies in Debatable Tumours. I. Lymphoepthelioma. (With Col. W.F. Harvey and Dr. Edith K. Dawson, Roy. Coll. Physicians Lab. Edin.)	(1937).	Edin. Med. J.
16.	II. Lymphosarcoma.	(1937).	" " "
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20.	VI. Meningioma.	(1939).	" " "
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28.	Multiple Malignant Neoplasma in a Dog with Portal Obstruction without Ascites. (With J. Whittick).	(1940).	J. Comp. Path. (In press).

A Preliminary Note on "Blood Tumours" (Haemangio-Endotheliomata) in the Fowl

BY

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A Preliminary Note on "Blood Tumours," (Haemangio-Endotheliomata) in the Fowl

By F. BLAKEMORE and J. R. M. INNES.

Introduction.

Angiomata in man and the lower animals are of frequent occurrence, but few references can be found in the literature regarding such tumours in the fowl. The condition has been described by Karetta (1928), Schürman (1928), Abels 1929), and Höve and Bornstedt (1931) in Germany. These authors classified he disease as *Haemangioma*, or *Haemangioma cavernosum*, indicating that he disease was specific and neoplastic in nature. In this country there has been a certain amount of misunderstanding regarding the nature of these growths to which the unsuitable name "blood tumours" has been given.

Reference was made to a disease of this type by the Adviser at the Harper Adams Agricultural College, in the Annual Report to the Ministry of Agriculture (1930–31), and in the same report the Adviser at Cardiff recorded an "outbreak" which occurred in a number of birds belonging to one owner in an gg-laying trial. *No* other birds in the trial developed the disease, but the wner of the affected birds had evidently stated that cases had occurred mongst his flock in previous years.

History of Cases.

1. Griffith. The condition was first brought to our notice by Dr. A. Stanley Griffith, of the Field Laboratories, Cambridge. In a flock of 22 White Leghorns (12 cockerels, 10 pullets), the ten pullets were affected. He had first noticed these growths in the form of small dark-coloured nodules about the size of a pea, which elevated the skin, rapidly increased in size and ultimately ruptured with consequent haemorrhage. Sometimes clotting followed, but invariably haemorrhage recurred until the bird became very anaemic; some of the fowls died, and the rest were then killed. All the birds had multiple subcutaneous tumours situated in different parts of the body. Latterly Dr. Griffith had ligatured the swellings, and in some birds managed to stave off rupture and haemorrhage, although usually the growths recurred elsewhere. Histological examination of several specimens from Dr. Griffith's cases showed that these "blood tumours" were subcutaneous angiomata.

One of us (F.B.), in the course of his duties, is in contact with the poultry advisers and farmers of the Eastern Counties, and by personal enquiry discovered several other cases, which are described in detail. Some of the flocks of birds in which cases were found were closely related by breeding.

As it is possible that these tumours may be dependent on some hereditary maldevelopment, the relation of the strains is, therefore, given. It is worthy of note that the cases to be described occurred almost simultaneously in each "outbreak" and all in one year old pullets.

2. "SMITH." In a pen of 30 White Leghorn pullets, four birds were found to be affected. They were in an exhausted condition and suffering from the effects of severe haemorrhage, two of the birds being unable to stand. The bleeding had been intermittent, and in each case had resulted from the rupture of one or several of these "blood tumours," situated in various parts of the skin. Post-mortem examinations were made, details of which will be given later. It was found that the pullets had been hatched from eggs obtained from a neighbouring farmer ("Brown").

3. "BROWN." An examination of the birds belonging to this owner revealed one case in a one year old White Leghorn pullet. This bird showed similar signs of severe haemorrhage from a ruptured tumour, situated subcutaneously on the under surface of the wing. It was discovered that cases had occurred amongst his flocks of birds over a period of four years, and were restricted to one year old White Leghorn pullets. Further enquiries revealed that Dr. Griffith had obtained his birds from "Brown." The breeding histories of these various flocks were traced, and it was discovered that "Brown" had originally obtained his strain from a breeder, "Jones," who had kept and bred from two pens of White Leghorn birds. "Jones" stated that no cases had occurred in his birds. The relation of these four flocks, "Jones," "Brown," "Smith" and Griffith is shown in tabular form (Table I). It is evident that a considerable amount of inbreeding had taken place between these birds over a period of 4–5 years. The average number of pullets kept by "Brown" each year was 26, and the number of cases which occurred each year is indicated in the chart.

4. "ROSE." One White Leghorn hen belonging to this owner developed the condition while competing at the Cambridgeshire Egg Laying Trials, and was under observation for about three weeks prior to death. A large tumour was present under the skin on the outside of the wing, and had ruptured; haemorrhage was intermittent, but became progressively more severe, until the hen finally died. Despite the severity of the haemorrhage the bird continued to lay fairly well, the last egg being laid 24 hours before death. An examination was made of the other birds at this owner's farm, but no cases were found and no history of any occurrence previous to this case was obtained. This farmer had originally obtained his birds from the strain B represented in Table I.

5. "FORD." Three cases occurred in a flock of 120 cross-bred pullets. These were the progeny obtained by the mating of Rhode Island Red hens with a White Leghorn cock obtained from the breeder, "Rose." All three cases proved fatal. No history of any previous occurrence was obtained.

6. "CELT." One Rhode Island Red was forwarded for examination. The bird showed one large subcutaneous tumour in the thigh and numerous smaller nodules in the skin of the neck.

TABLE I

ORIGIN AND RELATIONSHIP OF "BROWN" STRAIN.

"Brown" originally obtained 2 years' old hens from "Jones," who had bred from two strains A♀'s + B♂ and A♀'s + C♂.
"Brown's" birds or their progeny were mated each year with a cock supplied by "Jones" from each of his two strains alternately. The number of cases that occurred are given in brackets. The number of birds kept by the owners, "Smith" and "Griffith," was 30 and 22 respectively.

Post-mortem Examination.

Bacteriological examination was carried out in two birds immediately after death. Cultures were made from heart blood, liver and skin tumours and incubated under aerobic and anaerobic conditions for four days with negative results.

Autopsies were made of seven cases obtained from "Smith" (4), "Brown" (1), "Rose" (1), "Celt" (1). The lesions in all were similar in appearance, and to avoid repetition will be described in general. All the birds were in good condition, apart from the signs of general anaemia due to the haemorrhages. All cases showed the presence of tumours in the skin, which varied in size, but were always multiple. The size ranged from that of a small pea to a large hazel nut (Fig. 1). In one bird the nodules were very small (pin-head to pea size), numbered about 40, and were situated in the skin of the neck. The nodules were blue to black in colour, softish, freely movable from the underlying structures and on section were seen to be filled with blood. The skin over the growths was distended and thin and there was a tendency for the larger nodules to become pedunculated. Although several of the tumours appeared to be related to the feather follicles, this was not general. The commonest sites were the wing, neck, abdomen and thigh, very few being seen in the legs. Every bird showed evidence of rupture and haemorrhage from one or several of the large tumours. In five cases metastases (?) were found in the internal organs; the liver, lungs, spleen in one bird, the lungs and kidneys in two and the lungs alone in two. In the first case the *liver* was enormously enlarged, almost filling the abdominal cavity when viewed from the ventral aspect (Fig. 1). Its surface was studded with numerous soft haemorrhagic nodules which varied in size. The capsule had ruptured at several points, and the peritoneal cavity contained a considerable amount of clotted blood. The serous surfaces were covered with fresh fibrin, which matted coils of the intestine to the liver capsule. On section, the entire parenchyma of the liver was found to be infiltrated with nodules, which were irregularly spherical in shape, varied from a pin-head to a pea in size, and were similar in appearance to those seen on the surface. At one point a severe subcapsular haemorrhage had occurred resulting in the formation of a very large clot about 4–5 cms. in diameter (Fig. 2). The *spleen* of this bird showed a large haemorrhagic nodule in the centre of the pulp and rupture of the capsule had also occured, while a large, partly organised, clot was present on the outside firmly attached to the organ. Numerous nodules were present in the *lungs* of this bird, particularly on the dorsal aspect. The tumour nodules in the organs (lungs and kidneys) of the other four birds showed similar appearances, although the liver in each case was unaffected, but was pale and anaemic. No noteworthy changes were apparent in the other organs. No abnormalities were noted in the internal organs of those birds in which secondary (?) deposits were not found, apart from a general blanching of the viscera.

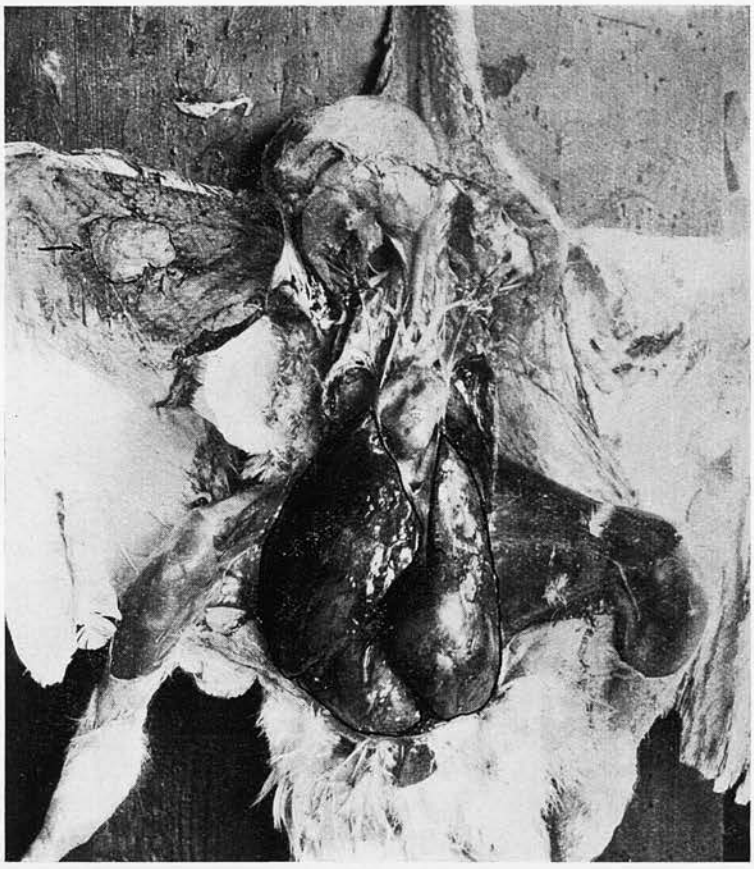

FIG. 1. Large tumour situated on surface of right wing; greatly enlarged liver, outlined by Indian ink.

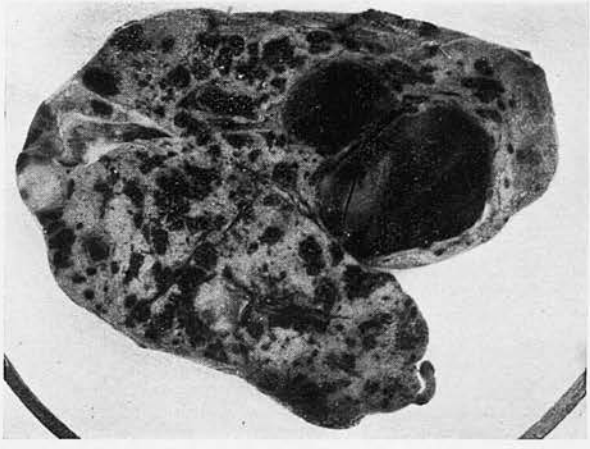

FIG. 2. Mounted specimen of liver from fowl (Fig. 1) with multiple subcutaneous tumours showing numerous haemorrhagic nodules.

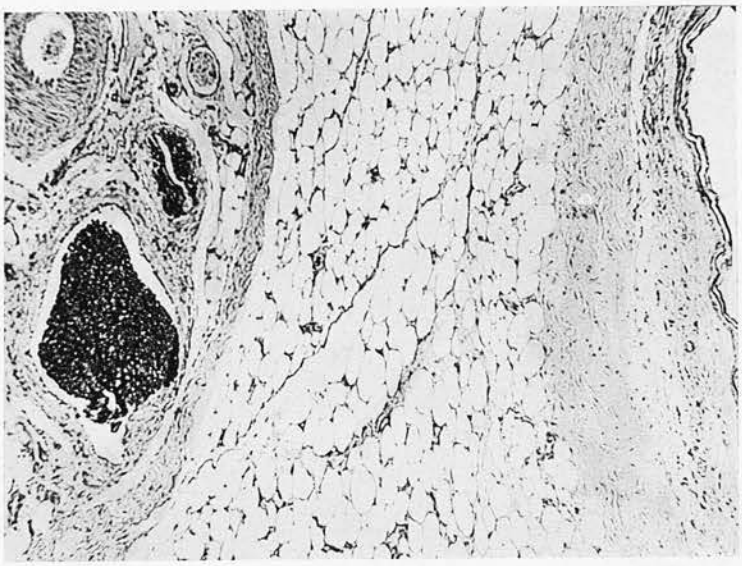

FIG. 3. Section of skin at margin of small subcutaneous tumour; the epidermal epithelium is on the right; two small veins and an artery in the dermis on the left were in continuity with the vascular channels of the tumour illustrated in Fig. 4. Haematoxylin and eosin. X.96.

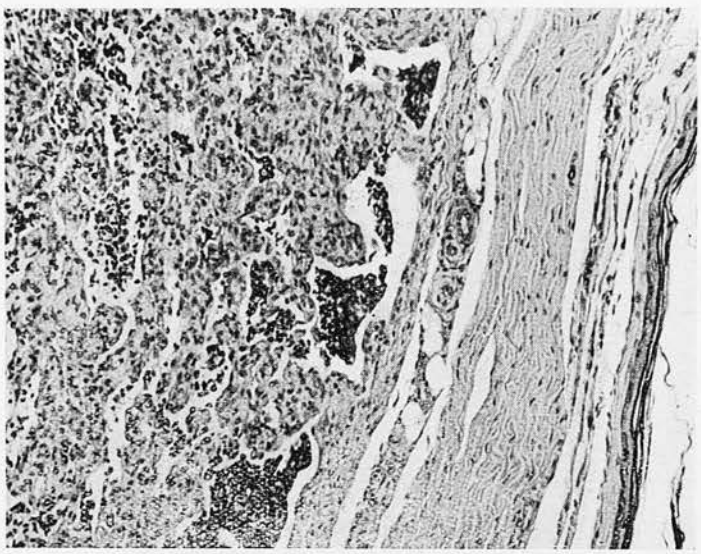

FIG. 4. Section through small nodule in the dermis; the tumour in this area resembles a capillary naevus; note irregularity in size of vascular channels. Haematoxylin and eosin. X.200.

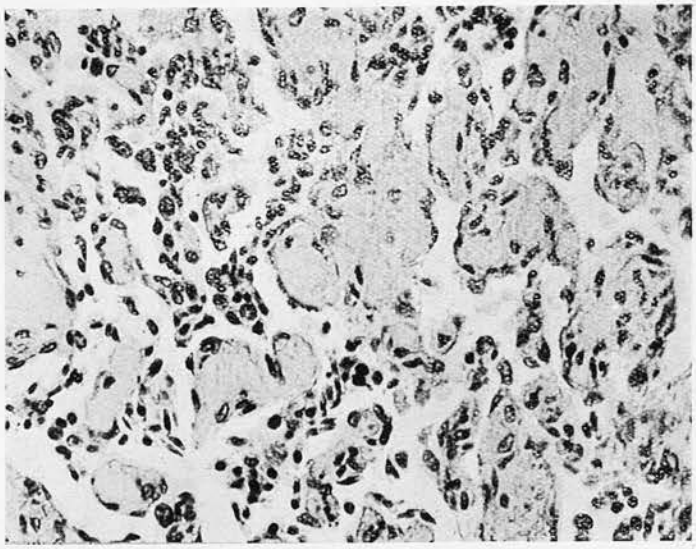

FIG. 5. High magnification of area from Fig. 4. The size, shape, the endothelial cells can be seen; the number and distribution of anastomosis of the vascular channels is demonstrated. Haematoxylin and eosin. X.400.

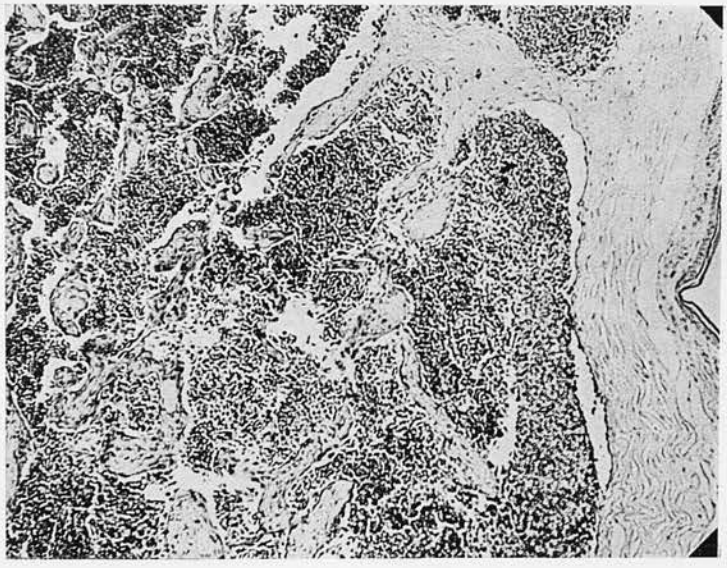

FIG. 6. Section of a subcutaneous tumour which approaches the cavernous type of angioma; epidermis on the right; large dilated sinuses filled with blood; papilliform ingrowths; compare with more cellular type of growth (Fig. 4). Haematoxylin and eosin. X.90.

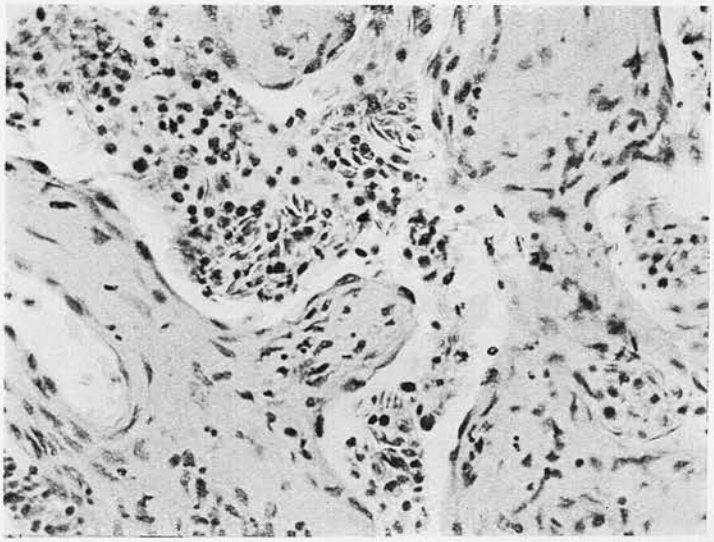

FIG. 7. High magnification of area from Fig. 6; note small papillary processes, and adherence of the endothelium to the walls of the vascular channels. Haematoxylin and eosin. X.400.

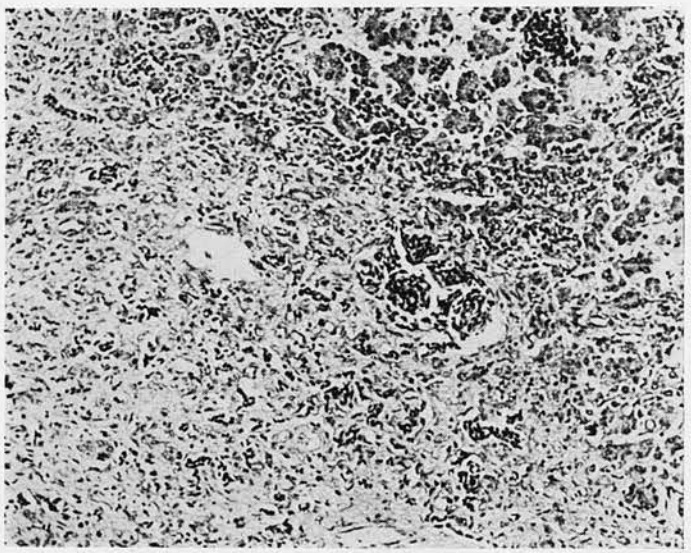

FIG. 8. Section showing metastases (?) in the liver; surviving hepatic cells are seen on the right; the nodules are of the more cellular type of tumour, the spaces being small. Haematoxylin and eosin. X.200.

The lesions described above were exactly similar to those observed by Dr. Griffith in the cases examined by him. Internal growths were observed in one only of his cases, but most of the birds in his flock were killed soon after the skin nodules appeared.

Histological Technique.

Numerous specimens of the skin tumours ranging from the smallest to the very large growths and pieces of tissue from the deposits in the liver, lungs, spleen and kidneys from the various birds were fixed in formol saline and embedded in paraffin. Sections were cut and stained by Mayer's haematoxylin and eosin, Weigert's iron haematoxylin with Van Gieson and Mallory's fuchsin-anilin-blue-orange G. method; other sections were prepared by the silver impregnation method of Laidlaw (1929) (a modified Hortega) for demonstration of the tumour reticulum. Some of the small skin nodules were cut serially.

Histology.

All the skin tumours conformed to the same morphological plan and type, and it will therefore be convenient to describe the appearances in general.

The growths were composed of anastomosing vascular channels containing blood, enclosed by thin septa and lined by endothelial cells (Figs. 4–7). The smaller tumours, which appeared to have their origin in the dermis of the skin, had gradually displaced the dermis and elevated the epidermis by the pressure of the circulating blood. No encapsulation was observed. The vascular channels exhibited great irregularity in size and shape in the same and different tumours which, therefore, showed a varied histological structure. In all the tumours, however, the one common and outstanding feature was the formation of these blood-containing channels. In some areas they were very small, the lumina being about the size of small venules and the tumour thus resembled a capillary naevus (Fig. 4); in other areas they were large saccular or tubular dilatations and gradations from these to very large cavernous sinuses were seen (Fig. 6). Serial sections of the small early growths showed that there was a free communication of the vascular channels of the tumour with the general circulatory paths by way of the small vessels of the dermis. The walls of these vascular channels were lined by a well-marked and regular endothelium (Figs. 5 and 7). The endothelial cells were closely adherent to the walls and showed variation in size and shape. Most were elongated and flattened, although not to the extent of the endothelium of normal vessels; the cytoplasm was very scanty, and the nuclei, which almost filled the entire cells, were pale staining, vesicular, with the chromatin particles arranged irregularly in clumps along the nuclear wall. Fine nucleoli were seen, but there were none which were acidophilic. Mitotic figures were not abundant. In a few places the endothelial cells were round with spherical nuclei and formed at some point on the wall not a single layer, but a solid group of cells. Many of the large

cavernous spaces showed papilliform ingrowths into the lumen (Figs. 6 and 7). These ingrowths consisted of a core of thin strands of collagen in which were imbedded a few small fibroblasts covered by a single or multiple layer of endothelial cells. Branching of the ingrowths was evident, because these papillary processes were cut in every possible plane; the sections showed variation from conical projections attached to the walls of the spaces to isolated circular and oval fragments which were lying free in the blood-filled spaces.

In some areas of the same tumour endothelial proliferation had been more active, the vascular channels were very small, and the area had almost a solid cellular structure. There was often a sharp transition from the structural appearance of the latter areas which resembled the capillary naevi to areas which resembled the cavernous type of angioma. All the channels contained blood, and in the larger spaces small fibrinous clots were frequently seen.

The stroma of the tumour was scanty in amount, and was composed of thin strands of collagen; elastic fibres were rarely seen. By the silver impregnation method of Laidlaw (1929) the intimate relationship of the tumour cells to the ramifying reticulum was well demonstrated. The epidermis situated above these growths showed no abnormalities apart from a thinning due to distension.

The larger skin tumours were identical in appearance, although there was a greater tendency for the vascular spaces to be of the cavernous type, suggesting that these had been formed by coalescence of the smaller spaces. All were filled with blood and papilliform ingrowths were commonly seen. In the very large tumours (e.g. Fig. 1) the spaces were enormously dilated, the endothelial cells were very flattened, and organised blood clots were present in some of the channels.

The growths seen in the *liver* of one bird (Fig. 2) were as vascular as the skin nodules, and were very extensive, the organ being riddled with small tumour nodules and haemorrhagic areas. The general structure of the nodules was the same as that described in the skin tumours. Encapsulation was not observed, and great variation in the size and shape of the vascular channels was again seen. Some areas were very cellular, the spaces being small, but in other nodules they reached the size of the channels, found in the common cavernous angioma (Figs. 7 and 9). The endothelial cells which lined these spaces were elongated, flat and closely adherent to the walls. Mitotic figures were present, but not in abundance. New growths infiltrated the liver tissue in all directions and atrophied but surviving hepatic cells were demonstrated in the tumour tissue. The reticulum of the tumour nodules was well developed and continuous with the normal reticulum of the liver, while the vascular channels were in communication with the hepatic circulation. The parenchyma, apart from the tumour nodules, was structurally normal except for a marked infiltration of eosinophil leucocytes, which had occurred at the margins of many of the growths.

The *livers* from the birds which macroscopically had not shown evidence of tumour involvement presented no abnormalities.

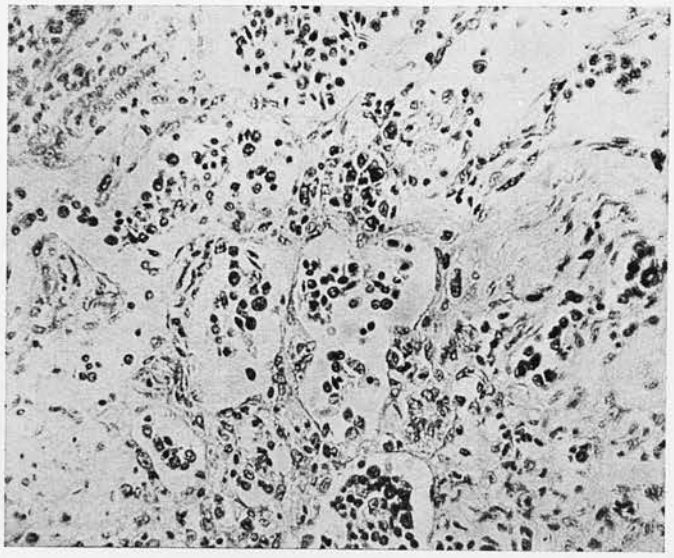

FIG. 9. High magnification of area from nodule in the liver which approached the cavernous angioma in type; note dilated and irregular channels containing blood, lined by endo-endothelial cells. Haematoxylin and eosin. X.400.

The *spleen* was involved in one case, and rupture of the capsule had occurred. In sections the normal reticular tissue of the pulp was recognised only in occasional areas and unaltered lymphatic nodules were rarely visible. Most of the structure of the organ, particularly in the centre, was obliterated by the formation of large dilated sinuses filled with blood, and many small organised clots were observed in these spaces. The general appearance resembled that of the cavernous type of angioma observed in some of the liver and skin nodules. The splenic capsule was ruptured at one point, and a large organised blood clot was firmly attached to the organ.

The growths observed in the *lungs* resembled the structure of those in the liver and skin, and were equally vascular. Many small nodules were observed in the lungs of all birds in which this organ was involved. The growths were of the more solid cellular type, the spaces being small and capillary-like.

The growths in the *kidney* which were observed in two birds showed similar structural appearances to those seen in the other situations. The vascular channels, however, were large and dilated.

Discussion.

According to Ewing (1928) the "determination of the scope of neoplastic processes affecting blood vessels presents unusual difficulties. Possessing less natural autonomy they may be expected to display pronounced neoplastic properties. Further, the growth of vessels is markedly influenced by the element of mechanical pressure of the circulation, which is absent in other tumours." Thus special standards are required in the interpretation of tumour processes of this type, and this has led to much difference of opinion as to what constitutes an angioma.

Malignant tumours of the blood vascular system in man which metastasize to other parts of the body are rare. Benign new growths, on the other hand, are not uncommon, and the literature abounds with descriptions of them and discussions of their origin and growth. Ewing classifies them as the *Haemangioma simplex* or the *Vascular Naevus*, of which he recognises several types. The most important are (1) the common localised non-progressive *naevus vinosus*, usually congenital in origin, (2) the *subcutaneous plexiform angioma*, which consists of a new growth of dilated capillaries in which the length of the vessel is increased, but the number of new cells is not in excess, and (3) the more hyperplastic type of growth in which the cells grow rapidly and show poor differentiation, and which may be designated *haemangio-endothelioma*. These tumours may be further characterised as being capillary or cavernous in type, according as the vessels of the growth have minute capillary-like lumina or appear as large distended spaces separated by thin walls. Mallory (1923) classifies all these varieties as well as the lymphangioma and dural endothelioma under the general heading of *Endothelioblastoma*, and states that the *haemangio-endothelioblastoma* are "often congenital and frequently, perhaps

always, arise from abnormalities of the blood vessels, especially from vascular naevi." Ewing also stresses this point of origin, which must be referred to "a developmental anomaly in the structure of certain vascular segments which do not fit with the circulatory system, and which retain embryonal characters." Further reference can be made to Ewing (1928), who deals with these points in detail.

What is the evidence that these growths in the fowl are truly neoplastic? Are the nodules observed in the various internal organs of some birds true metastases; or are they examples of tumour growth of multicentric origin?

All the tumours examined showed certain common outstanding features. Firstly, they formed atypical blood vessels and blood containing spaces; secondly, they grew fairly rapidly and invasively, and were locally destructive; thirdly, the type cell was the endothelial cell. The tumours can, therefore, be designated as haemangio-endotheliomata of an undoubtedly multiple nature in so far as the skin is concerned. The possibility that the growths in the internal organs were truly metastatic cannot be satisfactorily answered. The histological appearances of the nodules in these organs showed that the same structure as observed in the skin tumours was rigidly adhered to, and that growth of the tumour was by infiltration of the surrounding tissues. Nevertheless, most of the tumours showed no outstanding features which indicated any high degree of malignancy; in fact the general appearances were those of angiomata, which are usually considered to be of a benign character. A prominent feature, for example, was the regularity of the endothelial cells, which were quite well differentiated, and the comparative scarcity of mitotic figures. It was only in the neighbourhood of the papillary ingrowths that any semblance of rapid growth was evident; nor was there any evidence to show that the tumour had invaded the veins.

Nevertheless, the absence of those criteria, which are generally taken to indicate malignancy, does not necessarily eliminate the latter. Shennan (1914) described a case of multiple angiomata in man with extensive metastases which he considered to be histologically non-malignant. The fact that in many of the birds subcutaneous tumours were present without involvement of the viscera seems to suggest that the skin may be the primary seat of growth. The tumour may, therefore, be an example of malignant angioma; on the other hand, the deposits observed in the liver, lungs, spleen and kidneys in some birds may not be metastases, but may be the result of growth from a multicentric origin. The involvement of the kidney in two birds is of interest because of the extreme rarity of this organ as a site of angioma formation.

Several points of importance to the poultry breeder remain to be discussed. From this point of view the main question is that of origin and causation. It is improbable that a satisfactory answer can be given; nevertheless, there are some suggestions and possibilities. A number of single cases occurred (see pages 175–7) in birds which were in close contact, but no evidence of any subsequent cases was obtained. In other flocks where more

than one case was observed they occurred simultaneously. A preliminary transmission experiment has been attempted by us in this laboratory without success. Although these facts do not by themselves completely negative the possibility of infectivity, they are suggestive.

An interesting feature which is of importance to the breeder is the suggestion that the condition may be in the nature of a hereditary defect. Höve and Bornstedt (1931) suggest an inherited predisposition and injuries to the feather follicle capillaries during the moult. Schürman (1928) attributes the tumours to embryonic disturbances in the early formation of blood vessels. All the cases observed by us occurred with one exception in one year old pullets of the White Leghorn breed, which, according to our own and the finding of others (Schürman, Höve and Bornstedt), appears especially prone to the condition. The majority of cases occurred in birds which were all derived from the same original breeding strain (Table I). Apart from the cases occurring in birds directly derived from the "Brown" strain it is of interest that "Rose" (1 case) originally obtained his birds from the strain "Jones" B (Table I), and, further, that "Ford," experienced cases only after introducing male blood from "Rose." A considerable amount of in-breeding had undoubtedly taken place in the flocks of the different breeders in which cases were observed.

Summary.

Seven cases of multiple haemangio-endotheliomata of the fowl are described. In addition to the subcutaneous tumours, growths were also found in the liver, spleen and lungs in one bird, the lungs and kidneys in two birds, and the lungs alone in two. The data were insufficient to show whether the involvement of the internal organs was the result of metastases or an example of a neoplasm with a multicentric origin. The type cell was the endothelial cell, and the tumour in all situations mentioned was characterised by the formation of vascular channels. The latter were atypical and existed as small capillary-like vessels or as large cyst-like vascular cavities into which papillary processes projected from the walls. These variations may be observed in the same tumour. Neoplastic growth was active, invasive and locally destructive. The White Leghorn breed appears to be especially prone to the condition, and it is suggested that the tumour may have a congenital origin.

Thanks are due to Mr. H. Robinson, Poultry Adviser of Cambridgeshire, for help in obtaining information and material.

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THE COMPARATIVE PATHOLOGY OF TUMOURS.

By Dr. J. R. M. INNES.

IT is not possible to make a strictly scientific comparison between neoplastic diseases in domesticated animals and analogous conditions in man, since statistical data regarding the incidence of the different types of tumours in the various species are not sufficiently extensive, and there are many types of tumours in man which have not yet been shown to occur in animals. While tumours of the lower animals are perhaps of relatively minor importance, it cannot be doubted that the same laws govern their origin and formation as in man. Comparative encology is thus a matter of practical importance and not merely one of academic interest.

The following table, which has been compiled from a series of cases examined within the last few years, affords an indication of the types of tumour which occur in the domesticated animals, but the number collected is not large enough to indicate the influence and relation of age, sex and other factors. The growths have been obtained from the dog, horse, ox, sheep and cat.

Fibromata	17	*Epithelial tumours :—*	
Chondromata	1	Adenomata	4
Osteomata	4	Adeno-chondromata	2
Osteo-chondromata	4	Adeno-carcinomata	9
Myxomata	1	Fibrocystic adenomata (testis of dog)	2
Lipomata	3	Papillomata	9
Angiomata	14		
Leiomyomata	2	*Carcinomata :—*	
		Squamous-cell, ...	22
		Basal-cell, ...	—
Sarcomata :—		Transitional-cell carcinomata or lympho-epithelioma	1
Round-cell, ...	7	Embryonal carcinoma or seminoma. (Testis of dog)	5
Fibro-sarcomata, ...	8	Liver-cell carcinoma or hepatoma ...	1
Osteogenic	—	Dural endothelioma, psammoma, or meningoma	1
Gliomata	—		
Melanomata	18		
Lympho-sarcomata	2		

Total = 137

Fibromata.—Most of these were located in the skin and subcutaneous tissues, other sites being the vagina, tail, prepuce and ear. Considerable structural variation was encountered, from the soft cellular type with little stroma, to extremely dense hard growths in which collagen was abundant. *Osteomata.*—Included in this group are fibrous subcutaneous growths in which bone was present, but it is questionable whether these should be regarded as neoplasms or as examples of metaplasia. It was difficult to determine whether in these tumours the bone was the essential part of the growth or merely the secondary product. It has been observed that such growths in the horse arise as a result of trauma and chronic inflammation. *Osteo-chondromata* are of frequent occurrence in the mammary gland of the bitch, sometimes with a concurrent adenomatous process; their benign nature is well recognized. *Lipomata.*—These are frequently observed in slaughtered cattle in abattoirs, the common sites being the intestine and mesentery. *Angiomata.*—These include the cavernous and plexiform type, most of the former being located in the liver of the ox in which they are very common. The plexiform type were mainly located in the subcutaneous tissues of the dog. *Leiomyomata.*—These growths were extirpated from the uterus of a bitch, one case being in the nature of small multiple nodules. No case of rhabdomyoma has been encountered. *Sarcomata.*—These were located in the skin and subcutaneous tissues and, in one case, in the nasal cavity. In two instances metastases were present in the liver, the primary growth being in the skin. The same variety of cell types has been observed as in man, the small round cell,

large round cell and spindle cell. No authentic cases of any variety of the osteogenic sarcomata have been recorded. *Gliomata.*—In no instance has any one of the classified varieties of the gliomata been encountered, and it is generally accepted that tumours of this type are very rare in animals.

Melanomata.—Under this group are included all growths which showed an abnormal collection of melanin pigmented cells. All were located in the skin and subcutaneous tissues. It is well known that such growths are of common occurrence in dogs and horses, particularly grey horses. The types encountered have varied from the small pigmented nævi (single and multiple), warty and pedunculated in type with apparently benign histological characters, to large diffuse epidermal growths with metastases in the adjacent lymph glands. With the latter there is frequently a history of slow growth over a period of years in which trauma appears to play a part. Others apparently remain localized, but if interfered with may recur in a matter of months. An intensive study of this type in animals would perhaps help to elucidate many of the controversial points in connection with melanomata, e.g. the histogenesis of the melanoblast, the mode of origin and distribution of the pigment, and the relation of the simple pigmented nævi to malignant transformations. These cutaneous tumours offer an opportunity of studying pathological changes in the earliest stages from the simplest quiescent nævi to the gravest proliferation of the malignant forms. *Lympho-sarcomata.*—Two cases of this type have been observed in the dog. One was located in the mediastinum involving the mediastinal and bronchial lymph glands with secondary deposits in the lungs, the other was confined to the lower end of the ileum and mesenteric lymph glands with metastatic deposits in the kidneys. In both cases the regional invasive growth which is characteristic of this type in man was well demonstrated. The histology was that of the reticulum-cell or large-cell type. *Adenomata.*—These have been located in the mammary gland, intestine and liver. Anal adenoma in the dog is of frequent occurrence. *Fibrocystic adenoma.*—Two tumours of this type have been collected, being located in the testis of a dog. Both had a history of rapid growth but were apparently benign in nature. Periods of eighteen months and two years respectively have now elapsed since operation without there being any sign of regional involvement. Both were very large growths with much hæmorrhage and cyst formation. *Carcinomata.*—The *squamous* type was found in the skin, eyelid, mammary gland and clitoris and, in one instance, the heart of a cow, a very unusual site. No cases of the *basal-cell* type have been observed. One epithelial growth affecting the tonsils, with secondary deposits in the cervical lymph-glands and lungs, in a dog, closely resembled a type described by Ewing and others in man, and designated as *transitional-cell carcinoma*, or *lympho-epithelioma.* This type in man has a peculiar clinical course and is only located in the naso-pharynx. *Embryonal carcinoma* or *seminoma, of the testis.*—Five of these growths have been collected from the dog. This is a type of neoplasm which has not been recorded previously as occurring in this animal. The structure is identical with that of seminoma of man, namely, large polyhedral cells growing in cords and sheets, with a stroma richly infiltrated with lymphocytes. The tendency to alveolar formation has in the past led to its classification as alveolar sarcoma, but this is incorrect. In each of these cases there was a history of rapid growth. The epididymis was not involved and in no case has metastatic spread occurred after operation in periods up to two years. The affected animals were middle-aged or old dogs (5 to 11 years).

Dural endothelioma, psammoma, or meningoma.—Although only one specimen of this type has been collected, it is hardly a rare growth in the horse. The tumour, which probably develops quite slowly, is attached to and vascularized by the dura mater. It is never metastatic and only gives rise to symptoms as a result of the pressure it exerts. The commonest situation is in the falx.

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THE CHEMICAL IDENTIFICATION OF VITAMIN C

CONFIRMATION OF ACTIVITY OF A PREPARATION OF HEXURONIC ACID

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WHEN Szent-Györgyi [1] first isolated hexuronic acid in 1928, he concluded that it was identical with the reducing substance which is found in active vitamin C concentrates. The reducing substance, however, was thought by Zilva not to be identical with vitamin C. But recently Szent-Györgyi [2 3] has reported that hexuronic acid will protect guinea-pigs from scurvy (in doses of 1 mg. per day fed over the usual 90 days' test period), and he has concluded that "vitamin C is a single substance identical with hexuronic acid." This conclusion has been criticised by Zilva.[4] Independently of Szent-Györgyi, King and Waugh [5] have stated that a crystalline preparation of vitamin C which they have isolated from lemon juice has the chemical and physical properties of Szent-Györgyi's hexuronic acid.

Prof. Szent-Györgyi was kind enough to send a specimen of hexuronic acid (derived from suprarenal glands) to this laboratory for biological test, in addition to one which he submitted to Dr. Zilva at the Lister Institute. Dr. Zilva's report has just

appeared.[6] It states that while he had not enough material available for the usual 90-day preventive test, a daily dose of 1 mg. (other levels were not tested) kept scurvy from developing over a period of 55 days—even though the animals lost weight slightly (not from scurvy) during the last 15 days. Zilva concludes that the preparation contains vitamin C, but that the identity of hexuronic acid with the vitamin is not proved. Our own tests, concluded some weeks back, were carried out by quite different methods from the one used by Zilva, and, in addition, give some measurement of the approximate degree of activity of the hexuronic acid, and are now published at the request of Prof. Szent-Györgyi.

EXPERIMENTS WITH HEXURONIC ACID

The quantity of hexuronic acid at our disposal was so small (insufficient was available in our case for even the most curtailed test by the usual preventive method) that it was necessary to make use of methods which would give an indication of the activity with the most sparing use of the material. We chose two methods of test. The first, determination of the preventive action on microscopic structure of the teeth (based on Höjer's work), has been used satisfactorily in this laboratory since 1929. The second, a "recovery" test on scorbutic guinea-pigs, has been found in the course of two years' experience to give striking and quite unmistakable results, and needs only the smallest consumption of the material under test.

For the *tooth method*, graded doses of 0·5, 1, and 2 mg. per day of the hexuronic acid were fed in addition to a scorbutic basal diet (for composition, see below) to a series of normal young guinea-pigs of about 300 g. in weight, in duplicate. Reference animals received daily doses of 0, 0·5, 1, 2, and 5 c.cm. of orange juice, also in duplicate. After 11 days the animals were killed, cross-sections of the roots of the incisors made by the usual method, and the effect of the supplements on the preservation of normal structure determined, comparison being instituted between the appearance of the teeth in the hexuronic acid and the reference (orange juice) groups.

While, of course, a precise assay would have necessitated a larger group of animals (from which we were precluded by the small amount of material available), the results of the tooth test showed quite

definitely that 2 mg. of the hexuronic acid gave complete protection, 1 mg. practically complete protection, and 0·5 mg. only partial protection; 1 mg. of hexuronic acid roughly corresponded in potency with 1 c.cm. of orange juice, and 2 mg. were slightly superior to 2 c.cm. of orange juice. The numbers in the Table are given to correspond with those used

Substance and daily dose.		Degree of protection (Key and Elphick's scale).
Hexuronic acid, 0·5 mg.	.....	1, 2
„ „ 1 mg.	.....	3, 4
„ „ 2 mg.	.....	4. 4
Orange juice, 0 c.cm.	.....	1, 1
„ „ 0·5 c.cm.	.....	1, 2
„ „ 1 c.cm.	.....	3, 4
„ „ 2 c.cm.	.....	3, 4
„ „ 5 c.cm.	.....	4, 4

by Key and Elphick [7] to represent the varying degrees of protection; in reality there was a rather better demarcation between the different groups than is apparent from the numbering shown—i.e., a definite difference may sometimes be detected between two degrees of protection when both would be represented by the same form number.

In our "*recovery*" *method*, matched male guinea-pigs of about 250 g. are placed first for a preliminary period of about 10 days on the scorbutic basal diet supplemented with 15 g. per day of cabbage, and then on the basal diet alone (bran 80 parts, oats 720, egg-yolk 40, salts 8·4, cod-liver oil 1 per cent.). Within a further period of two to three weeks animals are chosen for test which are showing early symptoms of scurvy, and have begun to lose weight evenly and have dropped sharply 10 to 20 g. from their maximal weights (in the course of about three days) (see Figure). Any animals whose weight curves have been lacking in smoothness or who have shown a departure from the average normal response are discarded, as also are those giving indications of scurvy complicated by infection (a not infrequent occurrence).

Graded daily doses of the antiscorbutic supplement are then fed, and the recovery rates noted. A series of graded doses of orange juice (standard) is fed at the same time to another set of animals.* Negative controls are also taken and permitted to stay on the scorbutic diet, to demonstrate that in a given batch or litter the animals are behaving

* Like Key and Elphick,[1] we have found orange juice preferable as a standard of reference to lemon juice (the international standard).

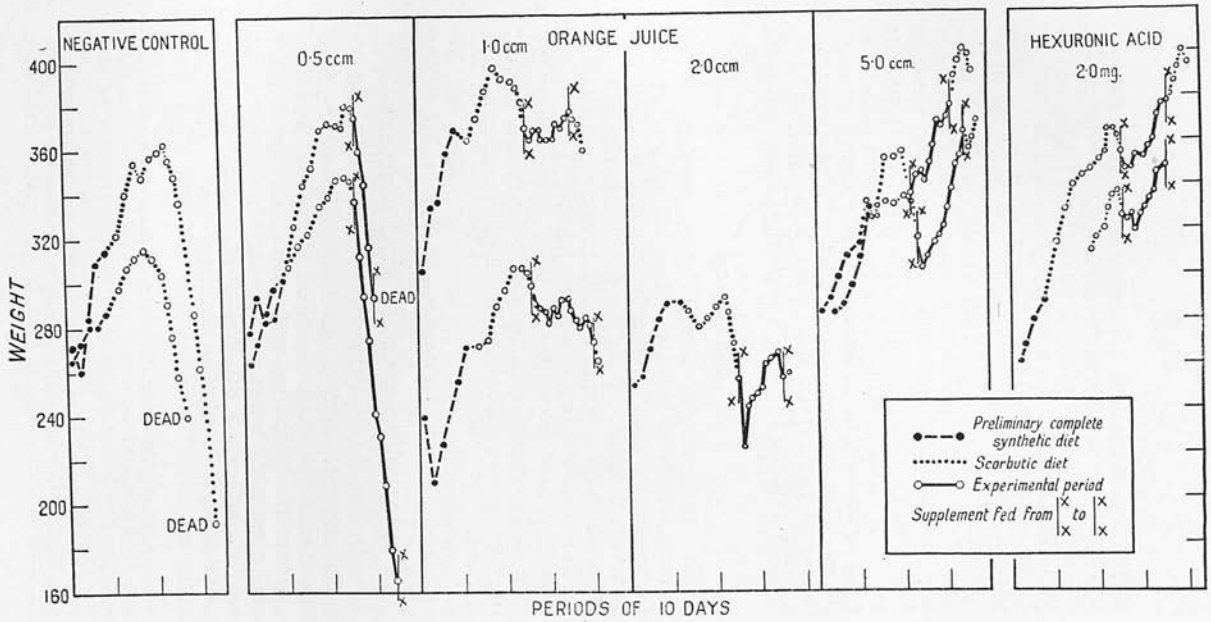
regularly, and would, in fact, continue to lose weight rapidly and die from scurvy but for the addition of the antiscorbutic supplement. After a day or two, growth is resumed at full rate, provided adequacy of vitamin C has been allowed. With partial adequacy subnormal growth results (see Figure), or the decline in weight is merely stayed. The minimum daily dose of the supplement needed to cause a given resumption in growth rate is thus determined, and a given dose of the unknown can be compared with the quantity of orange juice needed to produce an identical effect.

With a little experience the method is found to work most satisfactorily and conveniently. The demonstration of antiscorbutic activity is dramatic and unequivocal. Further advantages are that the method is economical of material, and it is of course far more rapid than the usual preventive method, since, assuming a continual supply of guinea-pigs developing scurvy, a test can safely be concluded within as short a period as ten days. Practically the only precaution is to make no use of animals which have declined too far, and would therefore fail to respond. One should of course omit from consideration animals which develop intercurrent infection or other complication to the scurvy, which likewise prevent a normal response.

We had in hand only enough material for three curative tests, but as to the conclusive nature of the result there can be no possible doubt. One animal we used for two successive tests; during the first curative period he broke his leg, and as a result showed for a time a somewhat diminished response, but this did not prevent a typical recovery in the second test. Only the second test is shown in the Figure. The results indicate that hexuronic acid at a level of 2 mg. had a curative effect equal or slightly greater than 2 c.cm. of orange juice, but less than 5 c.cm. of orange juice.†

The foregoing experiments confirm the antiscorbutic activity of the specimen of hexuronic acid in question. They throw no light on the question whether the activity is due to the hexuronic acid itself or, as is contended by Zilva, to some contaminating substance. In support of the conclusion that hexuronic acid itself is the vitamin, King brings forward the observation that in the final stages of separation of his vitamin C concentrates an approximate constancy of composition and of antiscorbutic

† As presumably some loss of activity had occurred during the process of isolation, and possibly also during subsequent storage (the specimen was not a new one), it is probable that the values obtained for antiscorbutic potency are *minimal* figures.



Growth curves of guinea-pigs showing cure of scurvy with doses of 2 mg. of hexuronic acid daily. (Weight in grammes.)

activity were reached. Further evidence as follows, consistent with the view (although by no means conclusive) that the vitamin is identical with hexuronic acid, has recently been obtained in this laboratory in the preliminary stages of an investigation by one of us (L. J. H.) in collaboration with Mr. S. N. Ray, a detailed account of which will be published later. The theory that the vitamin and hexuronic acid are one and the same substance demands that the raw suprarenal cortex should possess exceptionally powerful antiscorbutic potency, the organ being an even richer source of hexuronic acid than is orange juice. This we have determined to be the case, the degree of activity of the cortex being proportional, roughly at least, to its high content of hexuronic acid.

Summary

The antiscorbutic action of a preparation of hexuronic acid from suprarenals has been confirmed by means of a striking curative test and by the tooth-structure method. 1 mg. of the hexuronic acid (an old specimen) was found to have an activity somewhat greater than 1 c.cm. of orange juice.

Preliminary experiments have established the high antiscorbutic activity of raw suprarenal cortex, roughly proportional to its high hexuronic acid content.

Addendum

NEGATIVE EXPERIENCES WITH NARCOTINE DERIVATIVES

Experiments carried out in this laboratory,[8] in advance of the work described above, had failed to confirm the claim recently advanced by Rygh,[10] and apparently accepted in many quarters,[9] as to the identity with vitamin C of certain narcotine derivatives, methylnornarcotine, or irradiated narcotine.

In preparing these substances we followed the procedure described by Rygh as closely as possible. The vitamin C activity was tested (1) by the preventive method against scurvy in guinea-pigs; (2) by the microscopic tooth-structure method. The irradiated narcotine was tested at five levels—viz., 10, 1, 0·1, 0·01, and 0·001 mg. per guinea-pig per day—six animals being used at each level. Similar tests were

done on the methylnornarcotine. A second specimen of irradiated narcotine, kindly prepared for us by British Drug Houses, was tested at the 1 mg. level only. It was found that the incidence and development of scurvy were not affected by the narcotine derivatives, and the test animals died at the same time as the negative controls and with an equally severe degree of scurvy. The tooth method gave equally negative results—except for one anomalous result, that three animals receiving 10 mg. of methylnornarcotine showed considerably less severe tooth lesions after 10 days than the negative controls; little significance need be attached, however, to this irregularity, in view of the fact that after 18 days tooth lesions were now as severe as in the negative controls (no degree of protection), and that the survival period of animals receiving the same supplement was not increased.

On studying Rygh's account of his work a number of serious objections to his conclusions are apparent, as follows. The animals receiving his alleged active preparations survived no longer than the negative controls. Death, he supposed, was due to deficiency not of vitamin C but of some additional unspecified factor absent from his basal diet. The method he used for diagnosing scurvy had some unusual features, however ("looseness of teeth," "condition of suprarenals and intestines"), and the alleged inadequacy of the basal diet is hard to reconcile with the satisfactory results obtained by other workers on similar diets supplemented only with minute amounts of active concentrates (see present paper, for example). The improved results he obtained with addition of boiled orange juice were probably due simply to the presence in it of unchanged vitamin C. Particularly unsatisfying is Rygh's finding that the alleged active material, if fed in quite moderate excess of the supposed optimal level, again produces the same symptoms as the vitamin deficiency itself. His contention that sunlight protects animals from scurvy and synthesises the vitamin in the plant (deriving it as he supposes from narcotine) is negatived by earlier work,[10] as well as by the observation that scurvy has been rife in the tropics among people exposed to the sun.

Zilva [12] has already reported that he was unable to find any antiscorbutic activity for two hydrolysis products derived from narcotine; his negative conclusion is strengthened by our results obtained on materials prepared under the actual conditions described by Rygh.

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ON THE PATHOGENESIS OF AVITAMINOSIS A

VITAMIN A AS THE ANTI-KERATINISING FACTOR

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THE occurrence of infective troubles in vitamin-A deficient animals was noted by some of the earliest workers. This characteristic of the avitaminosis was specially emphasised by Green and Mellanby [1] in a study on rats, and led them to suggest that the vitamin be called the anti-infective vitamin. They believed that "a large number of common infective conditions are due to a deficiency of this substance in the diet of many people." (Inflammatory processes of the nasal sinuses, middle-ear disease, pneumonia, ulcerative conditions of mouth and eye, phthisis, bronchitis, acute rheumatism, and the common cold have been considered in this connexion.) On the side of therapeutic treatment, clinical trials of the effectiveness of vitamin A as an anti-infective agent were made by Mellanby and Green [2] in cases of puerperal septicæmia, and the apparently beneficial results aroused hopes that the vitamin might find wide application in the treatment of infectious diseases. In many quarters the view seems to have been adopted that vitamin A is an anti-infective agent in a quite generalised sense and presumably of value in combating all types of infections—i.e., that vitamin A is the anti-infective vitamin in the wide sense in which vitamin C is the antiscorbutic vitamin, vitamin D the antirachitic, or vitamin B the anti-beriberi. Commercial vitamin-A preparations are advocated "for the treatment of septicæmias and acute infections" and claimed to "increase the general immunity." The evidence

collected in the present paper is in support of the view that the infections which occur in vitamin-A deficiency are of a quite special type, localised in origin, and associated with the keratinisation of mucous membrane, which is regarded as characteristic of the deficiency ; and of the corollary that the anti-infective powers of the vitamin are similarly limited in scope.

Xerosis and cornification, or keratinisation, of epithelial tissues in vitamin-A deficiency has been described by Mori,[3] Wilson and DuBois,[4] and Wolbach and Howe.[5] But whereas Wolbach and Howe regard the epithelial keratinisation as the primary lesion, Yudkin and Lambert [6] conclude that the keratinisation is secondary to the inflammatory process and infection, and Wason [7] similarly supposes the xerotic changes in the cornea to be secondary to and not the cause of the infection. Goldblatt and Benischeck [8] believe that infection might be either coincident with *or* subsequent to the epithelial change, while Tyson and Smith [9] conclude that infection may occur in the absence of keratinisation. Further study of this aspect of the problem therefore seemed desirable.

It has been claimed that rats, a species it will be recalled normally highly resistant to tuberculosis, become susceptible when fed on a diet deficient in fat-soluble vitamins (attributed alternatively to vitamin A [10] or D [11]), or that they become sensitive to tuberculin shock under the same circumstances.[12] These conclusions would appear to support the theory that vitamin A has a real action in increasing immunity to pathogenic organisms, in addition to its function in maintaining the health of the mucous membrane. It was decided, therefore, to see whether confirmation could be obtained for this suggestion—with negative results, as will appear below.

Evidence from Past Work

If vitamin A possesses any generalised anti-infective action it is reasonable to seek for some change in immunological reactions in vitamin-deficient animals. All attempts in the past to show such a correlation have, however, yielded substantially negative results (examination having been made of: agglutinins, complement, amboceptor, precipitins, bacteriolysins, hæmolysins, and immunising power of vaccination). A second line of approach is to determine whether inoculation with suitable micro-organisms or toxins is more lethal to an animal on a diet low in vitamin A than to an animal on a normal diet. Several workers claim to have obtained indications of a positive effect of this kind. A serious difficulty of interpretation arises here however. It is obvious that an animal suffering from the double disability of toxæmia and vitamin deficiency is likely to fare worse than one suffering from either alone. That is, the effect may be merely additive, and indicate no genuine direct action of the vitamin upon resistance. In my opinion, the effects recorded in the literature have rarely, if ever, been sufficiently striking to dispose of this

criticism. On the other hand, there is some evidence that when micro-organisms are fed per os there is a tendency to greater absorption, and hence more severe ill-effects, in vitamin-A deficient as compared with normal animals (see below); which accords with the theory that in vitamin-A deficiency the integrity of the mucous membrane breaks down and ceases to function as an efficient barrier.[13] This consideration suffices to account for the many recent experimental findings which would otherwise appear to indicate a general anti-infective action for the vitamin, and have, in fact, been so interpreted. Against this trend in recent conclusions we may recall that, as long ago as 1924, Cramer and Kingsbury[14] found that vitamin-A deficient rats were no more susceptible than normal rats when the micro-organisms were injected subcutaneously; while Topley, Greenwood, and Wilson[15] have reported that large amounts of vitamin A added to a normal diet fail to reduce the severity of epidemic infections in mice.

Clinically vitamin-A therapy has yielded negative results in trials as a prophylactic against respiratory infections[16] and common cold[17] in infants, or the incidence of common infections generally[18]; it had a doubtful value in treatment of pneumonia[19] and failed to increase streptococcal immunity in pregnant women as judged by the Dick test.[20] In puerperal sepsis the occurrence of infection at the mucous membrane may provide a rational basis for vitamin-A prophylaxis, but, as pointed out by Mellanby, the number of cases so far treated does not yet permit of any final conclusions as to its value, having regard to the great variability of prognosis of individual cases.[21] The same difficulties as to natural variations in virulence and mortality, and the relatively small number of cases treated, make it even more difficult to assess the results of vitamin-A therapy in puerperal septicæmia.

Development of Lesions of Avitaminosis A in Rats

(*With* Dr. J. R. M. Innes)

Our object in this side of the investigation was to observe the slowly developing lesions of a- or hypovitaminosis A, with the hope of throwing light on their earliest manifestation, and order of appearance, in particular, if possible, to determine whether the structural changes in epithelia appeared to precede the infections.

Large groups of rats (26 animals were used in the principal experiment) were put on a vitamin-A free diet, consisting of sugar, 60 parts; purified casein (Glaxo brand), 20 parts;

hardened cotton-seed oil, 15 parts; marmite, 10 parts; salt mixture, 5 parts; with a daily supplement of Radiostol (vitamin D). In order to permit of a slow development of symptoms, a minimal provision of vitamin A (as red palm oil) was given once the animal had begun to lose weight, sufficient being allowed to prevent too early death or rapid loss in weight, but insufficient to enable good growth to be resumed. Representative animals were killed every few days, from 17 days after the beginning of the experiment, up to 90 days. After post-mortem examination the following tissues were prepared for histological examination by the usual routine methods: eye, tongue, submaxillary and sublingual glands, pharynx, œsophagus, trachea, lungs, stomach, intestines, liver, spleen, kidney, bladder, testes, and seminal vesicles (or uterus).

Perhaps the most important general observation was that in all instances where infection had occurred keratinisation of epithelium could without exception be detected, including generally in sites not far removed from the infection. It is important to note that the infections are of a highly distinctive character, apparently always originating in the neighbourhood of an epithelium. Thus small abscesses seen in the medulla of the kidney in the later stages appeared to have spread from the pelvis; infectious lesions in the lungs from the bronchi; and those in the tongue, from the surface of the mucous membrane. The probability that the infections are secondary to the structural changes in the epithelium which follow on vitamin-A deficiency seems obvious. But the hope that one might be able to demonstrate, in the group of animals taken as a whole, two definite stages, first keratinisation without infection, leading later to a secondary infection, was in general not realised.

It is true that in certain sites, notably in the sublingual (and submaxillary) glands, which are the seat of the most constant and characteristic lesions, the early condition is slight keratinisation (in the ducts) with little or no infection, and the later stages keratinisation accompanied by increasingly severe infection. Thus in one series of animals half way through a slowly developing vitamin-A deficiency, 5 animals in serial order showed slight keratinisation without infection, and the next 9 in order—the avitaminosis now reaching more advanced stages—showed definite infection. In many sites, however, no clear-cut division into two stages, keratinisation without infection and keratinisation giving rise to infection, could be demonstrated.

In general, keratinisation and infection both increased gradually and concurrently in severity. The failure to find two separate stages in the development of the avitaminosis, throughout the body as a whole and in the whole group of animals, may be attributed firstly to the irregular, almost haphazard, order in which the epithelia become keratinised in the different animals in the group, and second, to the fact that

infection may sometimes supervene almost simultaneously with the first detectable keratinisation.

When above we are using for convenience the term "keratinisation," we have of course in mind not one single factor but a series of structural abnormalities seen in the epithelia, comprising xerosis, keratinisation, desquamation. Secreting epithelium loses its normal character and becomes converted into a stratified, keratinised, desquamating structure, or in the cases of tissue like the epithelium of the kidney pelvis, or tongue, a hyperplasia is concerned in bringing about an analogous condition. It is easy to understand how these changes would conduce to local infections, and account for the observed picture. The xerosed membrane being deprived of its normal secretion, micro-organisms are not retarded from settling on its surface; apart from the more physical, or flushing, action of the secretion, its natural antiseptic properties, ascribed to lysozyme, must also be borne in mind. Secondly, the accumulation of a profuse debris of desquamated cells along the surface of a membrane will tend to set up irritation and cause severe obstruction, particularly in narrowly enclosed areas. Good evidence of ducts becoming in fact choked in this way and so rendered liable to infection is found in the experimental pictures of the ducts of the tongue, of the sublingual glands, and of the bronchi; these are commonly seen to be filled with desquamated epithelial cells, distended and infected. The pelvis of the kidney among other sites showed a similar condition.

The fact that vitamin A given to an animal not too far advanced in the avitaminosis will cure the diseased epithelia, and at the same time restore the animal to normal health, is further evidence for regarding the epithelium as the essential point in the pathogenesis of the infection. The local infection is, clearly enough, due to fortuitous micro-organisms which would normally prove harmless. If the condition is allowed to develop far enough, a general septicæmia may ultimately supervene. This can be attributed, beyond much doubt, to absorption of such micro-organisms through the walls of the perverted membrane which is no longer able to serve as an efficient barrier. Blood cultures confirm this conclusion, and there is little in common with acute clinical infections by specific pathogenic organisms to suggest that any benefit is likely to accrue from their treatment by vitamin A.

Influence of Vitamin-A Deficiency on Tubercle Infection in Rats

(*With* Dr. A. S. Griffith)

Two experiments were carried out, the first of a more general character, to test the suggestion that in vitamin-A deficiency rats lose their normally high resistance to tuberculous infection, and the second to examine more particularly the effects of the

increased absorption of the micro-organism from the alimentary tract which, on the grounds of the theory adopted in this paper, it was anticipated might be seen in vitamin-A deficient animals.

Expt. 1.—A large group of rats was put on a vitamin-A deficient diet consisting of casein (Glaxo brand), 20 per cent.; rice starch, 60 per cent.; dried yeast, 15 per cent.; salt mixture, 5 per cent.; radiostol 1 drop per rat per day; and after depletion, 6 animals which showed typical severe xerophthalmia were selected for test. These were then instilled in both eyes (one eye only in the case of an animal which had one eye completely closed), and in addition fed by pipette with an emulsion of a culture of bovine tubercle bacilli. Three normal control rats, which had been on a similar diet, but containing vitamin A in the form of cod-liver oil, were treated identically at the same time. The vitamin-deficient group were kept in a state of hypovitaminosis A, being given occasionally minimal allowances of vitamin A, sufficient to protect them from early death, but not sufficient to promote good growth. (The amount so needed was determined from controls in a third group, uninfected, but vitamin A deficient.) The infected vitamin-A deficient animals died after periods ranging from 20 to 93 days, and the controls were killed on the 47th and 97th days. Results were assessed on the basis of the following: macroscopic observation on autopsy, microscopical examination of lung, and cultures derived from the submaxillary and mesenteric glands. A similar picture was found in both the infected groups, vitamin A deficient and normal, and resembled that seen in previous experience in normal rats, after the same period of time after inoculation. In the lungs no more than two or three small greyish foci at the most could be detected, and there was little or no microscopic evidence of tubercle, while the average number of colonies cultured from the submaxillary and mesenteric glands was similar for the vitamin A deficient and the normal group.

It was concluded that under the conditions of this test the vitamin-A deficient rats showed no greater susceptibility than normal rats to tuberculous infection.

Expt. 2.—This experiment differed principally from the foregoing in the particular that the rats were not infected by a single exhibition of the micro-organism, but were fed it continually by mouth during the time they were suffering from the vitamin-A deficiency, with the aim of procuring a greater degree of absorption through the abnormal mucosa.

The diets were similar to that described for the preceding experiment. During a period of six weeks groups of rats on the vitamin-A deficient and on the normal diets received on eight occasions, with their food, in doses of 10 c.cm., mixed sputum from patients with phthisis. Several rats died after 82 to 116 days, and the others were killed at from 112 to 119 days for comparison. A comparison of the number of colonies obtained from the mesenteric glands suggested the possibility that there had been a somewhat greater absorption of tubercle bacilli in the vitamin-A free group, than in the group on normal diet. No macroscopic tuberculous lesions were observed at autopsy in the mesenteric glands or in the organs, in rats on either vitamin-

deficient or normal diet, and the failure to find bacilli in emulsions of the lungs indicated that there had been little if any dissemination of tubercle bacilli.

It was concluded therefore that absence of vitamin A from the diet had not lowered the resistance of this refractory species to any extent such as to allow the invading tubercle bacilli to produce macroscopic tuberculous lesions.

Summary and Conclusions

1. A study has been made of the development of the lesions of a- or hypo-vitaminosis A in rats. The metaplasia (or hyperplasia) and keratinisation of epithelium is confirmed, and its loss of normal structure and function regarded as the essential feature of the deficiency. The infections found in vitamin-A deficiency are of a special type, limited in origin to epithelial tissues, and not seen in the absence of neighbouring keratinisation. They appear, that is, to be attributable to the xerosis, deficient secretion of mucus, and desquamation (which may set up local irritation, and obstruction in ducts, &c.). With provision of vitamin A, the epithelium becomes normal again and the local infections disappear. If the avitaminosis is allowed to develop the local infections spread, and the destruction of the integrity of the epithelium is presumably responsible for absorption of the fortuitous infecting micro-organisms (normally non-pathogenic), and septicæmia may result.

2. The claim that rats become susceptible to tuberculosis when depleted of vitamin A, which appears to support the theory of a generalised anti-infective action for the vitamin, could not be substantiated. When the bacillus was fed by mouth some evidence was obtained suggestive of increased absorption in vitamin-A deficient rats, but not of any lowered general resistance to the spread of tuberculous disease.

3. The existing data afford no basis for the belief that vitamin-A therapy is likely to be effective in combating acute general infections due to specific highly pathogenic micro-organisms, or in those clinical toxæmias and infectious diseases which are unassociated with the peculiar structural breakdown of epithelial tissue, and the attendant localised infection, which characterises the vitamin deficiency.

The importance of ensuring that diets are adequate in vitamin A cannot, of course, be gainsaid. But measurements by my colleague Dr. Thomas Moore,

to be presented in a later paper, show that in various types of commonly occurring infections, terminated by death, there were often abundant vitamin-A reserves, so that any advantage to be derived from the administration of the vitamin in such cases, with the aim of rectifying a non-existing deficiency, would remain to be proved.

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Beitrag zur Wirkung des Cyanids auf die Schilddrüsen der Ratte

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I. Einleitung. Im Jahre 1928 beobachteten Chesney, Clawson und Webster bei experimentellen Arbeiten über Syphilis das Auftreten von Kropf bei Kaninchen, die längere Zeit mit einer großen Menge von Kohl gefüttert waren. Die Entwicklung dieses Kropfes war mit einer Herabsetzung des Stoffwechsels verbunden. Geringe Gaben von Jod hoben den Stoffwechsel wieder und verhinderten die Hyperplasie der Glandula thyreoidea. Histologisch war der erzielte Kropf eine Struma parenchymatosa diffusa. Daher sprach man das ganze Bild als einen einfachen Kropf an. Weitere Studien von Marine, Baumann und Cipra, Webster und Chesney in den Jahren 1929—31 dienten dazu, die Natur des kropferzeugenden Agens, das man im Kohl vermutete, zu bestimmen. Die Ergebnisse ihrer systematischen Untersuchungen können im folgenden kurz zusammengefaßt werden:

1. Man fand eine jahreszeitliche Verschiedenheit in der kropferregenden Eigenschaft des Kohles.

2. Die Möglichkeit, daß der Kropf allein durch absoluten Jodmangel bedingt sein könne, konnte ausgeschlossen werden: starke Unterschiede im Jodgehalt des Kohles blieben ohne Einfluß auf seine kropferzeugende Eigenschaft.

3. Die Ursache des Kropfes wurde auf einen relativen Jodmangel zurückgeführt, der wiederum von Faktoren abhängig ist, die im Kohl liegen und einen steigenden Bedarf für Jod enthaltendes Hormon verursachen.

4. Den größten Erfolg bei der Kropferzeugung erzielte man mit Cyanid, das ein charakteristischer Bestandteil aller Vegetabilien der Brassica-Art ist und eine Komponente ihrer Glykoside darstellt. Später konnten Marine, Baumann, Spence und Cipra (1932) zeigen, daß

tägliche subkutane Injektionen von geringen Mengen Cyanid bei jungen Kaninchen nach 21 Tagen eine Hyperplasie der Schilddrüse erzeugen. Und weiter, daß von allen Cyaniden das Methyl-Cyanid die stärkste kropferzeugende Fähigkeit besitzt. Jodgaben verhüteten das Auftreten der Hyperplasie ebenso leicht, wie es dasselbe bei anderen Kropfexperimenten tat. Später berichteten Spence und Marine (1932) über eine Vergrößerung und Hyperämie der Schilddrüse bei Ratten und Mäusen nach 28- bis 36tägiger subkutaner Injektion von Methyl-Cyanid. Sie bestätigten, daß die Ratte anscheinend eine höhere Toleranz für Methyl-Cyanid besitzt im Vergleich mit den Kaninchen, und daß die Erscheinungen, die sie in der Schilddrüse beobachteten, viel geringer waren als jene, wie man sie bei Kaninchen schon nach relativ kleineren Dosen erzielt. McCarrison (1933) in Indien bestätigte im großen und ganzen diese Befunde, doch war er nicht imstande, bei guternährten Ratten durch Methyl-Cyanid-Injektionen nach 35 Tagen Kropf zu erzielen. Er beobachtete keine Unterschiede im Gewicht der Drüsen der Versuchs- und Kontrolltiere.

Die Schlußfolgerung Marines und seiner Mitarbeiter bestand darin, daß er die Existenz von kropferzeugenden Substanzen in gewissen Pflanzen, Kohl usw. annahm, die ihre Wirkung durch Herabdrücken der Gewebsoxydation anzeigen, und diesen Stoff hielt er aller Wahrscheinlichkeit nach für das Cyanid. Freilich verliefen Versuche, Cyanide aus Kohl in hinreichender Menge zu isolieren, ergebnislos. Wenn ein Herabsinken der Gewebsoxydation auftritt, eben hervorgerufen durch Gaben von exogenen Cyaniden, so schien es, daß die Schilddrüse eine kompensatorische Anstrengung macht, um durch Überproduktion des Aktivators der Oxydation, nämlich des Thyroxins, einen Ausgleich herzustellen. Als weitere Folge hiervon gibt es einen relativen Jodmangel und als weitere Folge eine Hyperplasie der Schilddrüse. Diese Autoren behaupten, daß eine solche Auffassung gut mit der jetzigen Auffassung und Kenntnis des einfachen Kropfes übereinstimme. Nach ihnen erklären diese Beobachtungen folgendes: Bei geringen Jodgaben gibt es keinen Kropf, wenn die Produktion von Cyaniden im Körper unter der wirksamen Schwelle liegt oder wenn der Entgiftungsmechanismus für das Cyanid ausgezeichnet funktioniert. Weiter können auch hohe Jodgaben eine Kropfbildung nicht verhüten, wenn eine ausgedehnte Cyanidbildung im Körper stattfindet. (Eine Übersicht über alle diese Befunde wurde von Webster [1933] auf der Internationalen Kropfkonferenz in Bern gegeben.) Bei all diesen Studien fand man, daß gewisse Faktoren eine große Rolle spielen, vor allem, daß in bestimmten Futtersorten positiv kropferzeugende Faktoren vorhanden sind, im Gegensatz zu den Jodmangelerscheinungen des Kropfes. Dies führte dazu, daß man den durch Kohl erzeugten Kropf mit der Wirkung der Cyanide verband, und Marines Hypothese verdient erhöhte Beachtung bei der Diskussion der Ätiologie des Kropfes. (In gleichem Sinne verdient eine Beobachtung V. Suk's [1931] Erwähnung, der in den Kropfzentren der Karpathen eine einseitige Ernährung der Bevölkerung mit

Sauerkraut nachweisen konnte, die sich besonders bei den weiblichen Personen der armen Bevölkerung zeigte.)

Da diese Methode eine große praktische Bedeutung besitzt bei der künstlichen Kropferzeugung und bei dem Studium aller Phasen dieser Krankheit, hielt ich es für nötig, die Cyanidversuche an Ratten zu wiederholen, aber mit jüngeren Tieren, als sie Spence und Marine gebraucht haben, um zu sehen, ob sie vielleicht empfänglicher seien, und eine ganz genau und sorgfältig kontrollierte Diät anzuwenden.

II. Methoden. Synthetische Basis-Diät.

Kasein	20 Teile
Stärke	60 „
Salzmischung	5 „
Arachidis-Öl	15 „

Jede Ratte erhielt als Zugabe zu ihrem Futter täglich 2 Tropfen Lebertranöl und $^1/_2$ g trockene Hefe.

Zusammensetzung des Salzgemisches:

	Natriumchlorid	50 g
	Kalziumphosphat	165 „
	Kalziumlaktat	400 „
Merck	Eisenzitrat	35 „
	Magnesiumsulfat	80 „
	Mononatriumphosphat	105 „
	Kaliphosphat	290 „

Als das Futter fertig gemischt war, wurde wahllos ein wenig desselben genommen und von Dr. Widman an der Chirurgischen Klinik in Freiburg analysiert. Es stellte sich heraus, daß die Diät beinahe jodfrei war; der Analysenbericht zeigte, daß die Mischung in 3 Proben gar kein Jod enthielt und die untere Fehlergrenze der Methode 0,1 γ auf 1 g beträgt. Selbst wenn man annehmen will, daß die Ratte täglich 10 g ihres Futters fräße, so könnte dennoch die größte Menge Jod, die sie demnach in einem Tag zu sich nähme, 1 γ nicht überschreiten, in allen Fällen bliebe sie wesentlich darunter.

29 junge, weibliche weiße Ratten im Gewicht von 65—100 g wurden in folgende drei Gruppen geteilt.

Gruppe A. Jodfreie Diät, keine Zugaben von Jod. 9 Ratten wurden 5 Tage lang vor dem Versuch auf oben beschriebene Diät gesetzt. Sie erhielten dazu destilliertes Wasser in Spezialtrinkflaschen. Hiernach erhielten 6 dieser Ratten täglich subkutane Injektionen von Methyl-Cyanid, in $^1/_2$ ccm destilliertem Wasser gelöst, mit ansteigenden Mengen, zuerst 0,05 ccm und zuletzt nach 8 Tagen 0,1 ccm. Die restlichen 3 Ratten dienten zur Kontrolle, d. h. sie erhielten die Standarddiät und das destillierte Wasser, aber keine Cyanidinjektionen.

Gruppe B. Geringe Jodzugaben im destillierten Wasser. 9 Ratten wurden auf die gleiche Diät gesetzt, jedoch erhielten sie Na-

triumjodid dem Trinkwasser zugesetzt, und zwar 2 γ auf 10 ccm. (Nach Angaben von Remington [1932] entspricht diese Menge ungefähr dem Tagesoptimum an Jod, das die Ratte bedarf, in der Annahme, daß die Ratte täglich ungefähr 10 ccm Wasser trinkt.) Nach einer 5tägigen Fütterungsperiode wie in Gruppe A erhielten 6 Ratten Methyl-Cyanid-Injektionen mit destilliertem Wasser, genau wie Gruppe A. (0,05 ccm täglich in steigenden Dosen bis zu 0,1 ccm innerhalb 8 Tagen.) Wieder wurden die drei übrigbleibenden Ratten als Kontrolle benutzt.

Gruppe C. Jodreiche Diät. 12 Ratten wurden auf die synthetische Standarddiät gesetzt, zu deren Salzmischung vorher noch 0,5 mg NaJ auf 10 g Futter hinzugesetzt wurden. Dem Wasser wurde kein Jod mehr beigegeben. (Die Aufnahme von Jod muß bei diesem Futter den täglichen Bedarf weit überschreiten.) Nach einer Fütterungsperiode, wie sie oben schon beschrieben wurde, erhielten 10 Ratten täglich Einspritzungen mit Methyl-Cyanid (0,03—0,1 ccm), und die beiden übrigbleibenden Tiere wurden zur Kontrolle benutzt.

Alle Ratten konnten so viel fressen und trinken, als sie wollten. Wasser bekamen sie in Spezialtrinkflaschen, um äußere Einflüsse von etwa in der Umgebung vorhandenem Jod auszuschließen. Das Futter wurde für den gesamten Versuch auf einmal hergestellt, um Fehlerquellen, die in den verschiedenen Verunreinigungen der Chemikalien vorhanden sein konnten, auszuschließen. Das Futter der verschiedenen Gruppen wurde in verschiedenen Gläsern mit eingeschliffenem Deckel aufbewahrt (Eisraum). Die Versuchstiere wurden jedes für sich in einem besonderen Käfig mit Holzboden aufbewahrt, der vorher sorgfältig gereinigt und getrocknet wurde. Die Tiere erhielten keine Streu. Die Käfige wurden täglich gemistet. Täglich wurde Gruppe A zuerst gefüttert, um auch die Übertragung von ganz kleinen Mengen von Jod zu vermeiden. Alle diese Vorsichtsmaßregeln sind nötig und müssen peinlich beachtet werden, wenn man daran denkt, wie viele Mißerfolge es schon bei experimentellen Kropfarbeiten gegeben hat, wo man weiß, daß schon ganz geringe Mengen fremden Jodes falsche Resultate erzeugen können. Die Ratten wurden täglich gewogen. Einige starben, und ein paar wurden schon während des Experimentes getötet. 36 Tage nach den täglichen Cyanid-Einspritzungen wurden auch alle Überlebenden mit Chloroform getötet. Die Schilddrüse wurde dann sorgfältig herauspräpariert und vom umgebenden Gewebe befreit, darauf so schnell wie möglich gewogen und so rasch wie möglich in die Fixierlösung gebracht, die aus einer schwachen Lösung Formalin in Kochsalzlösung bestand. Da man nur sehr schwer einwandfrei das Gewicht eines so kleinen Organes, wie das der Schilddrüse, ohne Gewichtsverlust bestimmen kann — denn Wasserverluste bedeuten bei dem geringen Gewicht sehr viel —, muß diese Prozedur so schnell wie möglich ausgeführt werden. Die Drüsen wurden wie üblich in Paraffin eingebettet und Schnitte von beiden Lappen gemacht. Färbungen mit Hämatoxylin-Eosin.

III. Resultate. A. Wirkung der Cyanid-Injektionen auf das Körpergewicht. Bei der vorausgehenden Fütterungsperiode, wenn die Ratten von der gewöhnlichen Diät zu der synthetischen Diät wechseln, tritt ein leichter Abfall des Gewichtes auf (10—20 g). Aber am Ende der 5 Tage dauernden Futterperiode hatten die Tiere ihr altes Gewicht wieder erreicht und die Cyanidinjektionen begannen. Alle Ratten der drei Gruppen, die Cyanid erhielten, zeigten schon nach ein paar Tagen Verlust von Körpergewicht.

Ihre Gewichtskurven blieben stehen und blieben so bis zum Ende des Experimentes (s. Tab. 1). Im Gegensatz hierzu stieg das Körpergewicht der Kontrolltiere aller drei Gruppen, die kein Cyanid erhielten, stetig an und zeigte das Bild einer normalen Wachstumskurve. Die Menge der Cyanidgaben war also ganz ausreichend, eine toxische Wirkung zu erzielen, wie es sich in der Wachstumshemmung ausdrückt. Dies zeigte sich ganz unabhängig von den Jodgaben gleichmäßig bei allen Gruppen. Tab. 1 gibt die Körpergewichte im Anfang und am Ende des Experimentes an, ebenso die Gewichte der Schilddrüsen.

Tabelle 1

Gruppe	Versuchstiere					Kontrolltiere				
	Ratte Nr.	Tage	Körpergewicht g bei Beginn	Körpergewicht g am Ende	Schilddrüsengewicht mg	Ratte Nr.	Tage	Körpergewicht g bei Beginn	Körpergewicht g am Ende	Schilddrüsengewicht mg
A	1	36	65	70	10,1	1	36	100	165	12,7
	2	36	85	80	11,6	2	36	65	120	17,0
	3	21	95	75	11,8	3	36	65	105	13,6
	4	36	80	95	15,2					
	5	23	75	75	8,6					
	6	36	85	80	14,0					
B	1	36	80	90	12,5	1	36	70	95	10,1
	2	32	70	80	12,5	2	36	100	165	12,5
	3	36	80	90	11,7					
	4	36	85	100	11,3					
	5	36	80	100	12,9					
	6	33	85	100	12,8					
C	1	29	70	85	8,7	1	36	70	115	11,1
	2	36	80	75	8,2	2	36	75	120	8,2
	3	36	75	75	8,8					
	4	36	90	105	6,5					
	5	36	80	78	9,3					
	6	29	75	70	7,8					
	7	29	115	85	10,4					
	8	36	105	85	7,2					
	9	29	115	70	6,0					
	10	36	110	95	6,1					

Gruppe A = Jodfreie Diät und Aqua dest. Gruppe B = Jodfreie Diät und Aqua dest. mit 2 γ NaI pro 10 ccm Wasser. Gruppe C = Diät mit 0,5 m NaI pro 10 g Futter. Kontrolltiere = Dieselbe Diät ohne Cyanid.

B. Wirkung an dem Frischgewicht der Schilddrüse. Wie aus Tab. 1 hervorgeht, muß man mit einer individuellen Schwankung des Gewichtes rechnen. Vergleicht man das Gewicht der verschiedenen Grup-

pen mit ihren Kontrollen, so muß man folgendes beachten: 1. Es gibt grundsätzliche Schwierigkeiten, das Gewicht eines so kleinen Organes, wie das der Schilddrüse, zu bestimmen; Schwierigkeiten in der Präparation, Verschiedenheit des Wassergehaltes, Austrocknung; 2. Beziehungen von Körpergewicht und Schilddrüsengewicht und 3. die Tatsache, daß alle Ratten, die Cyanid erhielten, mehr oder weniger Wachstumsstillstand zeigten, der auch auf das Schilddrüsengewicht Einfluß haben kann. Ganz kleine Differenzen sollten jedoch völlig unbeachtet bleiben. Bei meinen Tieren zeigte sich kein deutlicher Unterschied zwischen 1. den Ratten aller drei Gruppen, die Cyanid erhielten, und ihrer entsprechenden Kontrolle; 2. kein Unterschied einer Gruppe zu einer anderen. Bei oberflächlicher Betrachtung sieht es so aus, als ob die Ratten, die am meisten Jod bekommen hätten (Gruppe C), die niedrigsten Schilddrüsengewichte aufwiesen, jedoch sind die Unterschiede im Gewicht so gering, daß man sie als bedeutungslos ablehnen muß. Wenn man überhaupt ein stärkeres Wachstum und Gewichtszunahme der Schilddrüse als Grund ansieht, diese als Kropf zu betrachten, so muß schon ein ganz bedeutendes Anwachsen im Vergleich mit den direkten Kontrollen zu sehen sein. Wir konnten das bei unserem Experiment nicht feststellen. Spence und Marine [1932] beschrieben nur nach Betrachtung mit bloßem Auge, daß ihre Schilddrüsen doppelt so groß wären als die ihrer Normaltiere.

Tabelle 2. mg Schilddrüsengewicht pro 100 g Körpergewicht

Gruppe	Versuchstiere	Kontrolltiere
A	15,1	11,0
B	13,1	8,6
C	9,5	8,2

Auf Tab. 2 sind die Drüsengewichte der verschiedenen Gruppen im Durchschnitt berechnet und dann mit dem durch 100 geteilten Körpergewicht in Beziehung gebracht. Tab. 2 führt zu folgender Überlegung: a) Das Schilddrüsengewicht der Ratten, die Cyanid erhielten, zeigt eine geringe Vermehrung, wenn man es mit dem der entsprechenden Kontrollen vergleicht. b) Steigende Gaben von Jod heben die Cyanidwirkung auf. Die Drüsen der Tiere mit jodfreier Diät (A) sind schwerer als die Drüsen in Gruppe B. c) Die verschiedenen Jodgaben der Kontrollgruppen lassen eine deutliche Wirkung an der Schilddrüse der Kontrollen erkennen, die Gewichte sind am niedrigsten in Gruppe C und am höchsten in Gruppe A. Wenn man überhaupt Werte wie diese als Beweis für eine Schilddrüsenhypertrophie bringen will, so gibt es viele Einwände hiergegen, und man mißt vielleicht den Unterschieden mehr Bedeutung bei, als sie wirklich verdienen. Bei meinen Versuchen sind in einigen Fällen die Differenzen so klein, daß man gar nicht daraus schließen darf, daß Cyanid wirklich eine kropferzeugende Wirkung bei Ratten besitzt. Dann sollte wenigstens das Anwachsen der Größe und des Gewichtes der Schilddrüse ganz klar zu sehen sein und sich nicht auf so kleine Unterschiede stützen.

Ich möchte daher betonen, daß Größe und Gewicht meiner Schilddrüsen nach 36 Tagen nicht deutlich verändert waren 1. trotz genau bekannter Unterschiede im Jodgehalt der Nahrung (siehe die Gewichte der verschiedenen Kontrollgruppen); 2. trotz täglicher subkutaner Injektion vom Methyl-Cyanid, unabhängig vom Jodgehalt, alles im Vergleich mit direkten Kontrollen.

C. Mikroskopische Untersuchungen. Die makroskopischen Befunde aller Tiere können im folgenden kurz zusammengefaßt werden: Die zum Versuch verwandten Ratten aller Gruppen, die entweder während oder am Ende der Versuchsdauer von 36 Tagen getötet wurden, zeigten völlig gesunde Organe. Alle Ratten zeigten einen schlechten Ernährungszustand, der sich im Fehlen von subkutanem Fettpolster, Fettdepotschwund zeigte und im Gegensatz zu der guten körperlichen Verfassung der Kontrolltiere stand. Ratte Nr. 3 Gruppe A, Nr. 2 Gruppe B, Nr. 7 und 9 Gruppe C starben nach 23, 32 und 29 Tagen. Keines dieser Tiere zeigte pathologische Veränderungen an den inneren Organen, woraus man auf die Todesursache hätte schließen können. Infolgedessen führe ich ihren Tod auf die Cyanidwirkung zurück, die den Organismus anscheinend sehr schwächt. Nr. 5 Gruppe A starb nach 23 Tagen Injektion und zeigte bei der Sektion eine hämorrhagische Nephritis. Nr. 6 Gruppe B starb nach 33 Tagen an Bronchopneumonie.

Die Organe der verschiedenen Tiere jeder Gruppe wurden geschnitten und gefärbt, jedoch zeigte sich, daß die Cyanidinjektionen zu keiner Gewebsveränderung geführt hatten außer zu einer Veränderung der Schilddrüse. Weder an Herz, Lunge, Leber, Niere, noch Milz konnte ich irgendeinen pathologischen Befund erheben.

D. Histologische Untersuchungen der Schilddrüsen. Beide Lappen der Drüsen aller Ratten wurden untersucht. In Gruppe A und B konnte ich keine Unterschiede zwischen den Drüsen der zum Experiment verwandten und der Kontrollratten feststellen und kann sie deshalb kurz gemeinsam beschreiben. Die Follikel waren regelmäßig, gleich an Größe, nicht vergrößert. Viele enthielten kein Kolloid, aber im allgemeinen waren sie doch kolloidhaltig. Das Kolloid färbte sich nur ganz schwach mit Eosin. Das Epithel zeigte keine starken Veränderungen. An einigen Stellen waren die Zellen höher, das Protoplasma zeigte Vakuolen; aber im großen und ganzen zeigte das Epithel kubische Form. Hyperämie war nicht vorhanden, dagegen an manchen Stellen umfangreiche Desquamation, dort, wo die Drüse während der Präparation und des Wiegens beschädigt war. In Gruppe C zeigte sich als einziger Unterschied zu den anderen Gruppen ein ganz geringer Unterschied im Kolloidtyp. Dies war homogen und färbte sich dunkel mit Eosin, jedoch beobachtete ich das gleiche bei den Kontrollen, auch hier keine deutliche Zellhyperplasie und keine Hyperämie.

Aus dem histologischen Bild geht hervor, daß subkutane Injektionen von Cyanid keine deutlichen Veränderungen in den Schilddrüsen hervorrufen, auch nicht bei Fehlen von Jod. Sicher treten keine Veränderungen

auf, die man als Kropf bezeichnen kann. Als einziges Ergebnis, soweit man bei diesen Experimenten überhaupt etwas beobachten konnte, kann man das Auftreten von schwach gefärbtem, follikulärem Kolloid betrachten, das sich in den Schilddrüsen der Ratten zeigte, die eine jodfreie oder jodarme Diät erhielten, im Gegensatz zu dem sich dicht und homogen färbenden Kolloid der Drüsen der Tiere, die mehr Jod erhielten.

IV. Diskussion und Zusammenfassung. Wir sind nicht imstande, die Beobachtungen Spences und Marines (1932) zu bestätigen, die behaupten, mit täglichen subkutanen Injektionen von großen Dosen Methyl-Cyanid bei Ratten einen Kropf zu erzeugen. Unsere Resultate stimmen überein mit den Befunden von McCarrison (1933). Weder das einzelne Gewicht der Schilddrüsen jedes einzelnen Tieres, noch das durchschnittliche Gewicht der verschiedenen Gruppen mit verschieden hohen Jodgaben, noch die histologischen Bilder berechtigen uns zu der Behauptung einer Hyperplasie der Schilddrüse. Der Unterschied, den wir im Kolloidtyp feststellen konnten bei Ratten, die eine jodfreie oder jodarme Diät erhielten, zu denen, die höhere Joddosen bekamen, ist auf den mangelnden Jodgehalt zurückzuführen. Dieselbe Erscheinung konnten wir an den Drüsen der Versuchstiere und ihrer entsprechenden Kontrollen feststellen. Die deutliche qualitative Wirkung auf das Kolloid der Schilddrüse bei mangelnden Jodgaben nach 36 Tagen ist vielleicht von einiger Bedeutung. Es wird von vielen Seiten bestritten, daß Jodmangel allein bei Ratten zu Kropf führen kann. Hellwig (1931) und Jackson und P'An (1932) gelang es nicht, nach langer Fütterung einer jodarmen Diät Kropf zu erzielen, dagegen erzeugte Tanabe (1925) relativ leicht mit dieser Diät einen Kropf. Wenn man Jod als Antagonist des joderzeugenden Cyanids betrachtet, so muß man erwarten, daß stärkere positive Resultate bei jodfreier Diät oder niedrigen Jodgaben bei Anwendung von Cyanid auftreten. Unsere Befunde zeigten aber, daß die Wirkung des Cyanids auf die Schilddrüse ganz unabhängig von den Jodgaben war. Natürlich ist es schwierig, die exakten Resultate von Spence und Marine (1932) genau zu bestimmen und sie mit unseren zu vergleichen, da sie in ihrer kurzen Mitteilung gar keine Angaben gemacht haben. Sie fütterten ihre Ratten mit einer Naturdiät mit inkonstantem Jodgehalt. Sie benützten keine Kontrollen, und über das Schilddrüsengewicht und das histologische Bild werden keine Angaben gemacht. Unsere Ratten dagegen waren jung und noch im Wachstum. Sie erhielten eine ganz genau kontrollierte Diät und wurden mit einer ganz bestimmten Technik behandelt. Wir gaben Cyanid an Gruppen mit verschiedenen Jodmengen in der Nahrung. Das Schilddrüsengewicht wurde nach dem Tode genau festgestellt, die Drüse nach dem Tode genau untersucht. Ich möchte darauf hinweisen, daß McCarrison dieselben negativen Resultate an einem ganz anderen Ort dieser Welt erhielt wie ich.

Bevor man deshalb die Behauptungen von Marine und seinen Mitarbeitern bezüglich der Rolle des Cyanids bei der Ätiologie des Kropfes allgemein annehmen kann, müssen die Diskrepanzen zwischen seinen und

den oben beschriebenen Resultaten erklärt werden, wie schön auch seine Hypothese sein mag. Weitere Experimente müssen ganz unabhängig von diesen Arbeiten noch an ganz verschiedenen Plätzen dieser Welt ausgeführt werden, um die Grundlagen dieser Hypothese zu festigen. Vielleicht sind wir berechtigt zu behaupten, daß bei der Ratte das Cyanid keine so starke kropferzeugende Kraft besitzt wie beim Kaninchen. Doch muß dann eine Erklärung gebracht werden, warum es so große Unterschiede in der Empfindlichkeit zwischen Ratte und Kaninchen gibt. Gleichgültig, ob man eine synthetische Diät oder eine Naturdiät anwendet, eine Beeinträchtigung der kropferzeugenden Wirkung des Cyanids ist kaum anzunehmen. Vor allem erscheint es mir unwahrscheinlich, daß eine Diät, die in jeder Hinsicht genau berechnet und bei anderen Ratten Wachstum und Gedeihen fördert, wie man das schon lange weiß, und auch bei unseren Kontrollratten deutlich bemerken konnte, eine deutliche Schädigung der Schilddrüse mit sich bringen soll. Es scheint mir wahrscheinlicher, daß es in der Ratte selbst einen Faktor gibt, der die kropferzeugende Stärke des Cyanids reduziert oder vernichtet. In Verbindung mit diesen Tatsachen muß man folgendes überlegen: Marine (1929, 1930) usw. glauben fest, daß neben einem kropferzeugenden Agens im Kohl und anderen Futtermitteln es auch einen Stoff mit der gegenteiligen, also kropfverhütenden Wirkung gibt. So wurde kürzlich ein Stoff als pflanzliche Hexuronsäure identifiziert, die, in genügender Menge gegeben, Kohl- und Cyanid-Kropf verhüten soll. Weiter konnte man zeigen, daß Injektionen dieser Hexuronsäure wirklich eine Involution der Rattenschilddrüse hervorrufen, eine Wirkung, wie sie auch durch Nebennierenrindenextrakte hervorgerufen werden kann. Die letzten Arbeiten von Szent-Györgyi (1927, 1928) und Svirbely und Szent-Györgyi (1932) haben es wahrscheinlich gemacht, daß die Hexuronsäure-Askorbutinsäure in Wirklichkeit das Vitamin C ist. (Bestätigt von Waugh und King [1932] u. a.) Harris, Mills und Innes (1932) und Harris und Ray (1932) konnten zeigen, daß diese Hexuronsäure starke antiskorbutische Fähigkeiten besitzt, und neuere Arbeiten zeigen deutlich, daß die Nebennierenrinde eine bedeutend stärkere antiskorbutische Kraft besitzt als irgendeine andere bis jetzt bekannte Quelle, wie z. B. Orangensaft, Kohl usw., und daß diese Fähigkeit parallel mit ihrem Hexuronsäuregehalt einhergeht. Es hat sich gezeigt, daß gerade bei den Meerschweinchen, die ja so empfindlich gegen Vitamin-C-Mangel sind, bei Skorbut der Nebennierenrindengehalt an Vitamin C deutlich herabgesetzt ist. Daneben gibt es bestimmte Tiere, die anders als Meerschweinchen, Affe und Mensch gegen Skorbut reagieren, bei denen es unmöglich ist, durch Vitamin-C-Mangel Skorbut zu erzielen, so z. B. der Hund (Innes, 1931), die Ratte (Parsons, 1920). Man konnte nachweisen, daß diese Tiere sogar nach einigen Monaten Vitamin-C-armer Diät in ihren Lebern reichlich Vitamin C hatten, mit anderen Worten, daß sie ihr Vitamin C selbst herstellen. Vor allem ist anscheinend die Leber das Reservoir und die Nebenniere bei diesen Tieren die Produktionsstätte des Vitamin C. Die Identität

der Hexuronsäure mit dem Vitamin C und die Tatsache, daß diese Substanz nach Marine usw. (1932) eine starke Antikropfwirkung haben soll, läßt eine einheitliche Erklärung der Tatsache zu, warum wir und ebenso McCarrison mit Cyanidinjektionen bei Ratten keinen Kropf erzielen konnten. Da die Ratte stets einen Überfluß an Hexuronsäure in ihrem Körper hat (in Nebenniere und Leber), tritt immer ihre Antikropfwirkung als Antagonist gegen die Cyanidwirkung auf und kann vielleicht so das entgiftende Agens des Cyanids darstellen, von dem Marine spricht. Die Tatsache besteht, daß McCarrison und wir keinen Kropf bei Ratten mit Cyanidinjektionen erzielen konnten (unabhängig von Jodgaben); daß weiter die Ratte ihre Hexuronsäure (Vitamin C) selbst produzieren kann. Es besteht eine hohe Toleranz der Ratte gegen Cyanid, wie es von Marine[1]) erwähnt und von Hunt und Seidel (1924) bestätigt wurde, und die nach Entfernung der Nebenniere, wie Perla und Marmorston (1933) erwähnten, abgeschwächt wird.

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[1]) Nach Beendigung dieser Arbeit schrieb Marine auf Anfrage Prof. Aschoffs wegen der Schwierigkeiten in Freiburg, bei Ratten Kropf zu erzeugen, daß auch ihm (Marine) jetzt Bedenken gekommen seien, ob seine Ratten wirklich Kropf zeigten, daß auch er schon inzwischen vergebliche Versuche angestellt habe, mit Cyanid bei Ratten und Mäusen Kropf zu erzeugen und daß er der Schwierigkeiten halber zu Kaninchen und Meerschweinchen übergegangen sei (Brief vom 14. XII. 33).

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Über Nebennierenveränderungen bei experimentellem Skorbut

Von J. R. M. Innes, Cambridge University, England
(Rockefeller Foundation Fellow)

Die Tatsache, daß die Vitamine vielfach über Drüsen mit innerer Sekretion wirken und auch chemische Verwandtschaft mit den Hormonen aufweisen, läßt die ganze Frage der experimentellen Avitaminose in einem neuen Licht erscheinen[1]). Es kann vermutet werden, daß die Avitaminose B 2, welche die pellagrösen Erscheinungen verursachen soll, eine Wirkung über die Nebenniere ausübt; ebenso nehmen viele Forscher an, daß Vitamin D über die Nebenschilddrüse auf den Kalziumstoffwechsel wirke trotz

[1]) Siehe auch W. Stepp und J. Kühnau in einer kritischen Betrachtung über Vitamine und Hormone, und ihre genetischen, synergistischen und antagonistischen Beziehungen (Klinische Fortbildung I. 1933).

mancher gegensätzlicher Ansichten. Ähnlich dem gibt es einige Meinungen, daß Vitamin C eine enge physiologische Beziehung zu der Nebenniere hat. Gerade die letzten Forschungen schienen dieser Idee eine Berechtigung zu geben und es möge mir hier erlaubt sein, eine kurze Übersicht über die letzten Arbeiten auf diesem Gebiete anzuführen.

Tillmans und Mitarbeiter (1930—32), Szent-Györgyi (1927—28), Svirbely und Szent-Györgyi (1932—33) konnten in ihren zahlreichen Experimenten die Identität von Vitamin C und Hexuronsäure oder Ascorbinsäure, wie sie es nannten, nachweisen. Ihre Forschungen wurden bereits bestätigt und so weitgehend von anderen Autoren erweitert, daß heute kein Zweifel mehr an der Identität der Stoffe besteht. (Waugh und King 1932; King und Waugh 1932 und andere.) Man konnte zeigen, daß ein Präparat der Hexuronsäure — aus Nebennieren dargestellt — in Dosen von 1 mg täglich Meerschweinchen über die Dauer von 90 Tagen sicher vor Skorbut bewahren konnte. Die ausgedehnten Experimente von Harris, Mills und Innes (1932); Harris und Ray (1932—33) konnten zeigen, daß diese Hexuronsäure starke antiskorbutische Fähigkeiten besitzt, daß die Nebennierenrinde eine bedeutend stärkere antiskorbutische Kraft besitzt, als irgendeine bis jetzt bekannte Substanz, wie z. B. Orangensaft und Kohl, und daß diese Fähigkeit parallel mit ihrem Hexuronsäuregehalt einhergeht. Die Leber mag wohl insgesamt mehr Vitamin C enthalten als die Nebenniere, doch ist sie ein bedeutend schwereres Organ. Die Nebenniere dagegen weist im Verhältnis zu ihrem Gewicht eine bedeutend größere Konzentration dieses Stoffes auf. (Mills 1932.) Svirbely (1933) bestätigte dies bei vielen Säugetieren. Man kann deshalb die Frage stellen, was bedeutet diese ungemein starke Konzentration von Vitamin C in der Nebenniere? Es besteht die Möglichkeit, daß die Leber ein Reservoir, die Nebenniere aber dagegen der Ort der Synthese des Vitamins C ist. Auf der anderen Seite benötigt vielleicht die Nebenniere diesen Stoff für ihre eigene funktionelle Tätigkeit, wie Harris und Ray (1932) gemeint haben. Harris und Ray (1933), Svirbely (1933), Siehrs und Miller (1933) zeigten, daß gerade beim Meerschweinchen, das ja so empfindlich gegen Vitamin-C-Mangel ist, der Nebennierenrindengehalt an Hexuronsäure bei Skorbut deutlich herabgesetzt ist. Daneben gibt es bestimmte Tiere, die anders als Meerschweinchen, Affe und Mensch gegen Avitaminose C reagieren, bei denen es unmöglich ist, durch Vitamin-C-Mangel Skorbut zu erzielen. So z. B. die Ratte (Parsons 1920), der Hund (Innes 1931). Man konnte nachweisen, daß diese Tiere sogar nach einigen Monaten vitaminarmer Diät in ihren Lebern reichlich Vitamin C hatten, mit anderen Worten, daß sie ihr Vitamin C selbst herstellen und daß die Nebennieren sogar eine sehr hohe Konzentration von Vitamin trotz vitaminarmer Diät haben können. (Siehe auch Harris und Ray, 1933.)

Wie aus obigem hervorgeht, gibt es viele Hinweise, daß die Nebenniere eine spezielle enge physiologische Beziehung zu dem Vitamin C hat, was schon von einigen Autoren durch vielfache Beobachtungen gestützt wird, die gefunden haben, daß die Nebenniere bei Skorbut hypertrophiert. Bei Betrachtung der Literatur ergaben sich so viele Unstimmigkeiten, daß ich glaubte, diese Experimente durch genauere Untersuchungen der Drüsen mit innerer Sekretion ergänzen zu müssen.

Die in der Literatur vorliegenden Untersuchungen von Iwabuchi (1922) einerseits und McCarrison (1919—20) andererseits zeigen nur, wie verschiedenartig die Veränderungen sein können, die an Meerschweinchen-Nebennieren bei

typischem Skorbut gefunden werden. Während der eine einen deutlichen Schwund des Lipoidgehaltes der Nebennierenrinde beschreibt, legt der andere hauptsächlich Gewicht auf die Verminderung des Adrenalingehaltes der Marksubstanz. McCarrison hebt die große Neigung der Nebennierenrinde zu Blutungen hervor und betont u. a. die deutliche Gewichtszunahme der Nebennierenbeim experimentellen Skorbut. Leider lassen sich diese grundsätzlichen Anschauungen durch die Befunde beim Menschen nicht überbrücken. Nach Aschoff und Koch (1919), die ein besonders großes Material von Leichen an Skorbut Verstorbener im Weltkrieg untersuchen konnten, fand sich in den Nebennieren sowohl bei makroskopischer wie bei mikroskopischer Untersuchung ein reichlicher Lipoidgehalt und sonst keine Besonderheiten. Die Arbeiten anderer Autoren zeigen große Unterschiede in ihren Befunden bei Tieren. So berichtet z. B. Peiper (1922), daß die Meerschweinchen-Nebenniere nur wenig oder gar kein Lipoid enthält, im Gegensatz zu den Veränderungen beim Verhungern, wo er große Mengen Lipoid fand; Morikawa (1922) zeigte dagegen auf der anderen Seite ein deutliches Ansteigen des Gewichtes und eine Vermehrung des Lipoides dieser Drüse bei Skorbut; La Mer und Campbell (1920) beobachteten ebenfalls einen Anstieg des Gewichtes um etwa 100%, und Bessesen (1923) und Quick (1933) fanden sowohl eine relative wie absolute Zunahme des Nebennierengewichtes bei Skorbut der Meerschweinchen. Jedoch lagen bei diesen letzten drei Arbeiten keine genauen histologischen Untersuchungen vor. [Siehe auch Brouwer (1927) und Lindsay und Medes (1926).] Später fanden Lockwood und Hartmann (1933) bei ihren Arbeiten, die sie sehr sorgfältig mit Kontrollen versahen, einen deutlichen Gewichtsanstieg der Drüsen und kamen zu dem Schluß, daß es eine fundamentale Beziehung zwischen besonderen Vitaminen (B 1 und C) und einem Stoff, der wahrscheinlich Cortin ist, gibt. Sie stellten einen günstigen Einfluß von Rindenextrakt, den sie vorher von Vitamin C befreit hatten, auf Meerschweinchen mit Skorbut fest. Dieses hielten sie für einen klaren Beweis der ständig wachsenden Auffassung vieler Autoren für die engen Beziehungen von Hormon und Vitamin[1]).

Morelli und Gronchi (1927) fanden bei Skorbut von Meerschweinchen eine Lipoidvermehrung im Anfangsstadium, aber eine Verminderung im Endstadium, die wichtigsten Veränderungen waren Rindenhyperplasie, viele Mitosen und Blutungen, trotzdem Findlay (1922) feststellte, daß bei Skorbut die Nebenniere beachtenswert frei von Blutungen war, obwohl Blutungen in anderen Organen bei Skorbut sehr häufig sind.

Bis heute gibt es gar keinen klaren Beweis für die Beziehungen des Vitamins C zur Nebenniere oder überhaupt eines Vitamins zu den Hormonen. Weder die Ähnlichkeit der Wirkung bestimmter Vitamine und Hormone (wie z. B. Vitamin D und Nebenschilddrüse) noch die verschiedenen Veränderungen, die in bezug auf Avitaminose (oder Hypervitaminose) beschrieben worden sind, lassen einen unbedingten Beweis für die Wechselwirkung von Vitaminen und Hormonen erkennen. Bei Avitaminosen werden die Krankheiten erzeugt durch einen Mangel von Vitamin, z. B. A-Avitaminose, Rhachitis, Beriberi, Pellagra, Skorbut usw., und sie werden oft eingeleitet und begleitet von einem Appetitverlust und Schwund des Körpergewichtes. Die Geschichte der Vitaminforschung zeigt deutlich die Gefahr, daß man alle möglichen erzeugten Veränderungen

[1]) Es ist aber dennoch möglich, daß der Rindenextrakt der Nebenniere noch Vitamin C enthielt, da es wohl bekannt ist, daß die Nebennierenrinde die stärkste Quelle für Vitamin C ist (siehe oben).

für spezifisch hält, jedoch muß man stets im Auge behalten, daß allein der Prozeß der Abmagerung mit all seinen Wirkungen auf den Stoffwechsel die Möglichkeit von sekundären Veränderungen mit sich bringt und damit Faktoren, die man als nicht spezifisch für Avitaminosen ansehen kann. Bedenkt man dies und die oben beschriebenen Veränderungen, die man unter verschiedensten Bedingungen bei den Nebennieren fand, so muß festgestellt werden, daß schon McCarrison Hypertrophie der Nebennierenrinde bei anderen Mangelkrankheiten feststellte, und zwar bei Meerschweinchen und Ratten bei Avitaminose B 1 und ebenso bei Tieren mit ganz gewöhnlichem Hunger. [Kon und Drummond (1927) berichteten auch über die Nebennierenrindenhypertrophie in experimenteller Beriberi.] Einen ähnlichen Befund geben Vincent und Hollenby (1920) bei Hunden, Meerschweinchen und Ratten nach Verhungern an. Desgleichen Krieger (1920) beim Menschen. Auf der anderen Seite berichtete Stephani (1923), daß der Fettgehalt der Nebenniere bei Inanition der Kinder vollständig verschwindet. Endlich muß ich in diesem Zusammenhang auch über die sehr ausführliche Arbeit von Kojima (1928) über die Struktur usw. der Meerschweinnebenniere berichten, der beobachtete, daß die Drüse im Hungerzustand vergrößert war, eine Verbreiterung der Zona fasciculata und Zona reticulata aufwies und der häufig Degenerationen bei allen Hungertieren fand. Er konnte in der Zona glomerulosa feine Lipoidkörnchen, in der Zona fasciculata bedeutende Vermehrung von Lipofusionen und manchmal Hyperämie im Mark nachweisen.

Aus allen diesen Arbeiten geht hervor, daß bis heute eine Einigung in bezug auf die Veränderungen der Nebenniere bei Skorbut und die Beziehung derselben zu Vitamin C nicht zu erzielen war.

Was die übrigen Veränderungen bei Skorbut (Avitaminose C) anbelangt, die eigentümlichen Veränderungen am Knochensystem, an den Gefäßen, an den Zähnen, so deuten diese darauf hin, daß irgendeine Veränderung der Kittsubstanzen durch die C-Avitaminose hervorgerufen wird. Daher die eigenartigen Veränderungen an der Knorpel-Knochengrenze, die Blutungen in der Gefäß- und Nervenscheide, die eigenartigen Veränderungen der Odontoblasten durch Bildung von Knochen im Pulpagewebe usw. Da ich mich selbst viel mit dieser Frage skorbutischer Veränderungen der Zahnpulpa experimentell beschäftigt hatte und dabei zu ähnlichen Ergebnissen wie Højer (1926) und Højer und Westin (1925) u. a. gekommen war, hielt ich es auch für meine Pflicht, dieser Frage der etwaigen Veränderungen der Drüsen mit innerer Sekretion beim experimentellen Skorbut noch einmal nachzugehen.

Methoden. 18 weibliche Meerschweine mit dem durchschnittlichen Gewicht von 270 g wurden während einer Zeit von 6 Tagen auf folgende Diät gesetzt:

Kleie . . . . 1 Teil, Preßhefe . . 9 Teile.

Jedes der Tiere erhielt außerdem 4 Tropfen Lebertranöl jeden Tag. Während dieser 6 Tage erhielt außerdem jedes Tier Vitamin C im Überfluß, und zwar 10 g Kohl täglich. Am Ende dieser Woche, als alle Tiere ein starkes Wachstum zeigten, wurden sie getrennt und in folgende Gruppen geteilt:

Gruppe A. 10 Tiere wurden mit der oben beschriebenen Diät, jedoch ganz ohne Koh , also vitamin-C-frei ernährt.

Gruppe B. 3 Tiere bekamen die gleiche Diät, erhielten jedoch als Zugabe täglich ½ g Kohl, d. h. sie bekamen nur ganz geringe Mengen von Vitamin C.

Gruppe C. 3 andere Tiere erhielten die gleiche Diät, jedoch täglich dazu 5 g Kohl, d. h. sie dienten als Normalkontrollen, die genügend Vitamin C erhielten, um Skorbut sicher zu verhüten.

Gruppe D. 2 Tiere erhielten überhaupt kein richtiges Futter, sondern nur Lebertran und 5 g Kohl täglich, um eine einwandfreie Verhungerung zu erzielen ohne die Komplikation eines möglichen Skorbuts.

Die Meerschweinchen wurden täglich gewogen, 2 Tiere der Gruppe A starben während des Experiments, die restlichen wurden getötet, entweder während oder am Ende des Experiments, zusammen mit den Kontrollen und den Gruppen B und C. Die Versuchszeit war nach 25 Tagen abgelaufen, eine Periode, in der man gewöhnlich beim Meerschweinchen ganz sicher Skorbut erzielen kann und oft Todesfälle sieht. Die Hungerkontrollen wurden nach 10 Tagen getötet. Alle Tiere wurden mit Chloroform umgebracht und auf die gewöhnlichen makroskopischen Veränderungen des Skorbuts untersucht. Die Nebennieren wurden so schnell wie möglich herausgenommen und gewogen. Sie wurden in Formalin eingebettet und mit Schilddrüse und Hypophyse untersucht. Eine Hälfte jeder Nebenniere wurde in Gelatine eingebettet und mit Sudan und Nilblau auf ihr Lipoid gefärbt. Die andere Hälfte wurde mit Schilddrüse und Hypophyse in Paraffin eingebettet und mit Hämatoxylin-Eosin gefärbt, ein Teil des Unterkiefers mit den unteren Schneidezähnen und die Rippen-Knorpelansätze wurden, ebenfalls in Formalin fixiert, entkalkt, in Gelatine eingebettet und in Hämatoxylin-Eosin gefärbt, um die Schwere des vorhandenen Skorbuts festzustellen, eine Methode, die auf den Angaben von Højers Arbeit fußt.

Resultate. Alle Tiere der Gruppe A zeigten während der experimentellen Periode die übliche Körperwachstumskurve eines Meerschweinchens, das von einem skorbutischen Prozeß befallen ist, d. h. nach einer kurzen Periode des Ansteigens des Körpergewichtes eine allmähliche Abnahme desselben. Nach 20 Tagen der Skorbut erzeugenden Diät fand ich einen Körpergewichtsverlust von mehr als 100 g. Als ich die Tiere dieser Gruppe tötete, waren sie sehr abgemagert. Die Meerschweinchen der Gruppe B zeigten keinen so starken Abfall ihres Körpergewichtes, Gruppe C, d. h. die normalen Kontrollen, zeigte das regelmäßige Wachstum der gesunden Tiere und bei ihrem Tode waren sie in gutem Ernährungszustand. Die verhungerten Tiere der Gruppe D hoben sich aber durch einen sehr starken Körpergewichtsverlust deutlich hervor (s. Tab. 1, Körpergewicht zu Beginn und Ende der Experimente).

Alle Meerschweinchen der Gruppe A, die nach 20 Tagen und später getötet wurden, zeigten die verschiedenen Grade von stärkerem makroskopisch sichtbaren Skorbut: die charakteristischen Veränderungen der Knorpel-Rippenansätze, Hämorrhagien in den Muskeln rings um die Rippen, um die Oberschenkelknochen und um die Gelenke. Die Tiere der Gruppen B und D (vitaminarm und Hungertiere) zeigten außer schwerer Abmagerung keine pathologischen Veränderungen. Die Kontrolltiere waren in gutem Ernährungszustand, ihre Organe und Knochen ganz normal.

Die Nebennieren der skorbutischen Meerschweinchen der Gruppe A zeig-

ten, verglichen mit den Drüsen der normalen Kontrollen, eine Vergrößerung und auf dem Schnitt erschien ihre Rinde breiter und das Mark kleiner. Das gleiche beobachtete ich bei den Drüsen der Hungertiere der Gruppe D und bei den Tieren mit vitaminarmer Diät der Gruppe B, jedoch in kleinerem Ausmaß. In Tab. 1 sind die einzelnen Drüsengewichte der Nebenniere eines jeden Tieres angegeben, mit Ausnahme der zwei Tiere, die vor der Beendigung des Versuches starben. Auf Tab. 2 ist das Durchschnittsgewicht der jeweiligen Gruppe ausgerechnet und zu 100 g Körpergewicht in Beziehung gesetzt, beides, das individuelle Gewicht der Nebenniere und die umgerechneten Werte, zeigen, daß die Nebennieren der Skorbuttiere schwerer sind als die der Kontrollen. Die Nebennieren der Meerschweinchen, die relativ vitaminarm ernährt wurden, sind nicht ganz so schwer; die Nebennieren der Tiere mit einfacher Verhungerung zeigen ebenfalls eine Gewichtszunahme, jedoch in geringerem Maße als die vorige Gruppe.

Tabelle 1

Tier	Nr.	Tage	Körpergewicht in g zu Beginn	Körpergewicht in g am Ende	Nebennierengewicht in mg	Nebennierengewicht pro 100 g Körpergewicht in mg	Stärke des erzeugten Skorbutes
Gruppe A	1	22	270	185	gestorben	—	++++
	2	25	265	170	188	110	++++
	3	10	300	195	gestorben	—	++
	4	25	265	145	323	220	++++
	5	10	240	160	250	156	+
	6	25	265	170	250	147	++++
	7	20	265	180	327	181	++++
	8	20	275	145	180	124	++
	9	25	470	290	804	277	++++
	10	25	465	285	454	159	++++
							Milderer Skorbut
Gruppe B	1	25	295	265	310	116	+
	2	25	215	230	323	160	+
	3	22	245	190	410	215	+
Gruppe C	1	25	250	360	178	49	Kein Skorbut
	2	25	260	370	191	51	„ „
	3	25	325	390	253	64	„ „
Gruppe D	1	10	440	290	308	106	Kein Skorbut
	2	10	350	270	308	114	„ „

Gruppe A = Vitamin-C-freie Diät; Gruppe B = dieselbe Diät mit ½ g Kohl pro Tag; Gruppe C = Normalkontrolltiere, 5 g Kohl pro Tag; Gruppe D = verhungerte Tiere.

Tabelle 2

Gruppe	Durchschnittliches Körpergewicht in g		Durchschnittliches Nebennierengewicht in mg	Durchschnittliches Nebennierengewicht pro 100 g Körpergewicht in mg	
	zu Beginn	am Ende		zu Beginn	am Ende
A	286	193	347	121	179
B	277	228	347	125	152
C	278	373	207	74	55
D	380	280	308	81	110

An den Zähnen und Knochen wurde jeweils die Schwere des Skorbutes festgestellt (Tafel 1). Es wurde ganz sichergestellt, daß jedes Tier der Gruppe A im Prinzip gleich stark an Skorbut erkrankt war, natürlich mit kleinen individuellen Schwankungen. Die typischen Veränderungen der Degeneration der Odontoblasten, die Verkalkung des Prädentins, die Dilatation der Tomesschen Kanäle und die Neubildung von Knochen in der Pulpa, Stauungen und Blutungen, Zysten in der Pulpa und die charakteristischen Veränderungen der Rippenknorpelgrenzen konnte ich bei allen Versuchstieren feststellen. Nummer 3, 5 und 8 zeigten etwas schwächeren Skorbut als die anderen Tiere. Die Zähne der Gruppe B zeigten die Veränderungen, wie sie von Højer selbst als ein Zeichen von milderem Skorbut berichtet werden. Die Zähne der Normalkontrollen zeigten normale Struktur an Zähnen und Knochen. Die der verhungerten Tiere zeigten normale Zähne und nur die Knochen zeigten anscheinend Wachstumsstillstand im Knochenwachstum.

Wenn ich also meine Ergebnisse zusammenfasse, so kann ich feststellen, daß

1. die Tiere einwandfrei Skorbut zeigten, Gruppe A;
2. die Kontrolltiere keinen Skorbut zeigten, Gruppe C;
3. die Hungertiere nicht die geringste Erscheinung von Skorbut zeigten, Gruppe D.

Alle Erscheinungen an der Nebenniere der Gruppe D können auf den Hungerzustand zurückgeführt werden, aber auf der anderen Seite können wir bei den Skorbuttieren die Veränderungen nicht so dogmatisch auf Skorbut zurückführen, da die Tiere in den letzten Stadien von Skorbut ihren Appetit verloren hatten und schlecht fraßen.

Bei der histologischen Untersuchung galt meine Hauptaufmerksamkeit den Nebennieren. Was mir besonders auffiel, war, daß bei allen Tieren mit Ausnahme von Nr. 8 eine sehr starke Lipoidanreicherung in der Nebenniere eintrat. Diese tat sich vor allem darin kund, daß die äußerste Schicht der Zona glomerulosa, welche beim gesunden Tier mehr oder weniger frei davon zu bleiben pflegt, mit Fetttropfen stark infiltriert war, so daß der Unterschied gegenüber der übrigen Rinde nicht mehr so deutlich wie normal oder gar nicht ins Auge fiel. Ich komme damit also zu einem gegensätzlichen Ergebnis wie Iwabuchi, auch könnte die Zunahme des Gesamtgewichtes der Nebenniere durch eine solche stärkere Lipoidspeicherung in der Nebennierenrinde erklärt werden. Da die Tiere auch regelmäßig an Gewicht abnahmen, würde das auch für eine typische Unterernährung sprechen.

Allerdings muß ich offen gestehen, daß diese starke Verfettung der Nebennierenrinde kein Merkmal des Skorbutes zu sein braucht, sondern auch ein Zeichen der Abmagerung sein könnte. Diese letztere Annahme wird um so wahrscheinlicher, als ich ganz die gleiche vermehrte Lipoidinfiltration der Nebennierenrinde bei den einfachen Hungertieren beobachtet habe, und dies trotz des Umstandes, daß die betreffenden Tiere das Vitamin C zugeführt bekamen. So hätten also die spezifisch skorbutischen Veränderungen in der Nebennierenrinde bei diesen Tieren nicht hervortreten müssen. Wenn sie trotzdem genau so wie die skorbutischen Tiere eine Zunahme des Nebennierengewichtes und eine Zunahme der Lipoidinfiltration der Rinde aufweisen, so muß man sich mit Recht fragen, ob etwa nicht andere Momente außer dem Skorbut bei den Veränderungen der Nebenniere eine große Rolle spielen[1]). Ich möchte nur kurz auf die Arbeit des Dr. Kojima hinweisen, welcher zeigen konnte, wie lebhaft die Nebennierenrinde, besonders die Retikularis, auf alle möglichen Störungen des Stoffwechsels reagiert. Dabei handelt es sich nicht etwa um experimentelle Störungen des Lipoidstoffwechsels, vielmehr hat Iwabuchi gezeigt, daß bei allen möglichen Schädigungen die Rinde, ganz besonders die Retikularis, in Wucherung gerät, und es zu eigenartiger Pigmentierung dieser Zone kommt. Auch ich habe in einigen Fällen derartige verstärkte Pigmentierung in der Retikularis gefunden. Ich möchte darauf hinweisen, daß die Kompliziertheit aller dieser Versuche uns hindern muß, aus bestimmten Veränderungen der Nebennieren sofort bestimmte ätiologische Schlüsse ziehen zu wollen.

In einem anderen Punkte freilich glaube ich den Ausführungen von McCarrison, Iwabuchi u. a. beistimmen zu müssen. Dies ist nämlich die schädliche Beeinflussung der Marksubstanz. Jedenfalls zeigt diese in der Mehrzahl aller Fälle von experimentellem Skorbut eine Auflockerung ihres Gefüges, eine stärkere Vakuolisierung ihrer Zellen, eine Schrumpfung vereinzelter Kerne bis zum pyknotischen Zerfall, kurz eine Schädigung, die sehr wohl erklären ließe, warum ein verminderter Adrenalingehalt in der Marksubstanz gefunden worden ist. Doch kann ich nicht sagen, ob das mit Skorbut ätiologisch zusammenhängt. Es könnte auch sein, daß irgendwelche andere Störungen des Nebennierenmarkes Veränderungen bedingt haben. Von größeren Blutungen, wie sie McCarrison, Iwabuchi u. a. beschreiben, habe ich in meinen Fällen nichts Rechtes feststellen können.

Die gleichen Einschränkungen, die ich bezüglich der Nebennierenveränderungen in bezug auf ihre rein skorbutische Genese machen muß, möchte ich auf die übrigen Drüsen mit innerer Sekretion ausdehnen. Ich hatte den Eindruck,

[1]) Die quantitative Bestimmung des Cholesterines (freies und gebundenes), Carotin, Vitamin A, und Phosphatide in den Lebern aller meiner Tiere bei allen Gruppen A, B, C, D ergaben keine wesentlichen Abweichungen von der Norm, (Breusch). Randoin und Michaux (1928) berichteten, daß der Cholesteringehalt der Leber bei Skorbut vermehrt war.

daß in der Mehrzahl aller Fälle die Schilddrüse nicht vergrößert war, obwohl mehr und dichteres Kolloid eingelagert erschien. Das sonst mehr kubische Epithel der Meerschweinchenschilddrüse, wie wir es in der Herbstzeit bei unseren Tieren beobachten, war unter dem Einfluß der skorbutischen Ernährung flacher geworden. Doch waren diese Unterschiede nicht groß. (Es gibt auch viele gegensätzliche Resultate über die Schilddrüsenveränderungen bei Skorbut [Jackson, 1929].)

Auch was die Hypophyse anbelangt, so hatte ich zwar bei einigen experimentellen Skorbutfällen eine Art Verkleinerung, besonders der eosinophilen Zellen, feststellen zu können geglaubt, jedoch habe ich im ganzen den Eindruck, als ob hier keine Veränderungen vorliegen, die man als sicher skorbutisch bezeichnen kann. Sedlesky (1294) hat ähnliche Veränderungen bei einfacher Inanition im Menschen gefunden.

Fasse ich also kurz meine Erfahrungen über den experimentellen Skorbut zusammen, so kann ich wohl sagen, daß es mir mit Hilfe richtig gewählter Nahrung gelungen ist, in allen Fällen, wo ich die avitaminotische Ernährung längere Zeit durchführen konnte, typische skorbutische Veränderungen an den Knochen-Knorpelgrenzen der Rippe und an der Zahnpulpa zu erzeugen. Daß es mir aber nicht recht gelungen ist, an den Drüsen mit innerer Sekretion (Nebenniere, Schilddrüse, Hypophyse) eine Veränderung zu erzielen, die ich als typische Folge bezeichnen könnte. Ich möchte daher auch annehmen, daß die bisher in der Literatur gemachten Angaben über das Verhalten der Nebenniere nicht auf den Skorbut als solchen zurückzuführen sind. Dies scheint mir um so wichtiger, als nicht selten gegenteilige Angaben über das Aussehen der Nebenniere, insbesondere den Lipoidgehalt derselben, gemacht worden sind. Wenn man berücksichtigt, daß in allen Fällen von Hungerzuständen eine stärkere Lipoidanreicherung in der Nebenniere stattfindet, so kann man damit auch die obengenannten Befunde erklären. Unterstützend tritt die Tatsache hinzu, daß bei einfachem Hungernlassen, aber mit genügend Vitamin C (um Skorbut zu verhindern), ganz die gleichen Veränderungen gefunden werden. Es gibt nur eine weitere Möglichkeit, die noch nicht untersucht ist, nämlich, daß die Hungertiere, die genügend Vitamin C bekamen, dieses Vitamin C als Energiequelle verbrauchen, so daß kein Vitamin in die Nebenniere gelangt. Die Skorbuttiere übernehmen in die Nebenniere ebenfalls kein Vitamin C (Harris und Ray u. a. 1932—1933), so daß in beiden Fällen vielleicht derselbe physiologische Störungsvorgang vorhanden ist (kein Vitamin C, aber starke Lipoidspeicherung).

Aschoff (1925) glaubt diese Lipoidablagerung in der Nebennierenrinde mit der Annahme erklären zu können, daß es sich hier, ähnlich wie bei der Neutralfettspeicherung in der Leber bei Phthisikern, Krebskranken usw., um eine Art Retentionsverfettung handelt. „Während auf der einen Seite unter dem Einfluß des Hungerns ein Gewebszerfall stattfindet, welcher auch Cholesterin und Cholesterinester freimacht und somit die verfügbare Menge an diesen Substanzen erhöht, wird auf der anderen

Seite die in der Nebennierenrinde zur Speicherung gelangende Lipoidsubstanz nicht mehr genügend für den Stoffwechsel und den Abbau neuen Gewebes verbraucht."

Zusammenfassung. Trotz charakteristischer Veränderungen am Knochen- und Zahn-System konnten von mir beim experimentellen Skorbut am Meerschweinchen keine sicher als skorbutisch zu deutenden Veränderungen an den Drüsen mit innerer Sekretion, insbesondere an den Nebennieren, gefunden werden.

Nachtrag. In dem Januar-Heft der Proceedings of the Soc. f. Biol. and Med. findet sich eine Arbeit von O. A. Bessey, M. L. Menten und C. G. King über „Pathological changes in the organs of scorbutic Guinea pigs". Diese Autoren kommen zu dem Ergebnis, daß bei schwerem Skorbut eine typische Fettentleerung der Nebennierenrinde stattfindet. Leider geben auch diese Autoren nichts über das Gewicht der Tiere an. Man kann sich also auch hier kein Urteil darüber bilden, wie die Nebennierenveränderungen mit dem Gesamternährungszustand der Tiere in Zusammenhang stehn.

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Vergleichende Untersuchungen der sog. Umgebungsreaktion der Tumoren und ihrer Metastasen

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Die **„Zeitschrift für Krebsforschung“**

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Vergleichende Untersuchungen der sog. Umgebungsreaktion der Tumoren und ihrer Metastasen.

Von

J. R. M. Innes,

(Rockefeller Foundation Fellow) Cambridge, England.

(*Eingegangen am 11. April 1934.*)

Schon seit man sich mit dem histologischen Studium des Krebses befaßt, hat man auch stets der Umgebung der Geschwülste und den darin auftretenden Zellelementen Aufmerksamkeit geschenkt. Überblickt man historisch die große Anzahl der Arbeiten über Tumoren, so wird man immer wieder die Angabe finden, daß sich in der Umgebung eine bindegewebige Reaktion oder nur zellige Infiltration von Lymphocyten, Plasmazellen oder polymorphkernigen Leukocyten finden. Die Frage, ob diese Vorgänge in der Umgebung der Entstehung der Geschwulst vorausgehen, ob sie gleichzeitig mit der Geschwulstentwicklung auftreten, ob sie sich erst bei stärkerem Wachstum und weiterem Vordringen des Carcinoms zeigen oder ob sie gar selbst zur Geschwulst zu rechnen sind, hat in den letzten 80 Jahren eine sehr verschiedene Auslegung und Deutung erfahren. Heute unterscheiden wir zwei große Gruppen, die sich mit ihrer Auffassung gegenüberstehen. Die eine vertritt den Standpunkt, daß die Umgebungsreaktion sowohl für den Tumor, wie für den Körper spezifisch ist und sie leitet aus der jeweiligen Umgebungsreaktion ein Recht zur Prognose des Tumors ab. Sie gibt ganz bewußt auf Grund einer morphologischen Beobachtung eine biologische Auswertung. Die andere Gruppe erkennt wohl die Infiltrate im Tumor wie auch in seiner Umgebung an, spricht ihnen aber jede Spezifität ab und führt sie als eine entzündliche Reaktion auf die Toxine, die durch die Zerfallsprodukte des Tumors entstehen, zurück. Sie hält eine Prognosestellung aus der Umgebungsreaktion, die nach ihrer Angabe an verschiedenen Stellen in der Umgebung des Tumors auch ganz verschieden ist, für unmöglich. Betrachten wir kurz historisch die Entwicklung beider Auffassungen. In Anbetracht des sehr stark angewachsenen Schrifttums läßt sich natürlich im Rahmen dieser Arbeit nicht auf jede einzelne Literaturgabe eingehen, sondern es können nur kurz die wichtigsten herausgenommen werden.

Waldeyer (1867) schreibt in seiner Entwicklung des Carcinoms ausführlich über das Bindegewebe. Er ist der Auffassung, daß dem Carcinom eine einleitende Bindegewebswucherung vorausginge, daß diese das Gerüst für das spätere Car-

cinom gäbe und daß sie nur eine untergeordnete Rolle spiele. Um die einzelnen Lymphdrüsen weist er kleinzellige Infiltrate nach. Im Jahre 1872 finden wir in seinem zweiten Artikel über die Entwicklung der Carcinome folgenden Abschnitt: „An der Grenze der Carcinome gegen das gesunde Gewebe wird man immer Veränderungen im interstitiellen Bindegewebe antreffen, welche sich bald mehr als akute, bald mehr als chronische entzündliche Prozesse anlassen. Man sieht eine starke Vascularisation des Stromas, die Gefäße dringen sogar in die Deckepithelien, z. B. bis in die Hornschicht der Portio vaginalis uteri vor. Daneben findet eine reichliche Produktion von Wanderzellen statt, welche sich zwischen die Epithelien aller Orte einschieben." Später sagte er, daß die Grenzbezirke der primären Carcinome in der Leber z. B. ganz den Charakter einer diffusen chronischen Hepatitis annehmen können.

Rindfleisch (1878) schreibt in seiner pathologischen Gewebelehre „das Stroma wird auseinander gedrängt und geteilt (von dem wuchernden Tumor), keine Bindegewebetextur widersteht auf die Dauer der stetig wirkenden mechanischen Gewalt, die die sich einbohrenden Epithelzapfen entwickeln. Trotzdem verhält sich das Bindegewebe keineswegs immer leidend. Vielmehr muß jenes Einbohren selbst als ein Entzündungsreiz betrachtet werden. Daher finden wir namentlich an den Punkten, wo die Epithelzapfen am meisten wachsen, vor der Spitze derselben eine üppige Zellwucherung, überhaupt ist das Stroma reich an jungen Elementen. Es ist auch zu eigenem Wachstum insbesondere zu papillären Excrescenzen geneigt." *Ribbert* (1894, 1911) lenkte die Aufmerksamkeit bei der Umgebungsreaktion besonders auf das Bindegewebe hin, faßte jedoch die Veränderungen als entzündlich auf und deutete die zellige Umwandlung des Stromas als Reaktion gegen das Einwachsen des fremden Epithels ins Bindegewebe, das aber das Eindringen nicht hindert, sondern im Gegenteil manchmal fördert. Auch glaubte er, daß Carcinom auf dem Boden vorausgehender chronischer entzündlicher Infiltrate entstünde, die primär das Bindegewebe durchsetzen. *Domergue* (1898) faßt die Bindegewebswucherung als Vorposten des Carcinoms auf, d. h. Bindegewebszellen des Tumors zerstören die gesunden Zellen und bilden eine entzündliche Zone, in die später die Tumorelemente eindringen (siehe auch *Bonney*, 1908). *Ribberts* schärfster Gegner *Hauser* (1890), desgleichen *Hansemann* (1893) und *Heidemann* (1892) legten der Bindegewebswucherung ebenfalls nur eine sekundäre Rolle bei. Es handelt sich nach *Hauser* um eine entzündliche Infiltration des Gewebes und um eine entzündliche Bindegewebsneubildung, die mit *Waldeyers* begleitender Bindegewebsbildung identisch ist.

Hauser (1890) konnte diesen Prozeß beim Magenkrebs genau verfolgen. Durch das Eindringen des biologisch veränderten Epithels in die Muscularis mucosae entsteht eine kleinzellige Infiltration des submukösen Bindegewebes. Die Epithelzapfen sind von einem Leukocytenwall umgeben. Es entsteht zuerst eine Bindegewebsneubildung von weichem, kernreichem Granulationsgewebe (medullärer Charakter), welches dann in ein festes übergeht, das so fest werden kann, daß die Epithelzapfen atrophieren. *Nothafft* (1895) erklärt die Bindegewebsreaktion ebenfalls für sekundär. Auch *Borst* (1902) bestritt sehr energisch die große Bedeutung der Stromareaktion und weist nachdrücklich darauf hin, daß sie nur sekundäre Reaktion auf die Krebszellen sei. Dieser Auffassung schloß sich *Petersen* (1902) an. Er hält die Bindegewebebeteiligung für einen koordinierten, daneben auch für einen subordinierten, aber nicht im *Ribbert*schen Sinn für einen supraordinierten Vorgang.

Die experimentelle Krebsforschung an Kleintieren brachte ein neues Moment auch in die Bewertung der Umgebungstheorien. So fand *Russell* (1908), daß bei Angehen der Impftumoren das Stroma mit einer Reaktion und Sprossung

der Capillaren antwortete. *Da Fano* (1910, 1912) stellte bei seinen experimentellen Studien fest, daß Lymphocyten, Plasmazellen und degenerierte Tumorzellen stets zusammen gefunden werden und schloß deshalb auf eine besonders wichtige Rolle dieser Zellelemente. *Levin* (1911) äußert sich in einer Zusammenfassung, daß das Studium der reaktiven Vorgänge sehr wichtig zum Studium der morphologischen Einteilung des Wachstums und der Genese des Carcinoms sei, er maß bei Impftumoren der Umgebungsreaktion eine große Bedeutung bei, er sprach sogar von einem spezifischen Bindegewebe zwischen Gruppen von Carcinomzellen und von einer besonderen Reaktion des Bindegewebes mit kleinzelligen Infiltraten, die den Tumor einkapseln und eine Wand zwischen diesem und gesundem Gewebe bilden.

Im folgenden möchte ich vorerst alle jene Autoren zusammenfassen, die sich gegen eine besondere Bedeutung der Umgebungsreaktion ausgesprochen haben. *Borst* (1902, 1924) bestreitet schon in seinem Lehrbuch (1902) die große Bedeutung der Umgebungsreaktion und erklärt sie als nur sekundäre Reaktion auf die Krebszellen. Man findet in einem Aufsatz über Geschwülste von ihm (1928), „daß die lymphocytären Reaktionen in der Umgebung bösartiger Geschwülste besonders der Carcinome in ihrem Wesen freilich noch nicht genügend erkannt sind. Sie können im Primärtumor vorhanden sein, in den Metastasen aber fehlen, ob sie als Gegenreaktion aufzufassen sind, ist nicht sicher." *Woglom* (1922), der bei Rückbildung von Mäusetumoren eine ausgesprochene Infiltration wahrnahm, beurteilt sie als unwichtig. Später (1923) äußerte er auf dem Krebskongreß (Straßburg): „Es gibt bis heute keine sichere Reaktion in der Umgebung des Krebses, weder allgemein noch lokal; der Körper reagiert nicht auf die Krebszelle wie etwa auf einen Infekt, manchmal habe der Körper einen Widerstand gegen das Carcinom, ob jedoch spezifisch oder unspezifisch, ist unklar." *Masson* (1923) differenziert die Umgebungsreaktionen in periphere und zentrale und möchte den Namen Stromareaktion nur für die peripheren Veränderungen angewandt wissen. Er unterscheidet grundsätzlich drei Typen von Reaktionen: 1. eine Reaktion, die vor dem Carcinom auftritt, „Stroma-réaction prècocé", 2. eine solche die mit dem Carcinom erscheint, „Stroma-réaction synchron" und 3. eine, die erst nach dem Auftreten des Carcinoms sich bildet, „Stroma-réaction tardive". Er erklärt die Umgebungsreaktion als entzündliche und führt sie auf die toxischen Zerfallsprodukte des Carcinoms zurück. Er beschreibt sie wie die Granulome mit multinucleären Leukocyten, die an Abscesse erinnern können. Doch herrschen meistens bei chronischen Formen Lymphocyten, Riesenzellen, Makrophagen und Rundzellen vor. *Greenough* (1925) nimmt Stellung zu dem Problem der verschiedenen Malignität der Mammacarcinome und gibt seiner Auffassung dahin Ausdruck, daß er nicht an die Widerstandskräfte des Körpers glaubt, die sich in Rundzellen und Hyalinisation gegen das Carcinom ausdrücken könnten. Er erklärt die kleinzelligen Infiltrate als Reaktion auf Degenerationsprodukte der Tumoren. Die Hyalinisation des Bindegewebes hält er für einen Altersfaktor, der individuell verschieden ist. *Plaut* (1927) gab eine kritische Übersicht über die histologische Prognose von Tumoren; er kann sich dabei zu keinem Urteil über das Verhältnis von Entzündung, Ödem, Nekrose, Hyalinisation und deren Beziehung zur Malignität der Tumoren entschließen und erklärt, alle histologischen Deutungen der Befunde als unsicher und hält weitere Forschungen auf diesem Gebiet für nötig. *Reimann* (1929) führt in seiner kritischen Betrachtung über Malignität der Tumoren und Prognose die Zellinfiltrate auf Nekrose und Hyalinose des Tumors zurück. Er bestreitet den hypothetischen Widerstand des Körpers. Nach seiner Auffassung hängt die Stromareaktion 1. von dem Organ und Gewebe, in dem der Tumor wächst, ab, 2. von dem physiologischen Zustand des Organgewebes, 3. von

den sekundären Veränderungen im Tumor. Er erklärt die Idee der Stromareaktion als von der Krebszelle primär erregt — als unsinnig und überholt. Nach seiner Auffassung kann man eine solche Reaktion bei jeder Gewebsdegeneration finden, und eine Tumorprognose an Probeexcisionen je nach der vorhandenen Umgebungsreaktion zu stellen, hält er für Zeitverlust, da ja der Körper an einer anderen Stelle wieder ganz anders reagieren kann. *Böhmig* (1929) weist in seinen umfangreichen Studien mit Nachdruck darauf hin, daß die Stromaveränderungen in der Umgebung der Tumoren unspezifisch seien, eine entzündliche Reaktion darstellen und erklärt über die Bedeutung der Lymphocyten und Rundzelleninfiltrate nichts Endgültiges sagen zu können, so lange ihre Funktion so wenig geklärt sei.

Wenden wir uns nun der Gruppe jener Autoren zu, die in der Umgebungsreaktion der Tumoren etwas Spezifisches erblicken, und deshalb aus ihr eine Prognose stellen wollen. *Murphy* und seine Schüler (1915—1926) betonen auf Grund seiner experimentellen Arbeit über Impftumoren an Mäusen die Wichtigkeit der Lymphocyten bei der sogenannten Krebsimmunität. *Murphy* stellt bei der größten Anzahl seiner angehenden Tumoren ein Fehlen dieser Zellen fest und schließt daraus, daß die Lymphocyten einen sehr spezifischen Widerstand gegen das Wachstum des Krebses im Körper leisten. Er behauptet, daß die Lymphocyten durch eine mäßige Röntgenbestrahlungsdosis angeregt, und daß durch eine größere Dosis eine Hemmung mit nachfolgendem Verlust der Widerstandsfähigkeit gegen das Fortschreiten des Tumorwachstums eintritt. *Powell* (1929) schreibt, daß Fibrose und Hyalinisation verbunden mit Lymphocyteninfiltraten günstig auf die Langlebigkeit der Patienten bei Plattenepithelcarcinom wirke. Er denkt, daß jeder Faktor ein Teil der Abwehrreaktion des Organismus sei. *Broders* (1920, 1926), *McCarty* (1922, 1930, 1931), *McCarty* und *Mahl* (1921) und *Sisstrunk* und *McCarty* (1922) beschreiben eine Zerstörung von Krebszellen infolge „Abschnürung" durch Bindegewebe und durch „Endotheliale Leukocyten" und Lymphocyten und lehren ganz dogmatisch auf Grund dieser Befunde eine günstige Prognose. Als Beweis führen sie die Anwesenheit dieser Elemente in der Nachbarschaft von wachsenden Geschwülsten an, und die Langlebigkeit der Patienten nach der Operation. *Flotow* (1928) ist der Auffassung, daß bei Mammacarcinom die Hyalinisation des Bindegewebes der wichtigste Faktor ist, der das Tumorwachstum hemmt; nach seiner Ansicht bildet es einen „unpassierbaren Wall" gegen die Krebszellen. Eine vorhergehende lymphocytäre Reaktion erklärt er für unbedeutend. *Huepper* (1928) beobachtete bei Cervixcarcinom des Uterus, daß fibröses Bindegewebe die lokale Widerstandskraft des Körpers gegen Krebszellen erhöht, hochvasculäres Stroma der Umgebung jedoch für die Prognose ungünstiger ist als gefäßarmes; eosinophile Infiltrate hält er für wichtige Faktoren und erklärt sie als schützende Reaktion des Körpers gegen die toxischen Produkte der Krebszellen. Sehr stark zellige Infiltration hält er von größter prognostischer Bedeutung und deutet sie als starke Abwehrkräfte gegen das Geschwulstwachstum. Er stellt eine Tabelle mit vielen Faktoren zur Bewertung der klinischen Prognose auf und erklärt den Tumor je nachdem, wieviel Punkte er bei der Bewertung erhält, für gut- oder bösartig. („Malignogramm".)

Ewing (1928) schreibt in seinem wohlbekannten Lehrbuch, „Neoplastic Diseases", „bei entzündlicher Reaktion besteht oft eine Invasion von Tumorzellen. Die entzündliche Reaktion ist gewöhnlich geringgradig und die Ursache des Auftretens von Lymphocyten, Plasmazellen und in gewissen Fällen von polymorphkernigen Leukocyten. Es ist ein äußerst wichtiger Zug des bösartigen Tumorwachstums und *muß* als Abwehrprozeß angesehen werden. Gut abgegrenzte Reaktion bedeutet eine ausgesprochene Fähigkeit, das Tumorwachstum zu begrenzen, aber die Wirkung kann ohne Erfolg sein. Sehr lebhaft wachsende bös-

artige Tumoren und die späteren Metastasen zeigen oft keine entzündlichen Reaktionen, aber die Mehrzahl der infiltrierenden Tumoren zeigt oft bedeutende celluläre Reaktion von seiten des durchdrungenen Gewebes. Infiltrierendes Wachstum ohne Stroma- oder celluläre Reaktion zeigen die Abwesenheit von Schutzkräften im Körper an. Das Entwickeln von gefäßreichem Stroma von seiten des Gewebes ist bis zu einem gewissen Grade günstig für das Wachstum des Tumors. Zu starkes Wachstum des Stromas mit viel intercellulärer Substanz bedroht das Wachstum des Tumors." In seinen weiteren Ausführungen führt er die polymorphkernigen Leukocyten auf Nekrose, Trauma, bakteriologische Vorgänge usw. des Tumors zurück und erklärt sie als bedingt durch chemotaktische Reize seitens der Tumorerzeugnisse. Lymphocyten und größere Mononucleäre bedeuten nach ihm eine Abwehrreaktion, die das Wachstum beschränkt, eine lokale Wehrimmunität herstellt und die Immunitätskräfte auf die Entfernung überträgt. Plasmazellen sind ein späterer Faktor in demselben Vorgang. Eosinophile Zellen häufen sich in Blutungen an. Endothelwachstum, wenn es gering ist, stellt einen Teil der Stromareaktion dar, wenn es jedoch stark ist, bilden sich Makrophagen und verhindern das Zellwachstum. Man sieht, daß er sich weder für noch gegen eine große prognostische Bedeutung der Stromareaktion entscheidet, in ihr aber doch eindeutig eine starke Abwehrreaktion des Körpers sieht. *Roussy* (1929) vertritt in seinem Lehrbuch die Auffassung, daß die lokale Stromareaktion an der Peripherie der Carcinome sehr häufig eine entzündliche Reaktion mit Gefäßdilatation und lymphocytärer und polymorphonucleärer Zelleninfiltration darstelle.

Er kann sich bezüglich der lymphocytären Infiltration zu keiner sicheren Auslegung entschließen, da die Ansichten der Autoren zu verschieden seien: seit der Röntgenbestrahlungstherapie hält er jedoch die Frage von Bedeutung.

Ähnlich gab *Hellwig* (1932) eine allgemeine Übersicht über die „Scientific Biopsie of Tumors" und erklärt, daß der verschiedenartige Aufbau der Geschwülste und die Verschiedenheiten der Reaktion an den verschiedenen Stellen eine Entscheidung bei dieser Prognose sehr schwierig mache. Zu einem abschließenden Urteil kann er sich nicht entschließen.

Aus allen diesen Arbeiten geht hervor, daß bis heute eine Einigung in bezug auf die Bedeutung der Stromareaktion nicht zu erzielen war.

Die vorausgehende Literaturübersicht ergab zwangsläufig die *Fragestellung*, wie ich mich bei der Untersuchung der Umgebungsreaktion zu verhalten hatte und worauf besonders zu achten war. Daß eine Zellenreaktion in den Tumoren und in der Umgebung vorhanden ist, daran kann nicht gezweifelt werden. Aber wie ist es mit ihrem zeitlichen Verhalten? Bei dem Auftreten von zelligen Elementen von Umgebungsreaktion zu sprechen, bevor der Tumor selber zu sehen ist, erscheint mir merkwürdig, und ich glaube kaum, daß man mit Sicherheit von einer dem Tumor *voraus*gehenden *Re*aktion sprechen kann. Es bleiben demnach folgende Fragen zu beantworten:

Wie weit ist die sog. Umgebungsreaktion eines Tumors der Nekrose zuzuschreiben oder ihren Folgen?

Ergeben sich lokale Unterschiede in den verschiedenen Wirtsorganen der Tumoren?

Gibt es in den einzelnen Organen eine organ-spezifische Reaktion gegen einen Tumor?

Tabelle 1.

Primärtumoren			Metastasen						
Wirtsorgan	Tumor	Fälle	Lymphdrüsen	Leber	Lunge	Zwerchfell	Peritoneum, Netz, Mesenterium	Darm	Niere
Magen	Adeno-Ca. = 7 Solides Ca. = 7 Scirrhöses Ca. = 4	18	12	7	2	2	5	3	—
Rectum	Adeno-Ca. = 7 Scirrhöses Ca. = 2	9	8	8	3	1	1	—	—
Niere	Hypernephrom = 6 Hypernephromartig = 3	9	2	2	6	1	—	—	1
Gallenblase	Adeno-Ca. = 3 Solides Ca. = 2 Scirrhöses Ca. = 1 Papilläres Ca. = 1	7	4	5	1	—	—	—	—
Pharynx, Gaumen usw.	Cylindrom = 1 Plattenzellen-Ca. = 3 Sarkom = 2	6	3	—	—	—	—	—	—
Mamma	Solides Ca. = 4 Scirrhöses Ca. = 2	6	6	4	4	1	—	—	—
Bronchien	Kleinzelliges Ca. = 5 Plattenzellen-Ca. = 1	6	6	1	6	—	—	—	1
Mediastinum, Pleura	Polymorphzelliger maligner Tumor = 1 Solider Endothelkrebs = 1	2	1	—	2	—	—	—	—
Pankreas	Solides Ca. = 1	6	3	2	1	—	—	—	—
Ovarien	Solides Ca. = 2 Papilläres Ca. = 2	4	1	3	—	3	3	4	—
Leber	Großzelliges Ca. = 1 Hepatom = 1	2	1	—	1	—	—	—	—
Uterus, Portio usw.	Plattenzellen-Ca. = 2 Solides Ca. = 1	3	1	1	—	—	—	1	—
Prostata	Kleinzelliges Ca. = 1 Solides Ca. = 1	2	2	—	—	—	—	—	—
Speiseröhre	Plattenzellen-Ca. = 2	2	1	1	1	—	—	—	1
Harnblase	Solides Ca. = 2	2	—	—	—	—	—	—	—
Schilddrüse	Struma maligna = 1 Spindel-polymorphzelliges Sarkom = 1	2	1	1	2	—	—	—	2
Total: Carcinome		85	52	35	30	8	9	8	5
Verschiedene Sarkome		5	1	1	2	—	—	—	—
Total: Fälle		90	53	36	32	8	9	8	5

Fälle.	
Primäre Carcinome	Metastasen
Primäre Sarkome	Metastasen
Total: Tumoren	Metastasen

Tabelle 1.

toneum, Netz, enterium	Metastasen: Darm	Niere	Pleura	Schilddrüse	Nebenniere	Herz	Perikard	Ovar	Milz	Blase	Haut, Muskel	Andere	Fälle ohne Metastasen
5	3	—	2	—	1	—	—	3	—	—	—	Hirn (1)	2
1	—	—	3	—	—	—	—	—	—	—	—	—	1
—	—	1	—	1	1	—	—	—	—	—	1	—	1
—	—	—	—	—	—	—	—	—	—	—	—	—	2
—	—	—	—	—	—	—	—	—	—	—	—	—	3
—	—	—	3	—	—	—	—	—	1	—	—	Sternum (1) Rippe (1)	—
—	—	1	1	2	1	1	1	—	—	—	1	—	—
—	—	—	2	—	—	—	2	—	—	—	—	—	—
—	—	—	—	—	1	—	—	—	—	—	—	—	1
3	4	—	1	—	—	—	—	—	—	—	—	—	—
—	—	—	1	1	—	1	—	—	1	—	—	—	1
—	1	—	—	—	—	—	—	—	—	—	—	—	2
—	—	—	—	—	—	—	—	—	1	1	—	Dura mater	—
—	—	1	—	1	1	1	—	—	—	—	—	—	1
—	—	—	—	—	—	—	—	—	—	—	—	—	2
—	—	2	—	1	—	1	—	—	—	—	1	Rippe (1)	—
9	8	5	13	6	5	4	3	3	3	1	3	5	16
—	—	—	1	—	—	—	—	—	—	—	—	3	—
9	8	5	14	6	5	4	3	3	3	1	3	8	16

Fälle.

. . . .	Metastasen 201
. . . .	Metastasen 8
. . . .	Metastasen 209

Gibt es in den einzelnen Organen eine organ-spezifische Reaktion gegen einen spezifischen Tumor?

Kann man Unterschiede beobachten in der Umgebungsreaktion gegenüber Primärtumoren und Metastasen?

Gibt es einen Unterschied in der Reaktion zwischen primären Tumoren einerseits und Metastasen eines anderen Tumors in demselben Organ andererseits?

Stimmen die Befunde der Stromareaktion der malignen Tumoren des Menschen mit den Befunden an transplantierten Tumoren im Tierexperiment überein? Kann man behaupten, daß transplantierte Tumoren eine große immunbiologische Bedeutung infolge Zellreaktion haben? Muß man nicht in Erwägung ziehen, daß transplantierte Tumoren keine primäre im Organismus entstandenen Gewächse sind, sondern gewissermaßen nur in einem anderen Wirt wie „in vitro" gezüchtete Kulturen von Gewächsen, die oft noch von einem anderen Organ des vorhergehenden Wirts stammen? Darf man eine Spontanheilung etwa eines Magencarcinoms eines Menschen mit dem „Nichtangehen" eines Impftumors der Maus vergleichen?

Mein Material erhielt ich freundlicherweise aus dem Pathologischen Institut der Universität München, dessen Chef, Herrn Geheimrat *Borst*, ich zu großem Dank verpflichtet bin. Alle von mir untersuchten Fälle stammen von Sektionen. Ich untersuchte Primärtumoren von Magen, Rectum, Mediastinum, Lunge, Mamma, Niere, Pankreas, Gallenblase, Leber, Pharynx, Ovarien, Uterus, Prostata, Speiseröhre, Harnblase, Hoden, Netz, Schilddrüse und verschiedenen anderen Organen des Körpers. Hatten diese Primärtumoren Metastasen gesetzt, so habe ich auch diese untersucht. Ich entnahm aus der Leiche von Primärtumor und Metastasen erstens ein Stück Tumor und angrenzendes normales Gewebe, zweitens ein Stück aus dem Zentrum des Gewächses. In vielen Fällen zwei bis drei Stücke. So untersuchte ich im ganzen 87 Tumorfälle (s. Tab. 1).

Technik.

Alle Gewebsstücke wurden in 10proz. Formalin fixiert, in Paraffin eingebettet und dann geschnitten. Ich färbte vor allem mit *Hansens* Hämatoxylin-Eosin, *van Gieson* und *Mallory*. In den meisten Fällen wandte ich noch *Bielschowskys* oder *Laidlows* modifizierte *Hortega*-Färbung an zur Darstellung von Reticulum in Tumoren. Bei meinen Untersuchungen richtete ich meine Aufmerksamkeit besonders auf die Unterschiede im Stroma des Tumors, in seiner nächsten und fernen Umgebung. Bei der Beurteilung des Stromas achtete ich besonders auch auf die Gefäßveränderungen, wie Dilatation, Degeneration, Blutungen, Thrombose und Entzündungen. Es interessierte mich besonders etwa vorhandene Fibrose, Sklerose, Hyalinose oder Nekrose usw. Desgleichen achtete ich auf vorhandene Zellinfiltrationen, auf ihre Lokalisation, ihre Elemente und ihre möglichen Beziehungen zu anderen Faktoren.

Es wäre natürlich viel zu weitläufig und würde zu ewig wiederkehrenden Aufzählungen gleicher Veränderungen führen, wollte ich alle unsere Befunde der Reihe nach aufzählen. Deswegen beschränke ich mich darauf, zusammenfassend die Ergebnisse mitzuteilen[1].

Ergebnisse.

Beantworten wir an Hand der Befunde die oben gestellten Fragen, so müssen wir uns zuerst der Frage zuwenden, wie weit die sog. Umgebungsreaktion eines Tumors der Nekrose zuzuschreiben ist oder ihren Folgen. Bei allen Tumoren, sowohl primären wie sekundären Carcinomen, wie den Tumoren der Bindesubstanzreihe finden wir besonders in der Umgebung oder unmittelbar in nekrotischen Herden stets eine ausgesprochene leukocytäre Reaktion. Demnach ist es ganz außer Zweifel, daß die Leukocyten eine Reaktion auf die Nekrose darstellen. Jedoch ist dies nichts Spezifisches für die Tumoren oder ihre Metastasen, denn wir kennen eine leukocytäre Reaktion auch bei allen anderen Nekrosen wie etwa bei Entzündungen oder nach Trauma. Für die weitere Beurteilung der Umgebungsreaktion müssen wir deshalb die polymorphkernigen Leukocyten als spezifische Reaktion ausscheiden.

Zu der Frage, ob sich lokale Unterschiede in den verschiedenen Wirtsorganen der Tumoren ergeben, komme ich an Hand meiner Befunde zu folgendem Resultat: In allen Organen fand ich die gleiche Reaktion von Lymphocyten, Plasmazellen, von mehr oder weniger starker Bindegewebsproduktion. Auch in der Leber gelang es mir, eine deutliche gleichsinnige Reaktion nachzuweisen, sowohl von Zellelementen des Blutes wie des Bindegewebes. Wenn man auf ganz kleine Unterschiede eingehen will, kann man vielleicht sagen, daß im Uterus eine deutlichere Beteiligung der eosinophilen Zellen vorhanden war. Im übrigen muß ich betonen, daß die Bilder bei den verschiedenen Tumoren in den verschiedenen Wirtsorganen prinzipiell die gleichen waren.

Zu der Frage, ob es in einem spezifischen Organ eine spezifische Reaktion *gegen* einen Tumor gibt, kann ich folgendes sagen: Es gelang mir nicht, eine organ-spezifische Reaktion nachzuweisen. Manche Tumoren zeigten etwas mehr oder weniger Fibrose oder Nekrose, doch waren die Bilder an den verschiedenen Tumoren nicht einheitlich und zeigten in dem gleichen Organe so unregelmäßige Befunde, daß ich nicht von einer spezifischen Reaktion in einem speziellen Organe sprechen kann.

Insbesondere scheint es mir unmöglich, von einer spezifischen Reaktion *in* einem spezifischen Tumor zu sprechen. In allen Fällen aller Tumoren in allen Organen fand ich im Stroma eine Wucherung des Bindegewebes, z. B. in Form von fibrösen Septen oder in Bildung von

[1] Protokolle der einzelnen Befunde sind im Archiv des Pathologischen Instituts München niedergelegt.

feinem Tumorstroma, dann als celluläre Reaktion in allen Fällen vorwiegend Lymphocyten, etwas weniger Plasmazellen, Histiocyten und wenig Eosinophile. Auf Grund dieser Befunde müssen wir es ganz entschieden ablehnen, von einer spezifischen Reaktion auf einen spezifischen Tumor zu sprechen, zumal weder Unterschiede im Stroma des Tumors noch seiner Umgebung oder des Organs, weder Gefäßveränderungen wie Dilatation, Degeneration, Blutungen oder Thrombosen und Entzündungen, noch Fibrose, Sklerose, Hyalinose des Stromas oder des Tumors selber irgendeine Organ- oder Tumorspezifität erkennen ließen. (Eosinophile Infiltration im Uterus s. o.)

Dies bestätigen vor allen Dingen die vergleichenden Untersuchungen von Primärtumoren und ihren Metastasen. Es gelang mir in keinem Falle, bei den Metastasen einen grundsätzlichen Unterschied zu den Primärtumoren nachzuweisen. Weder bei großen Metastasen noch bei eben beginnenden kleinen Knoten der Tochtergeschwülste konnte ich irgendeinen deutlichen Unterschied zu den Primärtumoren feststellen. Nie gelang es mir, eine deutliche Abkapselung des Tumors in Form etwa eines Walles, der den Tumor ersticken wollte, oder in Form von starker Bindegewebsreaktion, die den Tumor in seinem Wachstum aufhalten könnte, nachzuweisen. Im Gegenteil scheint mir gerade das einheitliche Bild von Primärtumoren und Metastasen mit ihrer Tumor- und Umgebungsstromareaktion, gleich in welchem Organ sie sich befinden, eindeutig dafür zu sprechen, daß man nicht von einer Spezifität der Reaktion sprechen kann.

Auch die Frage, ob es einen Unterschied in der Reaktion zwischen Primärtumoren einerseits und sekundären Tumoren (Metastasen) anderseits in demselben Organ gäbe, muß ich verneinen. Auf Grund meiner Befunde muß ich sagen, daß beide die gleiche Reaktion zeigen und dies wieder als Beweis der Unspezifität der Reaktion des Organs auf Tumoren hinstellen.

Zu meiner letzten Frage möchte ich folgendes sagen: Ich halte es nicht für richtig, ohne weiteres von Impftumoren der Maus auf die Spontantumoren des Menschen zu schließen. Ich glaube, daß dies eine ganz prinzipielle Frage ist und möchte betonen, daß eine Transplantation von Tumoren und ihr Angehen oder Nichtangehen bei der Maus prinzipiell nichts mit den menschlichen Tumoren, die ja Primärtumoren im engsten Sinn des Wortes darstellen, zu tun haben. Ein Impftumor stellt einen Fremdkörper in einem neuen gesunden Wirt dar und kann bei einem Organismus vorliegen, in dem ein Tumor langsam von Anfang an gewachsen ist. So muß ich es prinzipiell ablehnen, wenn man bei solchen an Impftumoren erhobenen Befunden von Umgebungsreaktionen irgendeine Beweiskraft bei analogem Schlusse zum tumorkranken Menschen zuspricht.

Wenn ich auf Grund meiner Befunde zu dem Problem der Prognosestellung aus der Umgebungsreaktion der Tumoren Stellung nehme, so geschieht dies, um jenen Autoren entgegenzutreten, die aus der Umgebungsreaktion eine biologische Wertung des Tumors und klinische Prognose ableiten. Nach meinen Befunden läßt es sich nicht aufrechterhalten, von einer spezifischen Reaktion auf einen Tumor, in seinem Stroma, in seiner nächsten Umgebung oder in dem befallenen Organ entfernt vom Tumor zu sprechen. Alle Versuche, eine solche Spezifität aufzustellen, erscheinen mir zwecklos und eine Prognosestellung aus dem histologischen Bild als zu gewagt. Wenn wir eines sicher sagen können, so müssen wir betonen, daß anscheinend jeder Tumor ein „Individuum" für sich ist, das an verschiedenen Stellen seiner Umgebung ganz verschiedene Umgebungsreaktion aufweisen kann, daß desgleichen seine Metatsasen keinen Anhaltspunkt für eine Spezifität der Umgebungsreaktion geben und daß deshalb eine Prognosestellung für das Leben des Patienten aus diesem Bild allein nicht möglich ist. Wie schon aus den Untersuchungen, vor allem *Böhmigs*, hervorgeht, ist es also heute auch nach unserer Ansicht nicht mehr richtig und kann als überholt gelten, durch Anwendung einer sog. Prognosestellung aus der Umgebungsreaktion Zeit zu verlieren. Ich habe mit Absicht keine Tabelle aufgestellt, da eine solche doch nur fragmentär die Befunde erläutern kann. Die Tumoren des Darmrohres bzw. Verdauungsschlauches zeigen, wie allgemein bekannt, mehr entzündliche Erscheinungen an sich als etwa die Tumoren der parenchymatösen Organe, was durch sekundäre Infektion hervorgerufen sein dürfte. Nach meinen Befunden ist jeder Tumor in sich verschieden und selbst ein kleines Stückchen gibt an verschiedenen Stellen ganz verschiedene Bilder. Das ist auch ein Grund, eine Tabelle über die Anzahl der Lymphocyten usw., die sich in der Umgebung finden, zur Prognosestellung für unzulänglich zu halten. Im Grunde stellen meine Befunde gar nichts Neues dar, denn schon die ersten histologischen Forscher der Tumoren berichten über genau die gleichen Bilder. Seit der Zeit *Thierschs* und *Waldeyers* wurde unendlich viel über die Krebsfrage geschrieben, so daß es heute fast unmöglich ist, die Literatur ganz zu beherrschen und sich ein sicheres Urteil bei der großen Zahl der Widersprüche zu bilden. Wesentlich neue Befunde gibt es seit *Thiersch* und *Waldeyer* nicht. Nur die Auslegung der Befunde war verschieden. Über Stroma und Umgebungsreaktion sind wir seit *Thiersch* nur ein ganz kleines Stück wirklich weitergekommen in der Forschung. Behauptet man das Gegenteil, wie z. B. *Murphy*, indem man sagt, daß die Lymphocyten eine starke Widerstandskraft des Körpers gegen Tumoren bilden und beurteilt man, wie verschiedene amerikanische Autoren die Malignität eines Tumors nach der Hyalinisation und cellulären Infiltration, so sind dies unbe-

wiesene Dogmen, für deren Richtigkeit bis heute durchaus noch nicht der Beweis gebracht werden konnte.

Gerade die Frage nach der Bedeutung der Lymphocyten kann am besten mit folgenden Worten *Borsts* charakterisiert werden: „Die lymphocytären Reaktionen in der Umgebung bösartiger Geschwülste, besonders der Carcinome, sind in ihrem Wesen noch nicht genügend erkannt, ob sie als Gegenreaktion aufzufassen sind, ist nicht sicher (1928)."

Vielleicht brauchen wir neue Methoden, um in diesen Punkten weiterzukommen — anscheinend stecken wir mit der reinen Morphologie in dieser Beziehung in einer Sackgasse. Unser Material stammt ja fast immer von Sektionen, von Fällen, wo der Tumor bereits sehr groß ist, häufig Metastasen hat und schon viele andere Faktoren eine Rolle spielen. Untersuchungen an jungen Tumoren, soweit solche überhaupt vorliegen, ergaben zwar die gleichen Resultate, doch müßte die morphologische Forschung ganz junge, eben entstehende Tumoren untersuchen, was leider nur allzu selten möglich ist.

Zusammenfassung.

Zusammenfassend komme ich zu einer Übereinstimmung mit allen den Autoren, die eine Umgebungsreaktion der Tumoren für unspezifisch halten. Nur die Nekrose wird anscheinend von einer starken polymorphkernigen leukocytären Reaktion begleitet. Sonst gibt es weder eine spezifische Reaktion auf Tumoren überhaupt noch auf einen speziellen Tumor, noch in einem speziellen Organ. Als Beweis dafür kann ich vor allen Dingen meine vergleichenden Untersuchungen der Primärtumoren und ihrer Metastasen anführen. Alle Tumoren sind begleitet von einer plasmacellulären, lymphocytären Infiltration. Die Beteiligung des Bindegewebes wechselt bei jedem Tumor. Es gibt keine Unterschiede zwischen den einzelnen Tumoren und ihren Metastasen im gleichen Organ oder etwa in anderen Organen. Eine Prognosestellung aus der Umgebungsreaktion für das Leben des Patienten muß abgelehnt werden.

Es ist unmöglich, auf Grund morphologischer Beobachtungen dogmatisch eine Bewertung der Umgebungsreaktion der Tumoren zu lehren, wie es von verschiedenen Seiten geschehen ist. Auch würden Vergleiche meiner Befunde mit denen anderer Autoren zu gar nichts Neuem führen, da wir mit unseren morphologischen Studien bei diesem Problem nicht weiterkommen, und wir uns in der Wissenschaft an Tatsachen zu halten haben. Im Grunde sind wir nicht weitergekommen, als die ersten Untersucher vor 80 Jahren. Einzig und allein können wir heute folgendes sagen: Es gibt eine Reaktion in der Umgebung der Tumoren, die teilweise auf Nekrose und sekundäre Veränderungen zurückzuführen ist. Doch gibt es keine Anhaltspunkte für spezifische

Veränderungen für oder gegen das Tumorwachstum. Mögen wohl geringe Unterschiede je nach Reife und Typus des Tumors beim gleichen Tumor oder seinen Metastasen gefunden werden, es handelt sich stets nur um quantitative, aber nicht um qualitative Unterschiede, darüber hinaus wissen wir nichts.

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(Die Zahlen beziehen sich auf die Seiten.)

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THE PATHOLOGICAL DIAGNOSIS OF SWINE FEVER.

It might appear that the first essential to any disease investigation would be to establish its exact nature and pathology - particularly of a disease of such great practical and economic importance as swine fever. Because, having obtained an accurate knowledge of the pathology of the disease it is then possible to assess the definite value of the various lesions as diagnostic criteria - as far as swine fever is concerned the necessity of making a quick and reliable diagnosis needs no emphasis.

At present the control and eradication of swine fever presents numerous problems and as in the case of foot-and-mouth disease, it is difficult to prevent its introduction from other countries. The fact that the clinical symptoms and epidemiology are confusing adds to the difficulties, since many outbreaks are not suspected in the early stages. These difficulties have not been lessened by the fact that an accurate diagnosis in many cases has not been possible.

Although for many years certain macroscopic lesions were accepted as diagnostic of swine fever, investigations into the detailed pathology and histology and significance of these lesions were never carried out and the individual parts played by the virus and secondary invading organisms remained therefore unknown. Even yet in a number of swine fever cases it is impossible to establish a diagnosis from the gross anatomical findings and this is especially true in cases which show at autopsy only a part of the typical complex of lesions such as haemorrhages in the lymph nodes, kidneys, bladder, large intestine and skin.

In 1930 Seifried, a German worker at the Rockefeller Institute, New York, and Röhrer working at the well-known Insel Reims Institute in Germany, independently of each other began an intensive investigation into the histo-pathology of swine fever, and since that date numerous publications[x] have appeared from both, which in England have not received the attention and credit so well deserved. Although much information of great practical interest has been obtained there are still problems to be solved, but the fact remains that these studies do indicate the possibility of acquiring a means to a rapid and accurate diagnosis of swine fever which has hitherto been denied. The technique and results of both Seifried and Röhrer were more or less similar and can be summarised together.

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Generally the recognised macroscopic lesions of swine fever such as haemorrhages, haemorrhagic and anaemic infarction, and necrosis so commonly found in lymph nodes, spleen and kidney, etc., were all shown to be dependent upon specific changes in the smaller blood vessels and capillaries. These lesions consisted of swelling, proliferation or degeneration of the endothelium subsequently followed by hyaline and necrotic changes in the vessel wall and often thrombus formation. (Endarteritis with a tendency to obliteration of the lumen.) A marked inflammatory cell infiltration occurred in the vessel wall. As a sequel to these vascular changes other lesions occurred in the form of (a) haemorrhages by diapedesis in the skin, muscles, serous membranes, lymph nodes, bladder, gastro-intestinal tract, spleen, liver, lung and bone marrow; (b) necrosis, e.g. the small necrotic areas in the intestinal mucous membrane, and (c) infarction, commonest in spleen and kidney, due to capillary thrombus formation. Under the influence of secondary bacterial infection these changes were intensified with the production of, e.g. the caseous type of pneumonia, ulceration and diphtheritic inflammation of the intestines. These observations indicate with emphasis that the virus primarily affects the endothelium and later the walls of capilliaries and smaller blood vessels throughout the whole body and give a satisfactory explanation of the pathogenesis of the other lesions. Seifried contends

that the magnitude of the vascular lesions depends on the virulence of the virus and somewhat on the presence of secondary bacterial invaders and that in acute cases degenerative lesions of the vessels predominate while in prolonged cases a proliferative type of vascular lesion is more marked. The lesions described have another interest from the comparative point of view inasmuch as they resemble those in pulmonary blood vessels in human influenza described by Oberndorder.

Finally, there must be considered the definite encephalitis which occurs in a large percentage of swine fever cases. Many workers in the past have noted cerebral symptoms although Hutyra and Marek state that the spasms, staggering and lethargy are due to haemorrhages in the meninges and brain substance. Both Seifried and Röhrer described the lesions in the nervous system and showed that these essentially represent an encephalitis similar in type to that of poliomyelitis and Borna disease. Briefly, the lesions consisted of a widespread perivascular cell infiltration, degenerative lesions in nerve cells in nearly all parts of the nervous system and glial proliferation; demyelinisation was absent and specific inclusion bodies could not be found; lesions could often be demonstrated in six days after infection.

It thus appears that in connection with the post-mortem diagnosis the following observations of these workers demand real consideration:-

(a) The encephalitis; this in the opinion of Seifried has only a limited diganostic value because in his series it only occurred in 60 to 80 per cent of cases and because it was considered doubtful whether the lesions were absolutely specific, the probability being that they might be confused with those of swine influenza and other inflammatory cerebral conditions of an unknown nature. A more recent paper by Röhrer (1934), however, gives results of work undertaken to clarify this problem. The brains from normal pigs and from those dying from diseases other than swine fever were examined histologically. The conclusion was that before lesions in the nervous system can be recognised as being part of the swine fever syndrome they must be indicative of an encephalitis in the true sense of the word and independent of other causes of cerebral inflammatory process such as softening, haemorrhage etc.

(b) The vascular lesions and the lesions in various organs resulting from them; these appear to be far more important as they are invariably present; especially in lymph nodes, kidney and spleen; when present the splenic infarctions are of

diagnostic value but they were only found in 40 to 60 per cent of Röhrer's cases.

In the light of these results it is pertinent to realise the fallacy of basing a diagnosis purely on the presence of such lesions as the "button" ulcer and diphtheritic type of inflammation of the intestines which are probably only produced under the influence of secondary bacterial infection. Further it must be remembered that there is the possibility that other aetiological agents may also produce lesions of a similar type. Apart from that, there is still the question of those suspected cases in which only part of the complex is present, and from the gross anatomical findings a diagnosis is impossible; the only resource left would then appear to be transmission experiments - a proceedure which would be costly and time consuming.

These results appear therefore of great importance and practical significance and only await further investigation into a large number of naturally occurring cases to confirm what has been observed in the disease experimentally produced. In conclusion, it is obvious that in this country, if the incidence of the disease is to be reduced, a thorough investigation should and must be carried out to determine the possible utility of the histological lesion as a means to diagnosis.

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A CASE OF SUPPURATIVE EPIDIDYMITIS IN A DOG CAUSED BY BRUCELLA ABORTUS.

By

T.L. WHITBY, T.J. BOSWORTH and J.R.M. INNES.

The subject was an Aberdeen terrier, twelve years old, in very fat condition.

History. - The animal, which had been blind for several years, had for some time suffered from polyuria and had shown a variable amount of swelling of one testis. It was killed on April 4th, 1935, when a post-mortem examination by one of us (T.L.W.) revealed an excess of fluid in the cavum vaginale on one side and the presence of pus in the epididymis; from the latter, infection had spread along the inguinal canal, at the entrance to which a small quantity of purulent material was observed. The testis, epididymis, kidneys, bladder and prostate were forwarded to the laboratory for examination.

Macroscopic Appearances. Testis. - No gross swelling of the organ; the tunica albuginea was smooth and the testicular substance showed a slight degree of post-mortem softening. Epididymis. - Apart from slight swelling no abnormalities were

visible from the surface; on cutting into the structure numerous small pockets of yellow pus were seen in the head, body and tail; the spermatic cord was not included in the specimen sent. Kidneys. - The capsule had been stripped; the surface was smooth and free from gross granulation but a few small scattered scattered scars were visible on it; the cortical pattern was distinct and the medulla and pelvis showed no changes. Bladder. - No abnormalities; the ureteral openings patent. Prostate. - No hypertrophy or cyst formation; one small soft yellow focus present in its substance.

Histological Findings. Epididymis. - The normal tubular character was completely obliterated except in a few areas where dilated tubules, showing partially disintegrated epithelium and containing cellular debris, were still recognisable. Throughout the gland there were numerous necrotic foci which in places had become confluent to form larger areas. At the margins of these foci and in the tissue between them there was an acute exudative inflammatory reaction; the vessels persisting in these situations showed hyaline necrosis of their walls, and haemorrhage had occurred into the tissue around. The interstitial tissue appeared oedematous with scattered fibrinous deposits. There was an intense cellular infiltration of polymorphs, lymphocytes, plasma cells and large phagocytes but without the predominance of any one type. In other areas cellular infiltration. was not so marked In one area the necrotic process had extended to the surface. Testis. - There were no lesions present comparable to those seen in the epididymis; the structure appeared to be similar to that normally found in the senile state; spermatogenesis was not evident. Around the vessels situated beneath the tunica albuginea were small collections of lymphocytes but the tunic itself appeared normal except near the epididymis where it showed inflammatory changes similar to those seen in that organ. Kidneys. - No changes corresponding in any way to those observed in the epididymis were present. There was no diffuse nephritis, but the scars which were evident macroscopically on the surface were found to correspond to small isolated foci in which the glomeruli showed ischaemic changes (atrophy of the tuft and thickening of the capsule) and in which there was atrophy of the related tubules, periglomular fibrosis, and lymphocytic infiltration of the fibrotic tissue. These foci were thus typical of chronic focal glomerulo-nephritis of a kind which is often seen in old dogs, and evidently bore no relation to the infection in the epididymis. Bladder. - Apart from post-mortem degeneration and desquamation of the epithelium no lesions were observed.

Prostate. - As in many such glands examined some time after death, post-mortem degeneration of the acinar epithelium was very marked; in the small focus observed macroscopically there was early new formation of small acini with cubical epithelium, diffuse interacinar fibrosis and marked infiltration of the latter by lymphocytes, plasma cells, and large macrophages.

Summary of Pathological Findings. - Acute suppurative epididymitis; focal periorchitis; chronic focal prostitis, with commencing adenoma; chronic focal glomerulo-nephritis.

Changes similar to those observed in the prostate and kidney are frequently seen in aged dogs and their presence in this case appears to bear no relation to the infection in the epididymis. The lesions in the latter are comparable to those which have been described in Br. abortus infection of the bull's testis and are of a non-specific character, predominantly exudative in type with a tendency to necrosis.

Bacteriology. A growth of Br. abortus was obtained from the lesion in the epididymis but the organ proved to be sterile apart from the presence of a few extraneous organisms. The culture was identified as Brucella by means of a known agglutination serum. When first isolated it grew only in an atmosphere containing an increased amount of CO_2 and was slow in becoming acclimatised to ordinary aerobic conditions. Growth occurred in the presence of methyle violet 1:50,000, basic fuchsin 1:25,000 and pyronin 1:100,000, but was completed inhibited by thionin 1:60,000. The production of H_2S from culture media occurred only during the first 48 hours of incubation under aerobic conditions. These results indicated that the organism was of the bovine type of Br. abortus and this was confirmed by comparing its serological characters with those of a freshly

isolated bovine strain. Agglutinating sera for each were prepared in rabbits and were then absorbed fractionally by adding successive small doses of organisms. It was found that either strain was capable of removing agglutinins, for itself and its opposite number, from both these sera to the same degree at the various stages of the process and this, according to the findings of Wilson and Miles (1932) would indicate that they were of the same type.

COMMENT.

There is evidence to show that the canine species possess a relatively high degree of natural resistance to organisms of the Brucella group other than bronchisepticus and that although cases of infection with melitensis and abortus may occasionally occur they usually pass unnoticed in the absence of clinical symptoms. There are on record a few instances in which aborting bitches have been found to react to the agglutination test, while Planz and Huddleson (1931) described a case of illness in a dog associated with a unilateral purulent orchitis from which Br. abortus var suis was isolated. Apparently, however, this is the only recorded example of a specific lesion in a canine animal caused by an organism of this kind and nearly all other reported cases of natural infection have been diagnosed solely on the presence of specific antibodies in the blood. In most instances these have been found in low concentration only. The reacting animals

have shown no signs of ill health and it has not been possible to isolate the organism from their tissues on post-mortem examinations. Exceptions to the latter rule are the recovery of melitensis from a mesenteric gland of one case by Kennedy and Eyre (1914) and of the bovine type of abortus from the blood of another by Van der Hoeden (1932). The evidence suggests that dogs in close contact with cattle are more liable than others to contract infection (Thomsen, 1932). For further information on the various aspects of the subject reference may be made to articles by Van der Hoeden (1932), Menzani (1932), and Feldman, Mann and Olson (1935).

Several workers have shown that it is possible to infect dogs experimentally with artificial cultures, the most certain method being by intravenous injection. Animals so treated develop agglutinins and the organism may be recovered from them for varying periods after inoculation. In most cases, however, the infection is rapidly overcome. Feldman, Bollman and Olson (1935) found that little or no disturbance of health resulted and that no lesions worthy of note were produced. Pregnant bitches carried their litters to full term and there was no evidence of spread from affected to healthy dogs. Van der Hoeden, however, believes that such spread may occur and

that infected dogs are a source of danger to man and other animals. Cardona (1935) has reported that the experimental disease in the dog may be associated with a temperature reaction resembling undulant fever in man together with marked loss in weight and the persistence of a high agglutinating titre for periods up to 15 months. He also noted the occurrence of intermittent lameness in afebrile cases.

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The Effects of Phosphorus Deficient Diets on the Metabolism, Blood and Bones of Sheep with Special Reference to Conditions Existing in Great Britain

By James Stewart

With Addendum

THE ANATOMICAL CHANGES IN THE BONES AND THEIR RELATIONSHIP TO RICKETS

By J. R. M. Innes

Diseases due to mineral deficiency are comparatively rare in Great Britain although in many parts of the world stock-owners suffer heavy losses from serious deficiencies of mineral in the soil and herbage. In the mountainous areas of Northern England and Southern and Western Scotland, however, the pastures on many of the hill farms are very poor in minerals. Sheep farming is the main occupation in these districts and the effect of the poverty of mineral in the pasture is a general unthriftiness in condition and a low calcium and inorganic phosphorus content of the blood. This has been clearly shown by Shearer and Stewart (1931) and Bosworth and Stewart (1933) for several districts in Yorkshire and Northumberland. In the latter paper it was shown that there was prevalent on several of the poorer hill farms a disease of sheep called *Cappi* which is characterised by certain peculiar changes in the skeletal structure. It was found that the blood and bones of affected animals and the pasture on which they grazed all possessed a phosphorus content bordering on what might be called deficient. In the light of this finding it was decided to carry out experiments to show if possible the relationship of the low phosphorus content of the diet to the aetiology of the disease. Although the calcium content and the nutritive value of the pasture were not high, they appeared adequate to allow the sheep to make a small but constant gain in weight. Therefore, in designing the present experiments it was necessary to compose a ration which would demonstrate the effect of a phosphorus deficiency *per se*.

In view of several admirable reviews of the literature dealing with phosphorus which have appeared quite recently, for example that of Theiler and Green (1932), it is not proposed to discuss many of the results of other workers here, except to mention several papers which have appeared more recently and which have a direct bearing on the present investigation. The first thorough investigations into the effects of phosphorus deficiency on livestock were

carried out by Theiler, Green and Du Toit in South Africa and they were continued over a number of years by their successors, a final report of the experiments carried out on phosphorus in the nutrition of the sheep having appeared under the names of Du Toit, Malan and Groenewald (1932). They concluded that sheep receiving 0·73 g. of phosphorus daily, which they aver is equal to the amount obtained by sheep on poor pasture, suffered severe set-backs from phosphorus deficiency, their final live weight being slightly less than their initial weight. A group receiving 1·53 g. of phosphorus daily, corresponding to the intake of sheep from a good pasture, did very well and were in prime condition at the end of the experiment. They also concluded that the low phosphorus content of the ration was reflected in a low inorganic phosphorus content of the blood. Martin and Pierce (1934) in an investigation carried out in Australia, fed lambs and sheep on a diet containing 0·6 g. of phosphorus per day, but adequate in respect of protein and calcium. They found that the live weight increase of the lambs receiving this low phosphorus diet was only 60 per cent. of that of lambs receiving 1·6 g. of phosphorus per day. The blood phosphorus of the animals receiving the low phosphorus diet fell to 2·5 mg. in the case of lambs and to 2·00 mg. in older sheep. Anatomical, histological and chemical investigation of the bones of the animals receiving the low phosphorus diet revealed varying degrees of osteoporosis and osteomalacia and one of the lambs showed evidence of rickets. In an investigation carried out by Riddell, Hughes and Fitch (1934) to study the problem of lowered food utilisation in phosphorus deficient cattle, it was shown that a shortage of phosphorus in a ration containing ample digestible protein and starch equivalent became a limiting factor in the economical utilisation of foodstuffs. A ration containing 0·12 per cent. phosphorus did not depress the digestive functions of the animals since those on the low phosphorus diet digested their food as completely as the normal controls. No abnormal losses of energy were demonstrated in the excretion of the phosphorus-deficient cattle, but by means of oxygen consumption measurements, a higher energy metabolism for animals in a phosphorus deficient condition was indicated. Although failure of appetite became general in the low phosphorus group, the loss of weight of this group could not be attributed to this factor, since the losses began whilst the animals were consuming sufficient nutrients to allow of an appreciable gain in weight. Turner, Kelly and Dann (1935) have adduced evidence that "peg leg," a disease of cattle in North Queensland characterised by under-development, relative infertility, lameness and various skeletal deformities, is essentially due to a deficiency of phosphorus in the pasture. The authors have also shown that this disease is associated with a decrease in the blood inorganic phosphorus, which fell as low as 2 mg. per 100 c.c.

Despite the numerous experiments carried out to establish the effects of phosphorus deficiency, little is known regarding the actual phosphorus requirements of livestock. A survey of the literature reveals that most workers have arbitrarily selected an amount of phosphorus in the diet which, for a variety of

reasons, they call a deficient amount. Moreover, most workers have made the diets of their experimental groups so low in phosphorus as to be of little use for correlation with that prevailing in actual farm practice in this country. In the South African work already referred to, the sheep in the very low phosphorus group received 0·47 g. phosphorus daily, whilst another group received 0·75 g. which the authors considered was common in phosphorus-deficient areas in South Africa. Martin and Pierce gave the low phosphorus group of their experiment 0·6 g. of phosphorus and their controls 1·6 g. of phosphorus daily. Cunningham (1933) in phosphorus deficiency experiments carried out in New Zealand assumed that a diet containing 0·38 per cent. phosphorus was deficient in phosphorus. Riddell, Hughes and Fitch kept their phosphorus deficient dairy cattle on a ration containing 0·12 per cent. phosphorus and their control group on one containing 0·34 per cent. phosphorus. Until recently much of the work done on phosphorus deficiency could be discounted on the grounds that workers paid little attention to the nutritive value of the ration fed, and consequently much of the deleterious effect of the ration was due to either too little protein or too little starch equivalent. Again many of the rations used were only phosphorus deficient inasmuch as the calcium phosphorus ratio was very large but possessed no absolute deficiency of phosphorus. The authors quoted above were amongst the first to pay attention to these details and to assume that a phosphorus-deficient ration was one which was phosphorus deficient *per se*, possessed an adequate amount of protein and starch equivalent for maintenance and growth, and enough calcium to satisfy the calcium requirements of the experimental animals. The experiments outlined below, however, were carried out to throw light on the effects of what might be called a "marginal phosphorus deficiency" rather than a gross deficiency as investigated by the above authors.

Description of Experiments.

Since in the present investigation it was desired to conform as much as possible to the natural conditions under which sheep grazed on the hill farms of Yorkshire and Northumberland, it was necessary to devise a ration which would provide adequate protein and starch equivalent for maintenance and growth as well as a sufficiency of the inorganic elements, with the exception of phosphorus, to satisfy the mineral requirements of the animals. It was decided that the plane of phosphorus in the diet of sheep used in the present experiments should be slightly under that which would be regarded as adequate. Difficulty was found in obtaining foodstuffs to compose a ration of this type. Ultimately use was made of a special brand of flaked maize prepared for brewers malt, the phosphate content of which was less than 0·3 per cent. At the start of the experiment the ration consisted of good hay, flaked maize, sugar beet pulp and locust bean flour. The limiting factor of the phosphate content of the ration was the hay which had a phosphate content of 0·69 per cent. Therefore the amount of hay fed was reduced to the minimum which would allow of a sufficient

supply of roughage in the diet. It was soon evident that the sheep would not eat their allotted portion of locust bean meal and this was withdrawn from the ration and replaced by an increased amount of flaked maize and sugar beet pulp without loss of starch equivalent or digestible protein. The animals receiving the low phosphorus diet actually consumed on the average between 1·00 g. and 1·50 g. of phosphorus per day. The CaO: P_2O_5 of this diet was approximately 3·5: 1. It will be seen from the results detailed below that the level of phosphorus in this ration was decidedly below that which was necessary for normal growth. The animals in the control group received, in addition to the basal ration, 100 c.c. of 10 per cent. sodium phosphate which added 0·8 g. phosphorus to their diet. This proved to be more than sufficient for maintenance and growth. The details of the experiments can be best discussed under three headings, namely, nutritional investigation, biochemical changes in the blood, and chemical and anatomical changes in the composition of the bones.

Nutritional Investigation.

In view of the findings of Shearer and Stewart (1933) it was considered possible that the presence of heavy infestations of nematodes might be a factor in inhibiting the assimilation of phosphate and thus increasing the deleterious effects of a phosphorus deficiency. Accordingly, in designing the present experiments, it was proposed to include a group which would be on a low phosphorus diet and have a heavy nematode infestation. This necessitated four groups in the experiment, but considerable difficulty was experienced in increasing the degree of infestation, and still more in maintaining it, by dosing the sheep with large quantities of larvae by the mouth. Despite the difficulties the work was continued over a period of some months, but was ultimately abandoned. On eliminating the parasite factor from the experiment the sheep were regarded as two groups, a low phosphorus group and a control group, and in the following descriptions and discussions the influence of parasites will be considered to be negligible unless otherwise stated. Sixteen sheep were placed on experiment on 1st January, 1934. The control group consisted of four ewes and four wethers and the low phosphorus group of three ewes and five wethers. At the start of the experiment their ages ranged from eight to ten months. For convenience of handling and feeding, the animals were placed in four boxes which provided access to a small open cement yard and free exposure to sunlight. In box A were placed five sheep, Nos. 4, 8, 12, 14 and 16, whose initial weights were 38, 44, 82, 77 and 78 lbs. respectively; in box B, Nos. 1, 5, 6 and 9, whose weights were 88, 86, 85 and 95 lbs.; in box C, Nos. 7, 10, 11 and 21, whose weights were 98, 89, 78 and 47 lbs.; and in box D, Nos. 13, 17, and 2, whose weights were 88, 61 and 75 lbs. The animals in boxes A and D constituted the control group, and those in boxes B and C the low phosphorus group. The basal ration consisted of hay, sugar beet pulp, flaked maize and locust bean meal. Analyses of the various foodstuffs used in the experiment are given in the following table.

The Anatomical Changes in the Bones and their Relationship to Rickets

BY

J. R. M. INNES

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ADDENDUM

The Anatomical Changes in the Bones and their Relationship to Rickets

By J. R. M. INNES.

It has been impossible to complete the histological examination of the bones in time for the results to be incorporated in this paper, as the process of preparation of bones from domesticated animals, even those showing porotic changes to a moderate degree, is a long and tedious procedure. Although all reference to this aspect of the subject must be reserved for a future paper, it is thought that a consideration of the gross changes observed in the bones may permit of some conclusions being drawn regarding the degree, extent and type of the skeletal disturbance which, as will be shown, has been caused by the diet.

In the absence of data regarding the presence and amount of osteoid tissue, the degree of osteoblastic activity and the manner, intensity and localisation of bone resorption, the interpretation of the nature of the lesions is certainly restricted, but all these features are, to a certain extent, mirrored in the gross anatomy of bone. The equilibrium between bone apposition and bone resorption which results in normal ossification and growth is reflected to the naked eye in the thickness of the corticalis, in the length and diameter of the shafts of long bones and the qualitative and quantitative appearances of the spongiosa at any given period of development (age of animal). For the purpose of the general conclusions of this paper (Stewart), therefore, the absence of histological data need not be considered of paramount importance. The main points to be considered are: (*a*) are lesions present in the bones of the experimental sheep? and if so, (1) can they be correlated with the prolonged deprivation of phosphorus? (2) do they bear any resemblance to those produced by a diet low in Ca, or to those seen in a naturally occurring bone dystrophy of sheep known popularly as "cappi" (Bosworth and Stewart 1932)? (*b*) do these experiments, as far as bone lesions are concerned, bear any relationship to those of Theiler (1934) regarding aphosphorosis and rickets of sheep in South Africa?

All experimental and control animals were either shot or killed by chloroform and autopsies carried out in the routine manner. As in no case were lesions present in visceral organs it will be unnecessary to mention this fact in the protocols below; the latter are confined therefore to skeletal changes.

The femur, humerus and skull were sawn in half longitudinally; one half of each of these along with portions of vertebrae were macerated in water or treated by antiformin for macroscopic examination. (Representative pieces of the remaining parts of these

bones along with rib junctions, and portions of scapulae were fixed in formalin for subsequent decalcification in Müller's fluid and histological examination.) It is a known fact that in generalised malacic conditions, e.g. rickets, osteomalacia and osteitis fibrosa the severity of the lesions varies in different bones of the skeleton and that the bones which are most prone to severe resorptive processes are those in which normal growth is most active. The bones selected for examination in this experiment are therefore considered as suitable types for determination of any possible widespread changes.

The macerated bones were examined by the naked eye and by the aid of a stereoscopic dissecting microscope. Schmorl (1931) and others have used this latter method to demonstrate gross quantitative and qualitative architectural changes in the spongiosa and compacta of various osseous dystrophies in man, and contend that it is a valuable supplementary method to any study of bone pathology. The bones of the experimental sheep were compared with

(*a*) bones from parallel control animals of the same age;

(*b*) bones from younger and older normal animals;

(*c*) bones from sheep which had suffered from prolonged low Ca diet;

(*d*) bones from sheep affected with the naturally occurring "cappi," previously described by Bosworth and Stewart (1932);

(*e*) bones (loaned by Sir Charles Martin) from sheep which had been fed on a low phosphorus diet in Australia (Martin and Pierce, 1934).

PROTOCOLS OF MACROSCOPIC CHANGES.

Control Sheep Nos. 16, 17. (Basal low phosphorus diet plus 0·8 g. phosphorus; 13 months on diet; 2 years old.)

No fragility or brittleness in long bones, vertebrae or calvaria; corticalis of long bones dense ivory-like in consistence; irregularity and partial absence of epiphyseal growth cartilages in femur and humerus (epiphyseal union commenced); periosteal and endosteal surfaces smooth and white; yellow marrow throughout medullary cavity; spongiosa reduced in amount at ends of long bones and composed of thick coarse white trabeculae, many being fused to form confluent plates and bars and continuous with partially ossified epiphyses; the delicate honeycomb-like arrangement of fine regular transverse, longitudinal and oblique trabecular bars seen in a young growing animal lost; thick calvaria of coarse spongy bone; similar coarse type in vertebrae; appearances compatible with age of animal and similar to those of any animals in which ossification has reached a low ebb (see Fig. 1).

Experimental Sheep No. 7. (Low phosphorus diet, 13 months; 2 years old.)

No beading of costo-chondral junctions; line of ossification in these straight and regular; all macerated bones comparatively lighter and smaller than in controls; no marked brittleness, but rather an all-round comparative delicacy of architecture; corticalis of long bones considerably thinned as compared with controls; average thickness in middle of femur shaft about 1 mm., compared with 3–4 mm. in control bones; no gross porosity; expansion of medullary cavity extending almost to epiphyses; periosteal and endosteal surfaces smooth; gross reduction in amount of spongiosa, the latter restricted to small irregular areas near partially ossified growth cartilage; yellow marrow throughout medullary cavity; the trabeculae of remaining spongiosa thin, delicate and extremely irregular, and the intertrabecular marrow spaces enlarged; the cranial and facial bones much thinned (see Fig. 2).

Control Sheep Nos. 12, 13. (Basal low phosphorus diet plus 0·8 g. phosphorus supplement, 18 months on diet; 2½ years old.)

Appearances similar to those of other control group except that epiphyseal union more advanced; corticalis of long bones thick and spongiosa exceedingly coarse in type with small intertrabecular spaces; bones of cranium, face and vertebrae show similar coarse type of bone (see Fig. 1).

Experimental Sheep Nos. 1, 11, 21. (Low phosphorus diet, 18 months; 2½ years old.)

No beading of costo-chondral junctions; several old standing simple transverse fractures in distal third of several ribs in No. 11; the changes in all the bones comparable to those already mentioned for experimental Sheep No. 7, but of greater intensity; the thinning of corticalis, expansion of medullary cavity, reduction in spongiosa of the long bones all more severe compared with their parallel controls (Nos. 12 and 13) than in No. 7; thin calvaria with some degree of fragility.

Experimental Sheep No. 9. (Low phosphorus diet for 14 months, extra phosphorus supplement for 4 months; 2½ years old at death.)

No beading of costo-chondral junctions; general delicacy of structure as in previous group; corticalis of long bones slightly thinner than controls, but thicker than previous experimental sheep; reduction in spongiosa still as marked as in others; as compared with Nos. 1, 11 and 21 the differences are slight and cannot with certainty be ascribed to additional phosphorus supplement.

Discussion.

Despite the apparently passive character of bone as a skeleton, it is becoming increasingly evident that it must no longer be regarded as a static, but rather as a plastic structure, which by acting partly as a reservoir for calcium and phosphorus is peculiarly susceptible to changes in the blood and prone to rapid alteration and reconstruction. The various factors which control normal ossification and growth, and which may therefore cause profound disturbance by their absence (or in certain cases by their excess), are now known to include vitamin D, Ca and P intake and absorption, the Ca/P ratio of the diet, the parathyroid hormone and an enzyme phosphatase, which is normally present beneath the periosteum and in zones of provisional calcification. The etiological significance of these factors has now been related to certain malacic diseases in man, and as a result certain differences in the lesions are more clearly understood (cf. rickets, late rickets, osteomalacia and osteitis fibrosa). A more recent development has been the application of this knowledge to analagous bone dystrophies in domesticated animals for which credit must be given to Marek and Wellmann (1931) in Hungary, and Theiler (1934) in South Africa. It is thus recognised that rickets and osteomalacia undoubtedly occur as pathological entities in domestic animals in those countries. Since no less an authority than Hess stated as recently as 1930 that "in spite of the numerous reports of rickets-like disorders in animals, the pathological identity of these with a true rachitic process in man had not been proved," the importance of this development in veterinary pathology need not be emphasised. Further, the view which is now accepted that rickets and osteomalacia in man are essentially the same disease (first suggested by Pommer as far back as 1885), and that the differences in the lesions can be related to the age of the patient and are merely quantitative, finds an analogy in these diseases of animals. The relatively early onset of ossification which occurs in animals *in utero* and their more rapid skeletal development after birth, preclude the possibility that the rachitic lesion as seen in them, except in the case of the dog, will conform to the classical picture described in the

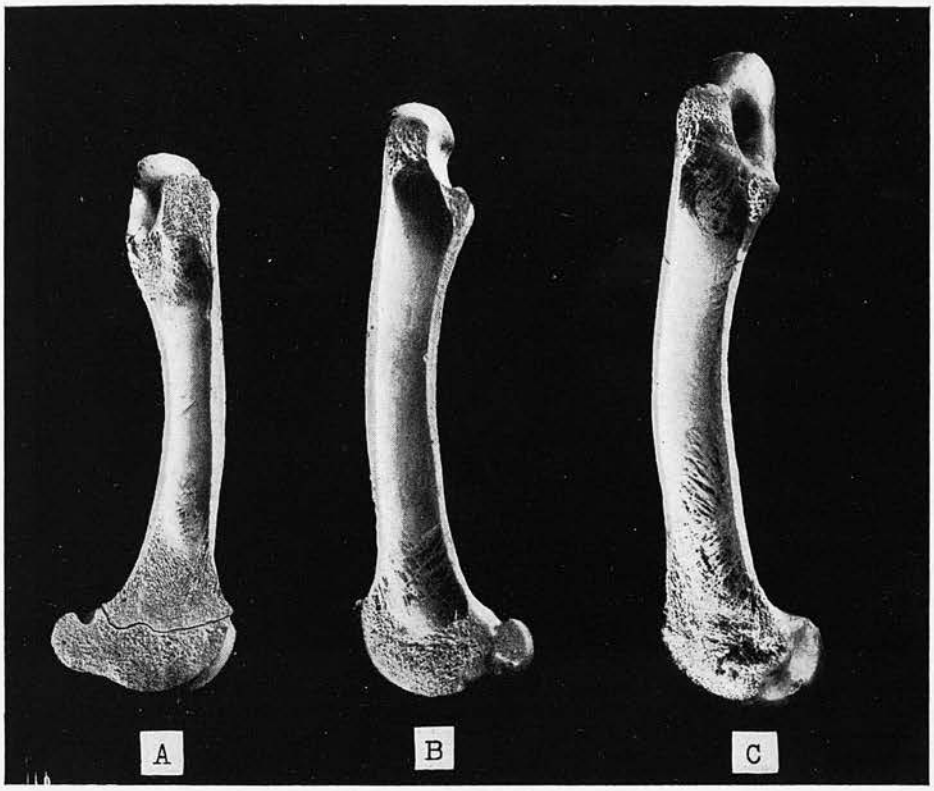

FIG. 1. Longitudinal sections of femora from (A), Lamb, 7 months old, actively growing; (B) Control Sheep No. 12; 2 years old; (C) Normal aged ewe about 4½ years old. Comparison of these three bones shows changes due to advancing age and slowing up of ossification.

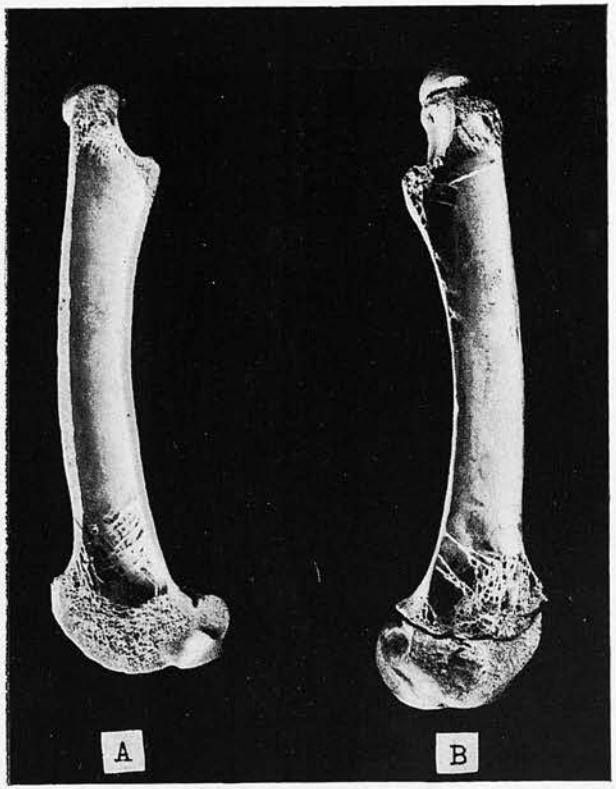

FIG. 2. Longitudinal sections of femora from (A), Control Sheep No. 12, 2 years old; (B) Experimental Sheep No. 7 (low phosphorus diet, 13 months, 2 years old when killed). Note thin corticalis, expansion of medullary cavity and relative absence of spongiosa in B compared with A.

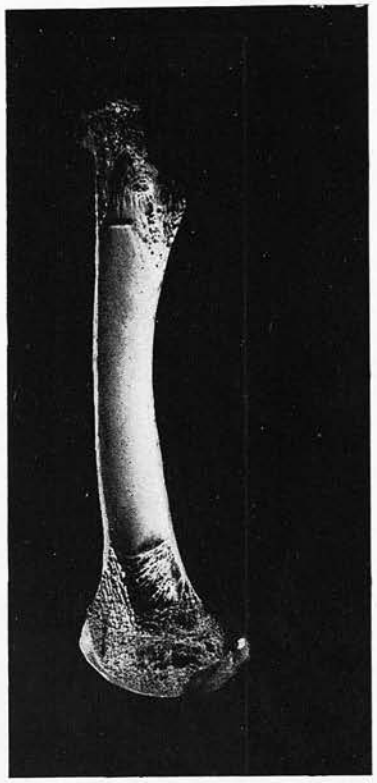

FIG. 3. Longitudinal section of femur from case of "cappi": note the similarity of the gross changes compared with experimental Sheep No. 7 (Fig. 2), although from a much younger animal (about 6 months).

child. It is more likely to resemble that of early adolescence in the human. Another important outcome of Theiler's work has been to show that the same factors which influence ossification and which are etiologically concerned with certain malacias in man, may exert their activity in a different manner in animals. According to this author, although rickets and osteomalacia are pathologically identical in the various domesticated animals, they are etiologically different: e.g. in pigs the condition is an acalcicosis, in bovines and ovines an aphosphorosis, and in dogs an avitaminosis.

The facts and explanation given by Theiler (1934) appear so indisputable that there is some hesitation in suggesting that it is still necessary to prove that they hold good for conditions in this country at least in the case of the sheep. It can be stated that no fully authenticated cases of rickets in sheep have yet been recorded in England, and therefore its existence as a disease entity in this animal in this country is still in doubt. (In this connection the term rickets is used in the more restricted classical sense.) It might be mentioned here that a number of bones (rib junctions and long bones) from sheep and lambs affected with a skeletal disease, e.g. "cappi" and the so-called "cripples" have been examined histologically. In these cases no lesions were demonstrated which could be classified as being truly rachitic in type. Osteoid tissue was conspicuous by its absence, gross endochondral changes were not present, and the essential features of the lesions appeared to be an exaggerated porosity and a corresponding fineness in bone trabecular structure which could be related to a deficiency in the development of the centres of ossification. While it may seem that such changes can hardly be ascribed to rickets in the ordinary sense of the term, it will be shown later that they might be included under that heading if a wider concept of the disease process was adopted. They would thus represent transient phases in a disease in which certain modifying factors have altered the capacity of the bone to react along truly rachitic lines. (In man, rickets and osteomalacia are two conditions arising from the same fundamental cause, but it cannot be denied that they show pathological differences.) All the above-mentioned facts must be clearly borne in mind when considering the nature of any naturally-occurring skeletal disease of sheep or one produced experimentally by dietary deficiency.

It would appear from the protocols that the subjection of sheep to a prolonged low phosphorus diet results in a widespread disturbance in ossification, exemplified in such bones as the femur, humerus, vertebrae and skull when compared with suitable controls. Corroboration of this fact is seen in the chemical analysis of the bones. In the long bones the outstanding features are the marked thinning of the corticalis without any gross porosity, the expansion of the medullary cavity and the decided quantitative and qualitative reduction in the spongiosa. The combined effect of these changes is seen as a general delicacy of bone architecture rather than a gross osteoporosis in the true anatomical sense. Further, these obvious lesions differ only in degree from those to be seen in similar bones of animals (same and different

ages) which were subjected to a low Ca diet, from those present in bones of sheep suffering from the naturally-occurring "cappi" (see Fig. 3), or from those observed by Martin and Pierce (1934) in their low phosphorus experiments.

It may be questioned whether there is any justification for attempting to interpret these gross changes in terms of pathogenesis and to relate them to those of the known bone dystrophies. However, the cessation of body-growth of the experimental sheep seen shortly after the diet was commenced, appears to indicate that the loss of phosphorus precluded further bone growth during the experimental period. In this light, therefore, the general thinness of the corticalis and the relatively smaller size of the bones as compared with the controls, would indicate that the process underlying the changes was essentially an incapacity of the animals to form bone; a complicating feature of this change is the fact that in a prolonged experiment of this nature ossification in general becomes progressively slower as the animal gets older. The condition of the bones seen after 18 months on the low phosphate diet would therefore represent a stage in ossification which has progressed little beyond that at the beginning of the experiment. In addition to this delayed and slow ossification the decrease of the spongiosa seems to indicate concomitant resorption of the bone reservoirs. The ultimate picture is thus regarded as due to decreased bone apposition, progressive slowing of ossification due to advancing age and a resorption of the spongiosa as the animal's general need for phosphorus becomes greater and greater.

Since Theiler in South Africa and Martin and Pierce in Australia have shown that rickets can be produced simply by feeding phosphate-deficient diets, is it possible to correlate the bone picture with that of rickets? It has been demonstrated that in the rat, bone lesions can be modified by slight variations of the etiological factors concerned. A condition almost identical with osteitis fibrosa, a simple generalised osteoporosis or rickets itself can be produced at will by alterations in the vitamin D and mineral content of the diet. Similarly the bone lesions of hyperparathyroidism (osteitis fibrosa), whether produced experimentally by excess parathormone or in the natural state in man as the result of a parathyroid tumour, can be simulated by simply feeding ammonium chloride to rats, thus causing intense decalcification (Jaffe 1933). It would appear, therefore, that these different types of lesions which are obviously related might be so modified under less drastic experimental conditions that phases might well occur in each which would show a similar picture. The imposition of more drastic conditions could thus be regarded as the cause of the gross differences in the bone reaction and the divergence in the final picture. The general conclusion from this would be that the final pathological picture in bone, either in a naturally-occurring disease or one produced experimentally, is modified by, and varies with, factors such as age, duration of the disease, and above all the speed and degree of bone resorption and probably the initial intensity of the process. (It need hardly be emphasised that the stage at which the lesion is examined is also of prime importance.)

These factors indeed might well account for the fact that all degrees of porotic and hyperplastic types of malacia may occur in the course of rickets in infants and children. In the case of the present experiments, where the dietary deficiency was one of phosphorus *per se*, it is probable that those factors which might have changed the bone picture from that of a simple retardation of ossification with concomitant slow resorption of the spongiosa to one of classical rickets were absent. If the experiments had been carried out under suitably modified conditions the same degree of phosphate deficiency might have resulted in true rickets. It appears, therefore, that as far as animals are concerned, the pathological and etiological concept of rickets propounded by Theiler might be regarded as even wider than hitherto recognised. The bone lesions produced by either an aphosphorosis or an acalcicosis, even if they do not conform to those of classical rickets, need not therefore be placed outside the scope of a rachitic process. They could include a simple osteopathy and a moderate degree of osteoporosis which might well be a manifestation of a phase in a rachitic disease in the wide sense, in which the end stages have not been reached or are in fact rendered impossible. In this case the lesions seen in these low phosphorus experiments, those seen in low Ca experiments, those of "cappi" and "cripples" (Stewart, W. L., 1933) and those of "peg leg" in cattle (Turner, Kelly and Dann, 1935) would be included in this category. To widen the concept of rickets in animals in such a manner is merely to bring it into line with those authorities who consider that rickets, late rickets, osteomalacia, hunger osteopathy, war, puerperal and senile osteomalacias in man are all manifestations of the same disease.

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Malignant Disease in Animals and its Implications as regards the Cancer Problem as a Whole

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The pathology, incidence, genesis, malignancy and surgical treatment of neoplastic disease of the lower animals has not been studied as intensively as in man. Nevertheless, it is to be stressed that such a study is a necessary adjunct for the study of the disease problem as a whole, as it is totally unscientific to regard cancer in man as worthy of so much attention and to regard it only as an object of academic interest in the domesticated animals. Much could be done by taking a wide view, and because of the enormous amount of material available, many points of great practical and theoretical importance could be investigated by utilising animal and human material as well as by the latter alone. Along these lines a series of papers will shortly appear in collaboration with Colonel W. F. Harvey and Dr. Edith Dawson, of the Royal College of Physicians Laboratory, Edinburgh, in which many debatable aspects of tumour pathology will be discussed.

In spite of orthodox teaching there is great difficulty in forming an accurate definition of neoplasia beyond the fact of autonomous growth. As Nicholson states, wherever we look we see that tumours exhibit no differences in kind but only in degree, often of the slightest, from other tissues of the body. Tumours are by no means the outcasts of the body, as so many pathologists would have us believe.

All tumours are composed of parenchyma and stroma, the amount and type of each varying in different classes of growths. Nearly all adult and embryonic tissues have their parody in some type of growth; it is therefore desirable that, at present, the classification of tumours should be on a histogenetic basis. For example, in the case of the mesenchymal tumours and gliomata (classification of Bailey and Cushing) it is possible in this way to find some analogy in neoplasia corresponding with every undifferentiated and adult type of mesenchymal and glial cell.

The distinctions between benign and malignant tumours were shown to involve questions of interest from both the theoretical and practical sides. The essential differences between them were reviewed. After many years of experience and co-operative work between the surgeon and pathologist it is possible in the case of man to draw some inference regarding the course of a tumour from its structure; in animal pathology this inference is in reality only surmise. In the case of animals it is assumed, often wrongly, that tumours which bear a structural resemblance to a tumour in man may be expected to take the same course. Exact data must be derived from studies of animal material alone and comparisons then made with the disease in the human. While the histology in many circumstances should override all other considerations in the estimation of malignancy or otherwise, this interpretation must be influenced by such factors as the anatomical location of the tumour, its attachments, the presence of a capsule, and the age and condition of the patient (human or animal). It is a common error to ignore these essentials and as a result is possibly the cause of so much difference in opinion regarding the relation of microscopical structure to the course and prognosis of neoplastic diseases.

The structural features indicative of malignancy were mentioned, stress being laid upon the feature of anaplasia. The more primitive and undifferentiated the type of cell the more malignant is a growth likely to be. As Adami has postulated, a cell cannot carry out at the same time its functioning and vegetative powers. This law has a wide application in all pathological processes, but more particularly to malignant disease.

The most impressive sign of malignancy is the metastatic spread of tumours. For the scientific study of the behaviour of tumours, metastases are of as great importance, however inconspicuous, as the primary growths. The description of a neoplasm is incomplete if it does not embrace the metastases. There are few comprehensive accounts of such studies in man apart from the recent publication by Willis (1933), "The Spread of Tumours in the Human Body," and none in animal pathology. This field alone in animal pathology has therefore much to commend it for study, as it may provide the solution to many problems of practical and scientific importance (*e.g.*, in the case of the spread of breast cancer).

The paths of neoplastic extension are (*a*) along the planes of tissue cleavage, (*b*) by lymph vessels, (*c*) by the blood vessels, (*d*) by coelomic spread, (*e*) along cerebrospinal spaces, (*f*) and along epithelial cavities. Along any of these routes tumours may extend *without loss of continuity* over wide areas, and by the same routes, except the first, by *discontinuous extension* and the production of remote metastases by the carriage of detached tumour cells. Regional extension and metastasis differ in the continuity or discontinuity of the spread; the term metastasis, therefore, should be confined strictly to discontinuous spread with the setting up of new secondary foci. Each method of spread was discussed and examples cited.

In the case of spread *via* the lymph vessels a distinction must be drawn between the entrance of tumour cells into the vessels and subsequent embolic transit, and lymphatic permeation. The latter has been claimed by Handley (its great protagonist) as the major mechanism involved in all carcinomatous dissemination, and in the case of the human female has been a feature in determining the surgical technique dealing with breast cancer. In view of the controversy raging around Handley's contentions it is apparent that here is a field in animal work which could prove of undoubted service in determining the extent which lymphatic permeation plays in the spread of malignant disease of the breast.

Although commonly taught to students it is erroneous to believe that carcinomata spread exclusively by the lymph stream and sarcomata by the blood stream. Some of the former class are notorious for their early invasion of the vascular system, *e.g.*, hypernephroma of the kidney, hepatic tumours, and bronchogenic carcinoma. In the case of breast cancer spread may occur simultaneously by both lymphatic and haemotogenous paths. In every tumour, irrespective of its type, subsequent to the involvement of the lungs by secondary deposits, invasion of the systemic circulation with resultant tertiary deposits in the visceral organs is almost universal. The intimate relation of sarcoma to the blood vessels and hence the common invasion of large and small veins, is repeatedly, perhaps too emphatically, stressed in text-books.

Tumour emboli present in the vessels of a primary growth do not necessarily signify metastases; many tumour emboli must undoubtedly perish in the blood stream or remain sterile in their new environment. Only if the transported cells survive, multiply and retain their growth characteristics can a secondary tumour be established.

In connection with coelomic spread it may be pointed out that, although primary tumours of the serous membranes probably do occur, the diagnosis of such is frequently made (in man and animals) on totally inadequate grounds, and without proper appreciation of the frequency with which small primary carcinoma in the neighbouring viscera may yield massive local extensions to the serous membranes (Willis, 1933).

The effects on the body of malignant disease were summarised. Emaciation can be said to be the constant effect of malignant disease. This may occur with or without anaemia and sometimes with the preservation of the fat deposits. Symptoms are naturally controlled largely by the localisation of the tumour.

The relation of neoplasia to inflammatory hyperplasia can be summed up in the words of Borst, that the latter results from exaggerated response to external irritants, while neoplastic growth arises from loss of normal restraints to growth. The one (inflammatory overgrowth) is purposeful, organised, self-limiting, typical accelerated hyperplasia (*e.g.*, callus formation in the repair of bone), the other (neoplasia) is baneful, lawless or progressive atypical degenerative growth excess. Nevertheless, such theoretical distinctions may fail when applied to processes of a doubtful nature.

Following the above general consideration of malignant disease, two examples of relatively common forms of malignant disease in animals —that occurring in the testis and the mammary gland of the dog—were discussed. As both of these will form the subject matter of separate communications in the future only the general outline of the remarks is given here.

Malignant Disease of the Testis.—The structure and embryology of the organ was reviewed to attain an understanding of the types of tumours which could and do occur. From the point of view of surgical treatment, it must be remembered that the lymph drainage of the testis is entirely through vessels in the spermatic cord to reach the lumbar and para-aortal lymph glands; the inguinal glands, although in close proximity to the testis, do not receive any lymph vessels draining from the testis. In 25 cases of testicular tumours (*post-mortem* and operation specimens) the seminoma or embryonal carcinoma were found to be by far the commonest type encountered (20 cases). In the case of man, the seminoma (also the commonest tumour) is still frequently and erroneously classified as a round-cell sarcoma. The seminoma is a rapidly-growing, soft tumour arising in the seminiferous tubules and invading the *rete testis*. The tumour may reach a considerable size and then undergo central necrosis and haemorrhage. The shape and outline of the organ is always retained, as the *tunica vaginalis* acts as an inhibitory barrier to lateral spread. The tumour occurs in dogs of any age, but usually in middle-aged animals, and the epididymis is almost never involved. Although the tumours in dogs are histologically indistinguishable from the seminoma of man (a malignant tumour), they appear to metastasise relatively late. From cases in which a follow-up history has been obtainable post-operative survival may be up to five years without recurrence or spread. Simple orchidectomy without any radical operation appears, therefore, in such cases to offer a reasonable chance of permanent cure. The occurrence of prolan in the urine of cases in man was discussed in relation to diagnosis and possible aetiology, as has been suggested by some workers. In a paper to be published shortly (Innes, Harvey and Dawson) dealing with the classification of testicular tumours in man and animals, it will be shown that in reality there are only two distinct types of tumour of the testis, *viz.*, the seminoma and the teratomata, the latter of which is undoubtedly more highly malignant.

Malignant Disease of the Mamma in Dogs.—The structure, embryology and lymphatic

drainage of the breast was next considered. It was emphasised, in relation to cancer of the mamma, that the breast is not a static structure, but one constantly undergoing periodic changes as a result of hormonal control, and that if this control becomes disturbed the changes will cross from the physiological to the pathological. To understand the genesis and pathology of malignant disease, one must bear in mind the variations in the structure and function of the breast from birth to puberty, pregnancy, lactation, involution and senile atrophy.

Whether the tumour is located in the anterior or posterior mammae secondary involvement of the axillary lymph node by carriage of tumour emboli in the lymphatic vessels draining from the mammae is almost inevitable. Naturally, primary tumours arising in the axillary or pectoral mammae will spread to the lymph node relatively earlier than those arising in the posterior glands. Secondary deposits may be set up in the lungs either by direct venous transport of tumour cells from the primary growth, or in the same way from the axillary node metastases or even *via* the subpleural lymphatics involved in direct lymphatic permeation from the primary growth. Subsequently to the formation of pulmonary metastases, invasion of the systemic circulation in the lungs will result in tertiary deposits in the visceral organs. All of these points regarding dissemination are of the utmost importance in the contemplation of post-operative treatment in the case of the human, and will form a separate publication at a later date.

The common occurrence of cartilage, and bone formation (often with haematopoiesis in the marrow spaces of the latter)—metaplasia—in chronic inflammatory conditions of the dog's mammary gland must be distinguished from true osteochondroma.

The various types of benign and malignant tumours of the mamma were briefly reviewed. The spread of malignant tumours in the bitch's mamma follows closely the paths of dissemination which occur in the case of breast cancer in women. In the case of the bitch malignant sarcoma of the mamma has been observed to disseminate by the same routes as carcinoma, *viz.*, primarily by lymphatic paths—a point which again emphasises that it is erroneous to believe that sarcoma spreads mainly by blood stream invasion.

The occurrence, genesis and histological changes of pathological conditions were discussed. It could be considered that in the mammae we might have increased lobular overgrowth (the adenosis of Dawson, normal when occurring during lactation but pathological when occurring outside the normal growth periods), increased epithelial proliferation remaining confined within the duct boundaries (the epitheliosis of Dawson) and interstitial fibrosis producing the fibroadenoma when combined with adenosis. Cystic ducts may complicate the picture at any time to produce what is often erroneously termed "chronic mastitis." A series of progressive changes might be followed in the duct epithelium until solid masses of atypical epithelium fill the duct to render the condition indistinguishable from carcinoma. The highly malignant condition is one of invasion of epithelium beyond the normal boundaries into the tissue spaces and lymph vessels of the mamma. In the bitch true malignant sarcomata are not infrequent.

The occurrence of lymphatic permeation in the deeper fascia of the breast, extending through the pectoral and intercostal muscles into the subpleural lymphatics, with the production of a true lymphangitis carcinomatosa in the pleurae, undoubtedly is common in mammary cancer of the dog. The practical importance of this possible mode of dissemination cannot be too highly stressed from the point of view of surgical treatment of mammary cancer in bitches and because of the criticism which has been directed at Handley's views regarding lymphatic permeation. It is wise to recall Handley's dictum regarding the operation involved in cases of breast cancer in women, namely—that "the problem is not the excision of an organ, but the extirpation of a diseased lymphatic area." The presence of miliary nodules near the area of the primary growth, in the area of the lymphatic paths draining the site, in the skin of the regional lymphatic glands or enlargement of the glands themselves in cases of breast cancer in bitches are all points of importance in determining the nature and extent of the surgical operation.

It is obvious that there is a great field awaiting investigation in this realm of animal pathology. More exact data are required about the incidence, types and genesis of tumours in animals; information is still required regarding the paths of dissemination of malignant growths; that regarding post-operative survival of animals following the extirpation of malignant tumours is unavailable. As one step in this direction the formation of a tumour registry would be invaluable. As Harvey and Hamilton have stated in connection with a similar idea in the case of man, this registry, if expanded, could be made into a similar collection to that of the National Type Collection of Organisms (Lister Institute). In this way it would be possible to obtain a type of tumour along with accurate information regarding the history of growth, malignancy and post-operative survival of the animal concerned either for teaching, research or museum purposes.

The paper was concluded by a demonstration of numerous specimens of malignant disease affecting the testis and mamma of the dog, including specimens to demonstrate the paths of dissemination.

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The Lesions Produced in the Organs of the Rabbit by Single and Repeated Intravenous Injections of BCG: A Preliminary Note

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THE aim of these experiments was to throw light on the development of immunity in calves vaccinated with BCG by the intravenous route. Buxton and Griffith (1933), and Buxton, Griffith, and Glover (1935), showed that calves resisted an intravenous injection of virulent tubercle bacilli better after an intravenous injection of BCG than after an intratracheal or subcutaneous one.

The intravenous route was therefore used in later experiments, and it was observed that BCG was rapidly eliminated from the body. For example, three calves died or were killed nine, fourteen, and twenty-five days respectively, after the injection of BCG; cultures were prepared from the lungs, and colonies were most numerous in the first animal and least in the last. In another experiment, five calves were inoculated intravenously with 100 mg. BCG; one calf was killed seventy-one days later and no acid-fast bacilli were found microscopically. Cultures were also prepared from various sites but only one colony of BCG was obtained from the left bronchial lymph-gland. From a second calf, killed at the hundred and twentieth day, no cultures of acid-fat bacilli were obtained. These results seemed to imply that, so far as cultural experiments were concerned, BCG disappeared from the tissues before the fourth month. The remaining three calves continued to react to tuberculin for several months although with decreasing intensity. Two were completely resistant to oral administration of virulent tubercle bacilli seven to ten months after vaccination, while the third, although it became infected after thirteen months, possessed a greater degree of resistance than unprotected controls.

In addition to the persistence of immunity after a primary injection, attention was directed to the effects of reinforcing doses of BCG. A second dose of 100 mg. BCG, given to calves at six, nine, and twelve months respectively, after the initial injection, definitely reinforced the immunity. Often, however, the second injection induced a severe reaction, which in two instances was followed by the death of the calf. It seemed, therefore, that if the pulmonary tissue was in this high state of sensitivity, a large proportion of the vaccinating dose might be rejected. It has not been possible to test this point in calves but the rabbit experiments described below, in which reinoculation was carried out at various intervals, suggest that this does occur.

The main object of this investigation, therefore, was to study the genesis and fate of the lesions produced in rabbits by single and repeated doses of BCG, with the hope that the results might throw some light on the immunity developed in the calf after vaccination with BCG. It was also hoped that additional information

might be obtained about the formation of the tubercle. Huebschmann (1928), Cunningham and Tomkins (1928 and 1931), Sabin, Doan, and Forkner (1930), believe that the entire development of the tubercle is an exudative process, all the cellular constituents being derived from the blood and that the lipoids of the tubercle bacillus contain a maturation factor for the monocytes and epithelioid cells. Krause (1927) contends that it is essentially a proliferative process, and states that although leucocytes, red blood cells, &c. may enter into the structure of the nodular tubercle, they are not an essential part of this lesion. Pagel (1932) has shown that some tubercles begin in exudation but later elicit a proliferative response, and that others are essentially proliferative from the beginning.

The origin of the fibrous tissue in the tubercle is also debatable. Does it grow in by proliferation of the surrounding fixed mesenchyme cells? Are the mononuclear phagocytes and their derivatives, the epithelioid cells, capable of forming it? (Maximov, 1935). Or is it a precipitation process arising from the production of a specific factor from the vascular endothelium and other mesenchyme cells? (Day, 1936).

Many workers have shown that BCG may produce tubercles. Lurie (1934) stated that BCG organisms are soon destroyed in the body after inoculation, for although the injection of 1 to 2 mg. produced typical tuberculous lesions in the lungs of rabbits, within two months the lesions and bacilli had disappeared. The destruction of the bacilli usually coincided with the development of hypersensitivity. Rosenthal (1936) made intracardiac injections of 10 to 15 mg. BCG into guinea-pigs and showed that distinct lesions could be found in the lungs after a few hours. The nodules which formed were initiated either by an accumulation of leucocytes or by a proliferation of epithelioid cells. By the end of the first week typical tubercles made their appearance. Their regression was accompanied by the entrance of lymphocytes and the lesions disappeared within one to three months. Fibrosis was rare and caseation and necrosis were absent. The histological differences of lesions after intracutaneous injection of BCG and virulent tubercle bacilli have been described by Pagel (1929).

Methods.—Ninety rabbits were injected intravenously with graded doses of BCG ranging from 0·005 to 10 mg. Most of the animals have been killed at intervals from 24 hours to 290 days, but the experiment is not yet finished. Various organs were examined histologically and attempts were made to recover the organisms from the lungs by cultural methods.

Synopsis of results.—Within twenty-four hours of the injection of 5 to 10 mg. BCG, innumerable cellular foci were discernible in the lungs. These foci, containing clumps of acid-fast bacilli, formed septal thickenings which had apparently followed the lodgment of the bacilli in the capillary walls. The cell aggregations consisted of polymorphonuclear leucocytes and phagocytes, and appeared responsible for the initial localization of the bacilli. The polymorphonuclear leucocytes took no further part in the formation of the tubercle. By proliferation of the fixed mesenchyme cells and/or by localization, and subsequent proliferation, of monocytes, these septal thickenings increased in size and by the sixth day formed considerable bulges abutting, but not exuding, into the alveolar spaces, so that at this stage the lesions were thus strictly intracapillary. By further cellular proliferation in an intramural direction the classical tubercle made its appearance between 12-21 days, when the lungs were riddled with such lesions. In the centre of the tubercles there was a conglomerate mass of epithelioid cells and at the periphery a well-marked zone of lymphocytes. Polymorphonuclear leucocytes were either scanty or absent. Necrosis was present in only a small proportion of the tubercles and Langhans's giant cells were few and not necessarily associated with necrosis. (Medlar (1926), Day (1934), and others have contended that giant-cells in a spontaneous tubercle are post-necrotic in development). The margin of the tubercle projecting into the

alveoli was composed of alveolar epithelium which, at a later stage, often showed a distinct metaplastic cubical transformation. Many of these mature tubercles, which were first seen within two or three weeks of the injection, persisted, and were present, though in comparatively small numbers, in the lungs of rabbits killed as late as 205 days after injection. Many must have regressed, therefore, at a relatively early stage. As Rich (1929) has stated, in connexion with the fate of some tubercles in the progressive disease, the cells appear simply to separate and wander off after the bacilli have died or been destroyed. Nevertheless, tubercles persist in the lungs of these rabbits long after bacilli can be recovered by culture, and the reason for this persistence is therefore at present unknown. No fibrosis was seen at any stage by ordinary stains, but by using a special silver impregnation method for reticulum, e.g. Wilder (1935), a well-marked argyrophile fibrillary network was seen enmeshing the tubercles in the lungs of rabbits killed as early as 21 days after injection. This formed a thick felt-work at the periphery of the tubercle (the fibres here anastomosing with the normal pulmonary and vascular reticulum), with numerous fibrils ramifying between the epithelioid cells and penetrating into the centre. In the later stages (e.g. in the lungs of rabbits killed 114-205 days after injection), this fibrillary basket-work was an outstanding feature of the tubercles. The subsequent fate of this process is still to be determined. As no small scar-like foci of such fibrils can be seen in the lungs in the late stages, this proliferation of argyrophil fibres may also be a reversible process. As other workers have suggested, most of these tubercles in the lungs probably resolve completely within a few months, but they may persist as long as 205 days after injection—much longer than has hitherto been suspected. In three animals which were killed 290 days after injection of 10 mg. BCG, no lesions could be determined in the lungs.

In those tubercles which had persisted, increased vacuolation of the epithelioid cells, the appearance of tissue crevices between these cells, and the entrance of blood-cells, appeared to be progressive changes. In such cases many epithelioid cells appeared to have wandered into the neighbouring alveoli.

The mechanism by which necrosis occurs in some of the tubercles is unknown. It is almost certainly not a case of tissue ischæmia caused by an avascular condition of the tubercles, as has been suggested by many workers in the case of tuberculosis.

The lesions in the tracheobronchial lymph nodes never reached the same intensity as in the lungs. In the early stage the nodes were macroscopically swollen, and histologically the essential change appeared to be an intense sinus catarrh, the sinusoids being filled with solid masses of macrophages (desquamated and proliferated littoral cells). No caseation occurred, and the glands in the late stages were unchanged.

Variations in the lesions in the lungs of rabbits injected with smaller doses of BCG than 5 mg. were purely quantitative. For example, no lesions could be seen in the organs of rabbits killed 20, 34 and 44 days after the injection of 0·005 mg. BCG.

The reinoculated rabbits varied considerably in their response to the second dose. Most of the rabbits reinjected as early as 21 days, and as late as 114 days, after the initial injections were not severely affected but a small number died. Some showed acute respiratory distress and died within 24 hours, but in others there was a latent period of about 7 days before this reaction. The lungs in all these animals were voluminous, dark reddish-grey in colour, and consolidated throughout. Histologically, the outstanding feature was the intense exudation into the alveoli of red blood corpuscles and cells indistinguishable from the epithelioid cells. The same type of pneumonic lesion has been observed in the lungs of calves which died under similar circumstances. In the lungs of those animals which survived the reinoculation and were killed at varying intervals, the reaction was similar in type to that which followed the initial dose.

Acid-fast bacilli could be demonstrated in the tubercles in sections of the lungs as late as 114 days after injection, but most of these must have been dead, as the organism could not be recovered by cultural methods after 62 days.

The experiments suggest that in the case of calves there is a considerable degree of resistance long after the "vaccinating" organism has disappeared, unless it is postulated that a few "vaccinating" bacilli persist but are not recoverable by culture. While the mechanism of this immunity is at present unknown, it might be tentatively suggested that the protection is associated with a tissue reaction like that discernible in the lungs of rabbits many months after the intravenous injection. In revaccination experiments in calves, it is possible that the BCG organisms are very rapidly eliminated from the body, and that the immunity results from the marked cellular response which they evoke.

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The Pathogenesis of Tuberculosis in Animals according to the Nieberle School*

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"This constitutes not merely an answer to a problem of scientific importance but equally permits us to draw practical conclusions concerning the individual and group prophylaxis of the disease." (Anon.)

At a previous meeting of this Society (1936)† I pointed out in vehement terms the arrant neglect in this country of authentic work relating to the pathogenesis and comparative pathology of tuberculosis. Considerable adverse criticism was received, which emphasised more than ever how bacteriological and immunological thought has dominated teaching appertaining to the disease to such an extent that the pathology has been deemed to be almost of only passing interest. In justification of these remarks, it was said that the work of Nieberle and his school in Germany on tuberculosis was apparently unknown. My aim is therefore to present a coherent essay regarding the pathogenesis of the disease in animals propounded by Nieberle, and thus vindicate the trenchant remarks made at the meeting last year.

In addition I view this occasion as a propitious one to plead for a more tolerant attitude towards the importance of, and a proper outlook on, pathology—a subject which justifiably can be termed the Cinderella of veterinary and human medicine. Boyd‡ states that "it has been the custom to regard morbid anatomy as a somewhat outworn creed, a science as dead as the material with which it deals." This is an apt reflection upon the undeniable neglect of veterinary pathology in this country, and upon the irrational attitude of workers in other fields towards a subject which is probably the keystone of modern medicine.

His next statement, that "it never has been dead except in the hands of those whose dull minds would take the very breath of life from the most vital subject" is a withering truism. As this was written in 1934 it is ironic to note that the death knell of pathology was supposedly rung in 1912 by Sir Frederic Goodhart as evident from the title of his paper —"The Passing of Morbid Anatomy"—surely, an impetuous and premature valediction. As Boyd again states, "the world of medicine did not think there was anything dead about the "Cellular Pathologie" when Virchow poured the new wine of his vital spirit into the old bottles of tradition. And the bottles are not yet full." The function of the pathologist is not merely to attach labels to the lesions which he sees, but to reconstruct the course of events from the earliest inception of disease to its fatal termination. (Boyd.) To paraphrase Handley, the picture of pathological anatomy so often given as one of a hollow and blasted trunk, leafless, and bearing but a few withered fruits in a last flicker of expiring fecundity, compared with the vigorous sapling of experimental sciences, is nothing but iniquitous disparagement. I reiterate my statement of last year that much has been done, and still can be done, when disease is rationally viewed through the eyes of the morbid anatomist, and that pathology must continue to act as the skeleton upon which all disease research must be built.

The final explanation of any disease problem must embrace that disease in its totality as it occurs in man and animals; to consider similar conditions in man and animals as separate entities is totally fallacious, and in reality means that distinctions are being made when only minor differences exist. Comparative pathology, in my opinion, thus offers one of the most hopeful prospects of the future of pathology which may resuscitate it from its apparent, but not real, state of decadence.

The crux of the problem of the pathogenesis of tuberculosis resides in the extent to which

*Presented to the Central Veterinary Society at the Conway Hall, London, on May 6th, 1937.

†Discussion on Tuberculosis in the dog. *Vet. Rec.* (1936.) Vol. 16. p. 788.

‡BOYD, W. (1934.) Text-book of Pathology. p. 6. Kimpton, London.

we can accurately answer two questions, (*a*) how does the bacillus gain entrance into the body, and (*b*), what is the subsequent fate of the organism?

It is not perhaps always understood that there are still many unsolved problems in connection with these questions. For example, to quote Boyd again, the death rate of tuberculosis is steadily falling, but many believe that this decline may be connected in some mysterious way with the discovery of the tubercle bacillus, and the institution of the sanatorium movement. Unfortunately for this hypothesis, the decline began at least 20 years before the discovery of the bacillus and about 40 years before sanatorium treatment attained any great degree of adoption. Regarding the actual mechanism of infection we are presented with a bewildering host of contradictory facts.

Before considering the disease in animals it is thus necessary to recognise certain valid and many irrefutable facts concerning the disease in man.

Rieux related that the three fundamental advances in the history of tuberculosis were made by Laennec who proclaimed its unity, Villemin who demonstrated it was due to an infective agent, and Koch who identified the tubercle bacillus. Surely, it is necessary to add to these the names of those workers such as Parrot, Kuss, Albrecht, Beitzke, Ghon, Ranke, Aschoff, Pagel, Schmorl, Rich, Opie, Cobbett and Griffith, who have contributed so much to our knowledge of the pathology and pathogenesis, as this undeniably influenced the whole outlook on the prophylaxis of the disease. The following is a composite account of a disease to which all these authorities have made outstanding contributions at various times.

The common pulmonary tuberculosis of the adult human being is not the first manifestation of the disease in the individual concerned. In nearly every person a primary infection, confined to certain sites, occurs in infancy, but in the large majority of cases the lesions heal, calcify and may even ossify, leaving a highly characteristic change which may be recognisable years afterwards. In a very small percentage of individuals the primary lesion progresses, and the child dies from its primary infection. In others, the infection may spread throughout the body, by direct extension, by the natural passages, and by haematogenous and lymphogenous paths. As Boyd says, "should the bacillus succeed in passing the Scylla and Charybdis of the lymphoid tissues it sets out in an Odyssey which may carry it far and wide into the body." My own view is that this question of spread has, to some degree, an analogy in the metastatic spread of tumours, and that the questions at issue may find a parallel solution.

In order to understand this problem of primary infection the law of Parrot and Cohnheim, that the portal of entry of the tubercle bacillus is indicated by the lesions present in the lymph nodes which drain the part, might be recalled to memory, a law which is as true to-day as when it was first enunciated. As an index of primary infection the lesion must be either the only lesion in the body or the oldest, and must at the same time be localised in a situation which is a possible portal of entry for the bacillus into the body from external sources. The primary focus can, however, be easily recognised even if there are post-primary lesions. The lymph node draining the path of primary infection always shows evidence of disease, and together with the primary infection forms the so-called primary complex of Ranke. Particularly in mucous membranes (especially in animals) and/or if the numbers of bacilli are small, the entry into an organ may be silent and only the regional lymph node is affected; this is referred to as the incomplete primary complex.

Work in the field of chronic consumption can be said to date from Behring, who stated that the disease in the adult was the end state of an infection begun in childhood. On this assumption Ranke, for example, believed that tuberculosis was comparable to syphilis, dividing the course of consumption into, (*a*) a primary infection period, (*b*) an anaphylactic stage with metastatic spread, and (*c*) a tertiary stage, of increased resistance expressed by limitation of the process to the lungs.

It is now universally recognised that a primary infection by the tubercle bacillus occurs in nearly every living person of civilised communities. The path of infection in about 80 per cent. is *via* the respiratory tract, in about 15 per cent. *via* the intestine, and the remaining 5 per cent. probably embrace such portals of entry as the nasopharynx, skin and middle ear. These figures naturally vary in different countries; for example, in Scotland the probability is that infection by the alimentary tract is higher than that given. As the genesis of the disease, subsequent to this primary infection is comparable irrespective of the portal of entry it will be advisable to confine our attention to the commonest type, namely, the pulmonary.

Primary pulmonary infection may occur as early as the first month after birth, usually in the first six years and less frequently after ten years. (The sequelae of a primary infection after this period will be mentioned later.) Opie, in a recent detailed study of autopsies of persons who had died from diseases other than tuberculosis, has given figures such as 43 per cent. of children up to five years, 80 to 90 per cent. of persons over 20, and nearly 100 per cent. of persons over 30 years. Subsequent to the

entry of the bacilli into the lungs, a rapidly caseating broncho-pneumonic focus results, which is small and sharply demarcated from the adjacent normal lung substance. This lesion may be located in any anatomical part of the lung, but is usually single and subpleural. This lesion is the so-called primary infection or Ghon lesion, and is always accompanied by a lesion in the regional lymph node (hilus, bifurcation or tracheo-bronchial). The transportation of the tubercle bacilli from lung to lymph node is probably conducted by means of the macrophages which abound in all tuberculous lesions. This primary pulmonary focus plus the lymph node lesion form together the primary complex. In the vast majority of cases the pulmonary lesion heals by fibrosis, followed by hyalinisation and calcification; with the passage of years this lesion may even ossify. Ossification of healed primary lesions occurs in about 40 to 50 per cent. of the calcified lesions found in people over 40 years, and this bony focus, with its myeloid components, may even in later life participate in systemic diseases affecting these elements in the body (*e.g.*, leukaemia). This permanent landmark of a solitary ossified lesion in the lungs thus constitutes a valuable index of the first infection of an individual. It is usually macroscopically demonstrable, but if not, its localisation may be revealed by X-ray examination. The associated lesion in the lymph gland may also heal and calcify and may also ossify. (This resistance to a primary infection of tubercle bacilli must be regarded as an inherited trait of the human race. The death rate is low at this period, then rapidly ascends, and maintains a high level throughout the rest of life.) Relatively rarely in children this primary infection does not heal, the initial caseous pneumonic process spreading by bronchial paths to other parts of the lung, and the child may die from its primary infection as a result of an acute tuberculous caseous pneumonia. Factors such as the virulence of the bacilli, size of infective dose and natural resistance of the host, play a key part in determining the course of the disease in every case, and at all stages.

Subsequent to the primary infection a state of allergy in the sense of Pirquet-Ranke, *i.e.*, hypersensitiveness, develops. The lesion in the lymph node may progress with regional infection of adjacent nodes, and with the formation of enlarged glands showing gross tuberculous caseous lymphadenitis—the so-called scrofula of days gone bye. So far, however, the lesions can be said to have remained localised. After the development of the allergic state, and by exacerbation of the pre-existing primary focus, the individual may enter the post-primary stage of generalisation. This is due to entrance of the bacilli into the blood stream (a tuberculous bacillaemia), and results in haematogenous spread of the infection to a few or many different organs of the body. Which organs of the body are attacked must be regarded as a fortuitous circumstance, and it is to be noted that the lungs are usually involved in this secondary spread. Almost every individual enters the post-primary stage in which lesions occur in the lungs, but in the vast majority this post-primary dissemination remains abortive and heals in the same way as the primary lesion. The post-primary lesions in the lungs are usually abortive and calcify, and are often referred to as Simon's foci. These lesions may, on the other hand, be progressive, and may then produce a special anatomical picture quite different from that found in the chronic isolated pulmonary tuberculosis. Punched-out cavities and cortico-pleural lesions are part of this process, and the type of disease may be referred to as the chronic disseminated pulmonary tuberculosis of Pagel. This type can also be distinguished serologically by the ability of the blood serum to retard the growth of tubercle bacilli *in vitro* (Pagel).

The post-primary lesions (haemic in origin) in the visceral organs may also be progressive —constituting the chronic post-primary disseminated tuberculosis. Various bones may be affected, usually in the vicinity of the metaphysis, with resultant contiguity spread from marrow to corticalis and to periosteum, and in many cases to the joints (tuberculous osteomyelitis, periostitis, and arthritis). The uterine tube in the female, the testicle, prostate, seminal vesicles in the male, one or both kidneys, the brain and the suprarenal glands are other organs which are commonly affected. In all, the subsequent course is the same, *viz.*, progressive caseation with contiguity spread of the infection and the lymph nodes draining the organs may also become infected. Gross invasion of the blood stream by the bacilli may occur with a resultant fatal acute miliary tuberculosis which may involve almost every organ in the body. Particular mention must be made of the involvement of the brain in this post-primary stage. Isolated foci may be set up in any locality of the brain which may caseate and rupture into the meningeal spaces, liberating showers of tubercle bacilli to produce the classical tuberculous meningitis so commonly seen in young people. Rich states that this is the constant process invoked in the genesis of tuberculous meningitis, and that the meninges are never affected by direct blood stream invasion. Chronic miliary tuberculosis, chronic proliferative lesions in the lymph glands, skin lesions, Boeck's sarcoid, and tuberculosis of the special sense organs are other forms of the disease which come into this category of post-primary dissemination.

We have now reached a point when the debatable problem of isolated pulmonary phthisis of the adult types can be discussed. In later life some adults, who certainly have a pre-existing primary infection focus (perhaps healed), develop progressive tuberculosis which is limited to the lungs. At one time it was thought that an individual with a primary infection could not be reinfected from without, just as a syphilitic is immune to reinfection, and that all manifestations of the disease in later life arose from an endogenous focus. Now opinion has veered to the other extreme, and adult phthisis is said to be due to a true superimposed new or exogenous reinfection; probably both endogenous and exogenous methods might be involved. Whether the reinfection is endogenous or exogenous the bacilli are arrested in the lung tissue to form again a characteristic lesion distinct in many ways from that of the primary infection, and which renders it easily recognisable at autopsy. This reinfection lesion of the lungs (Puhl's focus) is commonly situated in the apical lobe, and concurrent affection of the pulmonary lymph nodes does not occur (points in contrast to the primary complex). The percentage of individuals who are reinfected is probably about 100, but only a relatively small number (about 5 to 10 per cent. at the maximum) develop the progressive fatal disease. As in the case of the primary infection the reinfection lesion may also heal by slow fibrosis, and may calcify, but it rarely ossifies and extra-pulmonary lesions may again be only abortive. If healing does not take place, the progressive consumption of the adult man or woman develops entirely from this reinfection focus. The subsequent spread of the disease within the lungs in a progressive case is to a large extent by bronchial aspiration. The resultant anatomical picture seen in the lungs at autopsy depends, to some extent, on the manner of spread as well as the virulence of the bacilli and host resistance. When the infective dose is massive or more virulent, and/or resistance is low, breakdown of the caseous focus may occur with discharge into a bronchus, and cavity formation may result. The reinfection lesion, at first lobular in character, may ultimately assume lobar proportions in which all stages of progress of the disease can be seen. Erosion of the blood vessels or development of small aneurysms may lead to severe and even fatal haemoptysis. The overlying pleura may be thickened, and may show signs of an active pleurisy, and pneumothorax may be an additional complication. In the more chronic, slower progressive type of pulmonary disease productive inflammation predominates, and fibrosis is an outstanding feature. Finally, by coughing and aspiration, bacilli may reach the mouth and nasopharynx setting up tertiary lesions in the tonsils, pharynx and larynx, and if swallowed, may cause the common intestinal ulceration so often seen along with phthisis at autopsy: these latter lesions concomitant with chronic adult phthisis are thus all appendicular to the bronchial system. In this type of chronic pulmonary disease, the process is, therefore, truly limited to the lungs to a great degree. Acute miliary tuberculosis only occurs in the post-primary dissemination stage of the disease, and not in the isolated phthisis of the adult type, and it is necessary to emphasise that the two latter forms are mutually exclusive.

In individuals who receive a primary infection late in adult life the disease tends to pursue the course of the caseous pneumonia and acute generalisation seen in the child. Such cases were observed by Aschoff during the late war in Anatolian peasants brought up in a tubercle-free environment. Countless examples of this feature, however, could be given.

The disease in the human is thus one of primary infection, post-primary dissemination (acute miliary tuberculosis being a possible complication at this stage) and reinfection with the production of isolated pulmonary tuberculosis (*see* Fig. 1). The active tuberculosis of infants can therefore be compared to that of the experimental animal inoculated for the first time, while the isolated pulmonary tuberculosis of adults corresponds to the lesions produced by reinoculation of an animal which has already been previously immunised. From what has been said "It is evident that the ideal state will be that of an acquired immunity and not freedom from infection, for the latter is an unattainable ideal and the rarer the infection the more dangerous does it become" (Boyd). For that reason one might hope for advances along the path of preventative inoculation already indicated in the case of B C G.

I have refrained from discussing the cellular reaction which occurs in response to invasion of tissues by the tubercle baciilli, but it is perhaps necessary to mention that the classical tubercle is only characteristic for certain stages of the disease, and that there are lesions in tuberculosis which are no different from certain non-specific inflammatory reactions.

With this information at our disposal, it may now be asked to what extent the disease in animals is comparable with that in man. For an answer, attention must be directed to the work of Nieberle and his colleagues. In the time available it is impossible to give more than a synopsis of the pathogenesis and pathology, and for the same reason I shall confine my remarks to the dog, cat and bovine species.

Tuberculosis in the Dog and Cat

Tuberculosis in the dog and cat is undoubtedly a relatively rare disease; in the case of the dog, figures have been found in the literature indicating the incidence to vary

Fig. I. Tuberculosis in Man.

from 1 to 7 per cent. of all animals dying in clinics in Germany and France. There are no comparable data available regarding the incidence in Great Britain. It is obvious that there must be considerable variation in the incidence in different countries, in different localities, and in industrial areas compared with rural districts.

If the doctrine of primary infection in man is carried over into the realm of animal pathology, which it inevitably must be, my failure to find any old healed primary infections, in either the alimentary or respiratory tracts in a large series of autopsies on dogs, might indicate that this animal is but rarely infected in contrast to children. Further, it appears that when primary infection does occur, early spread and generalisation is almost the rule and not the exception as in older children. Important differences thus emerge almost at once in a consideration of the comparative pathology and genesis of the disease. (Isolated primary infection in an early stage of development can be only an accidental autopsy finding in dogs which have been killed.) Tuberculosis in dogs and cats can be said, therefore, to be comparable only with the tuberculosis of childhood in which primary infection is followed almost directly by regional spread and by dissemination of the infection to distant organs. In the large majority of cases, if the dog or cat is infected, the disease is probably progressive and fatal from its very outset, and there is thus *no* analogy of any stage of the disease with the adult phthisis of man, *i.e.*, with the chronic isolated pulmonary tuberculosis due to a reinfection. From the point of view of teaching it is thus thoroughly inaccurate to regard tuberculosis of the udder of the cow, lungs of the pig, kidneys of the horse, testicle of the dog, meninges of man, etc., as being all manifestations of an identical disease process. One might even be bold enough to state that the acute exudative inflammatory reaction seen in certain stages of the disease in man and in animals, and the chronic productive inflammatory reaction seen in other stages and in other animals, are two different manifestations of a body to infection by the tubercle bacilli.

As in man the main pathways of infection are *via* the respiratory and alimentary tract, *i.e.*, by inhalation and ingestion of the bacilli respectively. Primary infection through the skin, penis, vulva and vagina, such as has been described in the case of the calf, has not been described in the dog and cat. According to Nieberle, the commonest path of infection in both dog and cat is by inhalation, but before any final settlement can be made on this matter a much larger series of animals will have to be examined. In the small number of cases in the cat examined in Cambridge, I have failed to find any case in which infection has been by any route other than the alimentary.

The primary pulmonary lung lesion is comparable to the Ghon lesion in the human. This takes the form of a caseous pneumonic focus usually situated in the dorsal part of the main lobe, and which is subpleural in position. Transport of the bacilli to the regional lymph nodes results in a caseous lymphadenitis, and the complex is thus complete. It has been suggested that in animals the bacilli may pass silently through the primary portal of entry into the body, *e.g.*, the lung, to cause a lesion only in the regional lymph nodes.

The lesion in the lungs and lymph nodes is distinctive in its cellular reaction, and corresponds in no way to what is often regarded as the classical tuberculous reaction. (In the past this fact, no doubt, has been responsible in many cases for mistakes in diagnosis.) The classical tubercle is conspicuous by its absence, and the process is one of a diffuse slow coagulative necrosis. The predominating cell concerned in the cellular reaction is the large macrophage; the so-called typical tuberculous giant cell does not occur, and necrotic areas merge with normal tissue often without any definite demarcation zone of inflammatory cells. The absence of calcification in tuberculous lesions of both dogs and cats is an old observation, and is peculiar in view of the tendency of the dog tissues in other conditions to calcify and even ossify (for example, in chronic nephritis and mammary tumours). The regional lymph nodes (hilus and bifurcation) may become enormously enlarged, matted together and to the adjacent lung tissue, and may be a conspicuous feature immediately the thorax is opened. This massive tumour-like mass is probably the reason for the statement that such lesions may be mistaken for tumours (*e.g.* lymphosarcoma), but after histological examination there can be no confusion. The lesion in the lymph node is similar to that seen in the lung, *i.e.*, confluent foci of necrosis often not sharply delimited by cellular reaction from normal lymphoid tissue. Macrophages may be abundant, but giant cells and calcification are never present, and an early formation of highly vascularised fibrous tissue may be seen at the margins of the lesion. The inflammatory process is thus essentially productive in character.

Nieberle has observed healing of the primary lesion by fibrosis and hyalinisation, but this must be regarded as a relatively rare event and the natural sequelae to any primary lung focus may be as follows. Spread of the disease in and from the lungs is by (*a*) direct contiguity, (*b*) the blood stream, (*c*) the lymph stream and (*d*) bronchial paths.

Through breakdown of the lung focus or by

direct extension, showers of bacilli may be liberated into the pleural cavity to produce the diffuse or nodular type of sero-fibrinous pleurisy. In these cases the pleura may be enormously thickened and adherent to the lungs, and there may be an abundant serous or sanguineous pleuritic transudate. By the same route the diaphragm, pericardium and arch of the aorta may be involved. (In the case of the latter, gradual extension of the process through the walls of this vessel and the liberation of bacilli into the blood stream to cause acute miliary tuberculosis is not a remote impossibility.) This extensive type of coelomic disease in dogs has been an oft-repeated observation, and is perhaps one of the commonest types of spread which occurs from a lung focus. Whether this pleural disease, co-existent with pulmonary lesions, can occur by other paths than direct contiguity is problematical but spread *via* haematogenous and retrograde lymphatic channels has been suggested.

Spread of the infection within the lungs may occur along the bronchial paths by aspiration and along the peribronchial lymphatics. Tuberculous bronchitis, bronchopneumonia and peribronchitis are, therefore, common. Cavity formation has been described, but this is probably a manifestation of simple bronchiectasis , and not due to a true tissue breakdown such as is seen in the phthisis of the human adult. Sooner or later the initial lobular nature of the lesion assumes lobar proportions. At any stage in the process the bacilli may enter the systemic circulation to set up "metastatic" lesions in such organs as the kidneys, liver, spleen, testis, prostate and bones, etc. Dependent, no doubt, upon the size of the infective dose entering the circulation, the lesions in these organs may take the form of an acute miliary tuberculosis but more commonly isolated foci are set up in the organs mentioned (submiliary foci). In these isolated haematogenous outspreads, and in the miliary type, the structure of the classical tubercle is again missing.

Particular mention must be made of possible bone complications. A diffuse hyperplastic osteoperiosteitis ("Akropachie") has long been known to occur in dogs, but while some authorities contend that it is essentially tuberculous, others have stated that it is analogous to Paget's disease of bone in man. At present all that may be said is, that it is commonly observed in cases of tuberculosis in dogs, but that its tuberculous nature remains to be proved.

As in the other animals primary infection *via* the alimentary tract occurs in two main sites, (*a*) the pharyngeal region, which is relatively rare and need not be discussed, and (*b*) the ileocaecal region of the intestine. In the latter, infection in the majority of cases is in the nature of an incomplete complex, *i.e.*, lesions are present only in the lymph nodes draining the intestine and not in the intestinal mucosa. Subsequent spread from this nidus is comparable to the spread following a primary pulmonary infection. Breakdown in the node lesion may lead to generalised tuberculous peritonitis, while lymphatic invasion will result in the bacilli reaching the thoracic duct to be carried to the lungs. From the pulmonary foci which are set up, invasion of the systemic circulation must again inevitably lead to miliary and submiliary lesions in the viscera.

A more detailed publication concerning the pathogenesis of the disease in cats will be made at a later date, and in view of this many points of importance have been omitted, but the occurrence of tuberculosis of the uterus in the cat as part of the dissemination process may be mentioned, although congenital tuberculosis of either dog or cat has not so far been reported.

The pathogenesis of the disease in dogs and cats is shown in schematic form in fig. 2. (*See* page 790.)

Tuberculosis in Cattle

It will be impossible to do more than touch upon the surface of this subject; I intend, therefore, to deal only with certain aspects which might give some indication of the problems involved.

As in the case of man, dog and the cat just cited, the genesis of the disease in the bovine is founded on an understanding of the site, nature, and fate of the primary infection focus. While in man and the dog only a small percentage of primary infections occur in extrapulmonary tissues, in the case of the bovine it seems to be the unanimous opinion that alimentary infection is the more prevalent. In addition in the bovine there is the genesis of the not uncommon congenital tuberculosis to be considered.

Primary infection of the alimentary tract in the bovine species appears to be mostly in the nature of the incomplete complex, *i.e.*, the bacilli appear to penetrate the intestinal mucosa without leaving any trace and to affect only the regional lymph nodes. It is impossible to say in what percentage of cases this is true because the search for a lesion in the mucosa, which might be microscopic in its dimensions, must be a formidable task, and many intestinal lesions must be overlooked. On the other hand, primary infection of the respiratory tract in the majority of cases is comparable to that in man, *i.e.*, a pulmonary focus (Ghon lesion) plus a lesion in the regional lymph nodes

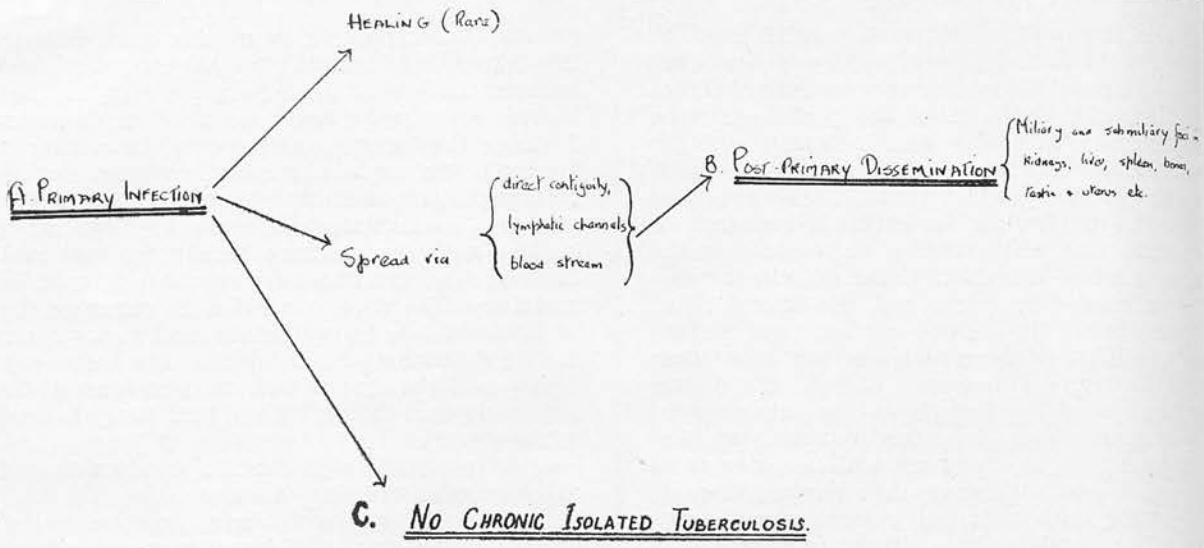

Fig 2. TUBERCULOSIS IN DOGS and CATS.

forming a complete complex. It is pertinent to note that such observations could only have been made in the case of young animals, not suspected of being tuberculous, which have been slaughtered and in which primary lesions have been found in an uncomplicated state. Primary infection occurring in the young bovine by other routes than those mentioned have been described—for example, by way of the penis, vulva and vagina—but these will not be discussed.

In the cow the lesions which follow the entrance of the bacillus are similar irrespective of their portal of entry. These changes are also closely allied to the type of cellular reaction commonly recognised as being typically tuberculous. Tubercle formation is here seen in its classic form of central caseation bordered by abundant epithelioid cells, and with numerous lymphocytes at the periphery of the nodule; multinucleated giant cells are always present, and as in man calcification occurs relatively early and is often an outstanding feature. The caseation in the lymph nodes may, however, not take the form of the homogeneous type seen in man, but often may form multicentric foci which may partly coalesce and the gland has a coarse streaky appearance. Whether or not healing of the primary infection takes place in any great number of animals is unknown but ossification of the primary infection such as is seen in man is apparently not observed.

In contrast to the case of the dog and cat, but in some degree analogous with man, tuberculous in the bovine can be divided into, (1) primary infection, (2) post-primary dissemination and (3) late generalisation in which chronic tuberculosis of the lungs and other organs is an outstanding feature.

In calves, post-primary dissemination follows directly after the primary infection and irrespective of the location of the primary focus the disease spreads by four paths, (*a*) by direct contiguity, (*b*) by intracanalicular channels, (*c*) by the lymphatic vessels and (*d*) by the blood stream. The first mode needs no explanation and the second is best exemplified by the bronchial spread which occurs within the lungs. If the primary focus is situated in the lungs invasion of the systemic circulation will result in isolated foci in some or many of the visceral organs. In the case of a primary infection of the alimentary tract (where lesions may only be present in the regional lymph nodes), the bacilli will reach the systemic circulation with the same end-result by way of the thoracic duct and lungs. Lympho-haematogenous paths thus represent the main channels by which infection is spread from one part of the body to another. The organs most frequently found to be affected in this early generalisation are given by Nieberle as spleen, peripheral lymph nodes, lungs, kidneys, serous membranes and liver.

The lesions of this early post-primary dissemination can be divided into protracted progressive generalisation, large nodular generalised tuberculosis and the so-called acute miliary type; these will not be discussed any further. All "metastatic" lesions in the visceral organs are inevitably followed by infection of the lymph nodes draining the organs concerned; for example, lesions in the testis will be followed by infection of the iliac and para-aortal lymph nodes, and liver lesions by corresponding infection in the portal nodes, etc. It is almost certain that a

haemic origin is responsible for the widespread infection of the peripheral lymph nodes so often seen, and of those visceral nodes which appear to be infected without a corresponding lesion in the organ which is drained by that node.

So far the disease in the young bovine resembles closely that in the human being—the mode of infection, the spread of the disease and the fact that primary infection may be followed directly by early dissemination are all parallel features. In the disease affecting the adult bovine animal there is, however, a further feature to be considered, namely, the stage of late generalisation in which there is some resemblance to the chronic isolated pulmonary tuberculosis of the human adult. In the case of the cow chronic disease of the late generalisation period affects not only the lungs but also the udder, and Nieberle even states that certain chronic forms affecting the uterus, kidneys, and bones, may also be included in this category.

According to this authority, proof of the analogy of the chronic "organ" tuberculosis of cows with the isolated pulmonary phthisis of man resides in three pathological characteristics, (*a*) the predominating intracanalicular manner of spread, *e.g.*, bronchial in the lungs and *via* the ducts in the case of the udder, (*b*) the absence or minor degree of calcification which occurs in the lesions, and (*c*) the absence of lesions in the regional lymph nodes of the organ concerned. It must again be stressed, if such chronic organ tuberculosis of the late generalisation stage in the cow is to be compared with the chronic isolated pulmonary phthisis of the adult human, then such forms must be limited to one organ (*e.g.*, lungs, udder, etc.), or at the most only abortive lesions must be present elsewhere in the body. [The term chronic "organ" tuberculosis used by some German authors is rather confusing (in this case by Nieberle for forms occurring in the late generalisation period) as Aschoff originally used the term to denote the spread of the disease in the post-primary period.] The percentage of cases in the cow in which this chronic form of the disease is truly isolated remains to be discovered.

It is impossible to deal with chronic "organ" tuberculosis in its entirety; therefore, I intend to confine my remarks to one organ, namely, the udder.

According to Nieberle, it is possible to subdivide tuberculosis of the udder into three distinct anatomical forms, all of significance in any consideration of the pathogenesis of the disease, (1) miliary tuberculosis, (2) caseous tuberculous mastitis and (3) chronic udder tuberculosis due to a reinfection—the so-called infiltrating lobular tuberculosis.

(1) *Miliary Tuberculosis.*—One quarter, one part of a quarter, or even the whole organ may be riddled irregularly with small typical tubercles, between which normal udder tissue is clearly discernible. This miliary process is no different from that which will have affected many other organs of the body, and which is simply a complication of post-primary dissemination. Histologically, the lesions take the form of the classic tubercle, caseation and calcification being the rule. The tubercles are nearly always intralobular in position, and are obviously the result of a heavy bacillaemic invasion of the blood from some extra-mammary tissue with haemic spread to the udder. The regional lymph nodes are always infected.

(2) *Caseous Tuberculous Mastitis.*—This form of the disease may affect large areas of the udder to the extent of a whole quarter. The margins of the lesion are irregular, but delimited from the normal udder tissue by a hyperaemic zone, and to some extent the lesion rather resembles the appearance of an infarct. The caseation is not homogeneous but in the form of multicentric streaks and patches; the supramammary lymph nodes are nearly always macroscopically enlarged, and may show a similar appearance to that of the udder. Microscopically the lesion is one of a diffuse coagulative necrosis, the margins of which are demarcated by an acute inflammatory reaction.

(3) *Chronic "Isolated" Udder Tuberculosis—Infiltrating Lobular Tuberculosis of Nieberle.*—This type, according to Nieberle, is the commonest form of disease in the adult cow, representing about 75 per cent. of all udder tuberculosis. The anatomical picture is one which might not be regarded as typically tuberculous in nature, and the supramammary lymph nodes are seldom affected. Macroscopically the udder shows dense nodules of greyish red granular tissue, often occupying the deeper parts of the gland, which stand in contrast to the lobulated fleshy appearance of adjacent normal udder tissue. Caseation in these foci may not be marked, and thickened septa are outstanding landmarks. Histologically, the picture is not that of the classical tuberculous reaction; the normal architecture of the udder lobule may be obliterated by a dense cellular infiltration of the large macrophagic type of cell, and the ducts are filled by plugs of these cells and detritus; the walls of the ducts may be markedly fibrosed. The supramammary lymph nodes may show no changes or only abortive tubercle formation. This is again an essentially productive type of inflammation.

As mentioned, the miliary type is part of a miliary process affecting the body in general due to haemic transport of infection. The caseous mastitis is viewed by Nieberle as being the reaction of the udder to an infection, in a body in which allergy has been developed to

a high degree. The chronic lobular infiltrating type is regarded by him as analogous to the isolated pulmonary phthisis of the human adult due to a reinfection, and occurring in a body in which resistance is relatively high. The anatomical character of the lesions, the proliferative type of inflammation, the minor degree of calcification, the essentially intracanalicular manner of spread within the udder and the freedom of the regional lymph nodes from infection are points directly comparable with chronic pulmonary tuberculosis of man. It must be emphasised, however, that unless the udder disease is indeed isolated, *i.e.*, if only abortive lesions are present elsewhere in the body, this comparison cannot be made. Finally, it may be said that udder tuberculosis develops entirely as a result of haematogenous spread whether it occurs in the stage of early dissemination or of late generalisation, a primary ascending infection *via* the teat and lactiferous ducts being a highly improbable event. The possible infection of the udder by way of retrograde lymphatic channels is similarly an untenable proposition.

If this chronic tuberculosis of the late generalisation period occurring in extra-pulmonary organs in the cow is indeed identical with the isolated pulmonary phthisis of man, it is obvious that it must be due to an endogenous reinfection; only in the case of the lungs of the adult cow can an exogenous reinfection be possible.

Congenital Tuberculosis in Calves.—All congenital cases arise as a result of a placental tuberculosis of the mothers; *i.e.*, the foetal membranes act as the ante-natal source of primary infection of the calf. There are thus two possible modes of entrance of tubercle bacilli into the foetus, (*a*) by aspiration into the lungs of infected amniotic fluid and (*b*) by passage of the bacilli along the umbilical vein into the foetal circulation. The former need not be considered apart from mentioning that in the rare event of an isolated primary pulmonary focus being seen in a new-born calf or in one only a few days old, such an origin can be the only one. In the second event, the bacilli reach the liver and from thence pass to the portal lymph node. Lesions may be present in both the liver and portal lymph node or in the latter alone, and the condition is comparable in every way to the complete or incomplete primary complex of the lungs or intestine caused by post-natal infection. Nieberle stated formerly that in calves over 14 days old, in which such isolated portal gland lesions are seen, it is impossible to decide whether infection has been truly congenital or has been a case of very early post-natal primary infection via the duodenum. Latterly he has produced evidence to show that nearly every case of isolated portal lymph node tuberculosis (with or without liver lesions) is congenital in origin.

This concludes a relatively brief account of the comparative pathogenesis of tuberculosis. Owing to the immensity of the subject, the paper of necessity has been abbreviated, and I have tried as far as possible to exclude much discussion of detailed pathology; cellular reaction and the histology of the lesions thus have been almost omitted. I think, however, sufficient facts have been presented to show what has been accomplished in studies of the comparative pathology of tuberculosis, mainly through the arduous labours of Professor Nieberle and his pupils. Many prob-

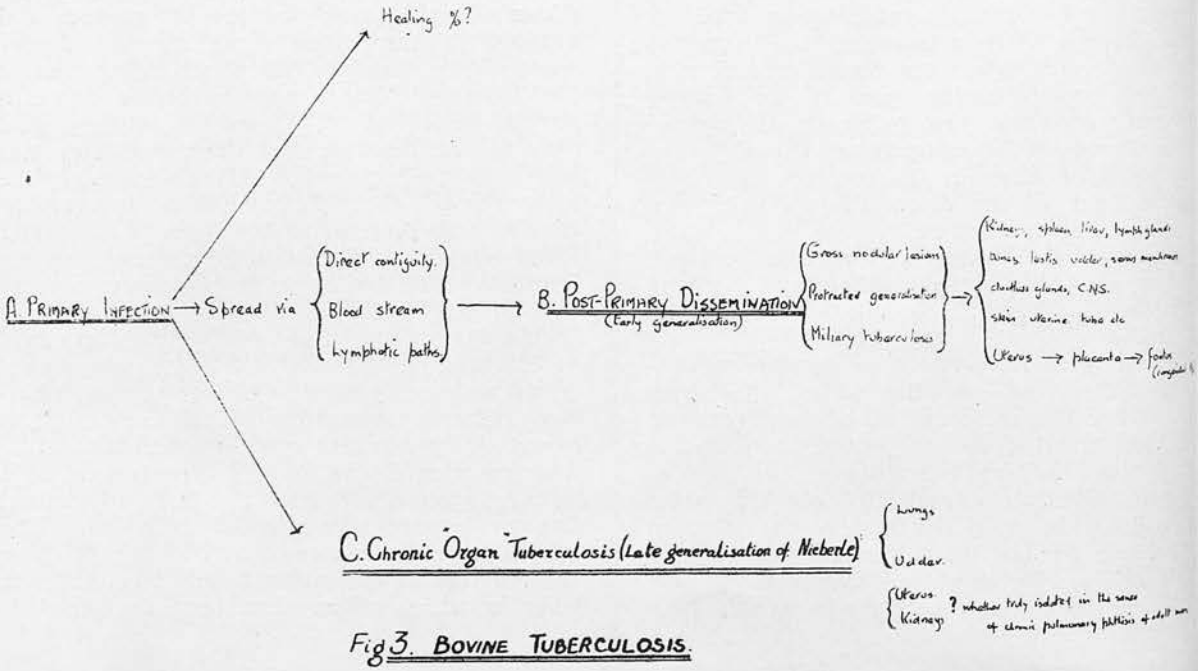

Fig 3. Bovine Tuberculosis.

lems still remain to be solved, and with the vast amount of material at the disposal of the animal pathologist much could be done by anyone willing to devote the time necessary for minute and careful *post-mortem* work. In the future more reliance must be placed on detailed observation and data derived by pathological studies of animal diseases alone, and not as in the past on simply transferring facts concerning diseases of man to parallel conditions in animals without any attempt at serious and critical analyses.

As far as tuberculosis concerns the animal pathologist, it must not be considered sufficient simply to give a positive or negative diagnosis at autopsy; the origin of the infection, mode of dissemination and reaction of the host are of equal paramount importance. Only in this way will any complete understanding be obtained of the variation in the pathology of the disease in different animals, and in the same animals when caused by the different types of bacilli.

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XII. THE ASSIMILATION OF THE STEENBOCK-BLACK DIET IN NORMAL AND VITAMIN D-DEFICIENT RATS WITH AND WITHOUT CAECUM

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(*Received 19 November 1936*)

THE rat, like other species, needs vitamin D for optimum health. It is generally accepted, however, that the vitamin is not necessary for the prevention of rickets in rats, provided that the diet is furnished with a well balanced Ca/P ratio; provided also that these two elements are present in the diet in a readily available form.

The lessened susceptibility of the rat to rickets compared with other species, even in absence of vitamin D, has been explained by the considerable ability of this animal to absorb ingested Ca and P [Harris, 1932]. Its cause has, however, never been investigated experimentally. In the rachitogenic rations most generally used in experiments on rats, cereals, such as maize and oats, form a regular and essential component of the diet. In the Steenbock-Black diet 76 parts are yellow corn. Both in maize and oats the essential source of P is phytin (inositolhexaphosphoric acid). The significance of this form of P in the pathogenesis of rickets has been emphasized by Bruce & Callow [1934]. Phytin is not attacked by any enzyme secreted in the digestive tract. The utilization of P from this source is therefore limited to the amount which can be split off by bacterial action. Now it is well known that parts of the ingested food may be recovered from the caecum of rabbits more than a week after the ingestion. During preliminary studies of the passage of the intestinal content through the digestive tract of the rat, a similar stagnation of ingested food was observed in the rat's caecum. Carmine ingested with the food was found to stain the content of the caecum bright red several days after ingestion. In rats starved for 24 hours the caecum is full, whereas the rest of the gut is nearly empty. This stagnation of the remnants of ingested food in the caecum will favour any bacterial action. It seemed possible therefore that the utilization of the phytin P might be favoured by this action, which would explain the better ability of the rat to absorb Ca and P. The content of the caecum is also rather acid, which naturally will tend to increase the solubility of Ca and P. A study of the Ca and P assimilation of the Steenbock-Black diet by rats with and without caecum was therefore made, both in normal and vitamin D-deficient animals.

These experiments served also a second purpose. In order to study the action of vitamin D on the secretion and absorption of Ca and P in the digestive tract, a basal diet free from Ca and P had to be used. Such a diet is deficient also in other respects and metabolism experiments with it must therefore be of very short duration, with intervals during which a more sufficient diet is given. In

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short-time metabolism experiments, the large caecum of the rat might retain previously administered food rich in Ca and P and in this way upset the results. It was therefore necessary to amputate the caecum in the rats used for such short-time metabolism experiments, but first the effect of this operation on the animals had to be studied, to see how far results obtained could be applied to intact rats. (The operations were performed by J. R. M. I.; the planning of the experiments and the analytical work by R. N.)

Experimental

Young rats were used, bred on stock diet until they weighed about 50 g. Half of the animals were then operated on. The caecum was amputated 3–5 mm. from the ileum and the stump closed with two layers of suture. The one important point in this operation is to use thin enough silk. Carell's artery needles with silk No. 00 can be used, but artery needles with attached silk No. 000000 are still better. The rats received milk and sugar on the day of operation and again the day afterwards. Then they were put on the rachitogenic diet for 10 days before the experiments were started.

It was found that the animals recovered quickly from the operation, kept their weight constant for about a week and then recommenced to gain in weight

Table I. *Weekly metabolism experiments on rats with and without caecum, fed on a Steenbock-Black diet with addition of 50 I.U. vitamin D daily*

Rat no.	Food intake g.	g. dry faeces per g. food	Ca intake mg.	Ca in faeces mg.	mg. Ca in faeces per g. food	Net absorbed Ca mg.	P intake mg.	P in faeces mg.	mg. P in faeces per g. food	Net absorbed P mg.
Rats with caecum 1st week:										
1	58·0	0·108	700	507	8·7	253	151	104	1·8	47
2	43·5	0·121	553	365	8·3	188	113	80	1·8	33
3	49·5	0·124	630	498	10·0	130	129	105	2·1	24
4	45·0	0·110	572	405	9·0	167	117	81	1·8	31
5	48·0	0·113	610	390	8·0	220	125	82	1·7	43
Rats with caecum 2nd week:										
1	59·5	0·102	758	500	8·4	258	155	134	2·3	21
2	67·5	0·100	860	568	8·4	292	175	147	2·1	28
3	53·0	0·100	675	485	9·2	190	138	117	2·2	21
4	58·0	0·088	740	424	7·3	316	151	109	1·9	42
5	51·0	0·100	650	425	8·3	215	132	112	2·2	20
Rats without caecum 1st week:										
1	56·3	0·099	718	446	8·0	273	146	89	1·6	57
2	56·3	0·134	718	475	8·4	244	146	100	1·8	46
3	56·8	0·129	722	465	8·2	257	148	96	1·7	52
4	48·8	0·134	621	430	8·8	191	127	95	1·9	32
5	46·5	0·118	592	340	7·3	252	121	85	1·8	36
6	56·0	0·133	713	495	8·8	218	146	100	1·8	46
7	53·5	0·133	682	453	8·5	229	138	106	2·0	33
Rats without caecum 2nd week:										
1	57·0	0·100	725	367	6·4	358	148	92	1·6	56
2	54·5	0·140	695	490	9·0	205	142	116	2·1	26
3	63·0	0·130	802	540	8·6	262	164	152	2·4	12
4	59·0	0·123	751	500	8·5	251	154	121	2·1	33
5	50·5	0·106	644	342	6·8	302	132	90	1·8	42
6	60·0	0·135	755	500	8·3	255	156	142	2·4	14
7	52·0	0·131	662	472	9·1	190	135	119	2·6	16
					Av.	238 = 34 mg. daily			Av.	33 = 4·7 mg. daily

Table II. *Weekly metabolism experiments on rats with and without caecum, fed a Steenbock-Black diet without any addition of vitamin D*

Rat no.	Food intake g.	g. dry faeces per g. food	Ca intake mg.	Ca in faeces mg.	mg. Ca in faeces per g. food	Net absorbed Ca mg.	P intake mg.	P in faeces mg.	mg. P in faeces per g. food	Net absorbed P mg.
Rats with caecum 1st week:										
1	48·9	0·101	622	521	10·7	101	128	122	2·5	6
2	53·6	0·101	681	565	10·6	116	139	132	2·5	7
3	65·5	0·105	835	728	11·1	107	172	158	2·4	14
4	68·0	0·103	865	747	11·0	118	177	165	2·5	12
5	54·2	0·108	690	566	10·6	124	142	129	2·4	13
Rats with caecum 2nd week:										
1	50·2	0·102	640	580	11·6	60	132	125	2·5	7
2	54·8	0·105	698	620	11·4	78	143	140	2·6	3
3	70·8	0·107	901	817	11·5	84	184	175	2·5	9
4	72·9	0·106	929	787	10·9	142	190	180	2·5	10
5	54·0	0·108	688	620	11·5	68	140	131	2·4	9
Rats without caecum 1st week:										
1	70·0	0·113	890	765	10·9	125	182	173	2·5	9
2	69·8	0·125	887	754	10·8	133	182	172	2·5	10
3	64·2	0·123	819	762	11·8	57	167	156	2·5	11
4	62·7	0·132	800	710	11·3	90	166	160	2·6	6
5	61·8	0·122	786	630	10·2	156	163	149	2·4	14
Rats without caecum 2nd week:										
1	69·7	0·115	887	755	10·9	132	182	161	2·4	14
2	68·6	0·136	874	775	11·1	99	179	170	2·5	9
3	68·2	0·127	870	820	12·0	50	177	166	2·4	11
4	61·3	0·127	780	640	10·5	140	159	153	2·5	6
5	68·2	0·115	870	640	9·5	130	179	169	2·6	10
					Av.	105 = 15 mg. daily			Av.	9·5 = 1·4 mg. daily

at the same rate as the control rats. About 100 rats in all were operated upon, roughly 10% of which died. In all cases where a post-mortem was performed an invagination of the ileum into the colon was found. No secondary dilatation of the rest of the caecum was found in rats which had been living 6–8 weeks after the operation. Later a slight dilatation was observed.

Four groups of rats were used: (1) intact rats on rachitogenic diet + vitamin D; (2) rats without caecum on rachitogenic diet + vitamin D; (3) intact rats on rachitogenic diet; (4) rats without caecum on rachitogenic diet.

The experiments performed to investigate the role of the caecum lasted two weeks in each rat. The urine was absorbed on thick blotting paper, so that faeces free from contamination could be obtained, and any food which was spilt could be recovered. The food intake in one week was measured to the nearest 0·1 g. The experimental results are given in Tables I and II.

Discussion

The findings may be briefly stated as follows. (1) No difference was found in the assimilation of P and Ca as between the rats with and without caecum, either in the normal or in the vitamin D-deficient rats. This is clear from the figures which give the Ca and P in the faeces per g. food intake (see columns 6 and 10). It can therefore be concluded that the caecum has nothing to do with the greater ability of the rat to assimilate ingested Ca and P. (2) A difference was

seen, as was to be expected, in the assimilation of Ca and P as between normal and rachitic rats. (3) There is a difference in the output of dry material in the faeces as between the rats with and those without caecum, but no difference between comparable rats (see Tables I and II). The faeces excreted by the rats without caecum were only slightly more moist than the faeces excreted by the intact rats. An increased output of salts could not therefore account for the output of more dry material in the faeces excreted by the caecum-free rats. The cause of this increased excretion has not been ascertained. Considering that the chief biological processes in the caecum are of bacterial origin, it is probable that some part of the food, hardly attacked by the enzymes secreted into the digestive tract, but split up by bacterial action, is less digested by the rats without caecum. The increased output of dry material in the faeces corresponds to only 1–2 % less utilization of the food in these rats. It is thus established that caecum-free rats are well suited for metabolism experiments, where the purpose is to study the Ca and P metabolism. The question of the Ca and P requirement of rachitic rats is raised by the observations recorded above. This question is dealt with in an accompanying note.

Summary

The assimilation of the Steenbock-Black diet has been studied in normal and vitamin D-deficient rats with and without caecum. The absorption of Ca and P from this diet is quite unaffected by extirpation of the caecum, the only effect being a slightly decreased (1–2 % less) utilization of the diet. The slow rate of passage of the intestinal contents through the caecum of the rat does not play any role in the smaller susceptibility of the rat to rickets.

My (R. N.) thanks are due to the Medical Research Council and to Dr L. J. Harris for their kind hospitality during this work.

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NO. 15.

DEBATABLE TUMOURS IN HUMAN AND ANIMAL PATHOLOGY.

I. Lymphoepithelioma.

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DEBATABLE TUMOURS IN HUMAN AND ANIMAL PATHOLOGY.

I. Lymphoepithelioma.

By W. F. HARVEY, E. K. DAWSON, and J. R. M. INNES.

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Introduction.

OUR aim in embarking on this work is to give our views on certain tumours of the body which, in the course of our routine reporting work, have frequently raised difficulties in histological diagnosis. It may be well to insist that we do not imagine that we, or anyone, are at present qualified to make final settlement of the subjects of which we shall treat. The advantage of only taking up the debatable tumours is that we shall be able to deal, in any desired sequence, with any localities and tissues, without expending efforts of description upon those types of tumour which do not require it.

The sources of our material are the card records, sections and paraffin blocks of over 13,000 tumours available in the Royal College of Physicians' Laboratory, the records and sections of the Tumour Service Follow-up Department of the Royal Infirmary, Edinburgh, and the pathological material of the Institute of Animal Pathology, Cambridge.

Considerations of space demand that we restrict ourselves in our descriptions, with the certainty that we shall be unduly dogmatic and shall be unable to give credit, historically and by acknowledgment, to previous workers upon any one tumour. This dogmatism will, we hope, be discounted by a study of the directional literature accompanying each paper.

As examples of the type of tumour, set out at random, which we consider to be debatable, we may mention :—lymphoepithelioma ; reticulum-cell sarcoma ; thymoma ; mixed tumour of salivary glands ; sarcoma of thyroid, breast, prostate, muscle, fat, etc., basal-cell carcinoma ; seminoma ; meningioma ; hepatoma ; granulosa-cell tumour ; craniopharyngoma ; adamantinoma ; endothelioma of bone ; bronchiogenic carcinoma ; neurogenic sarcoma ; carcinoid

tumours; angioendothelioma; hypernephroma; chorion-epithelioma; pleural endothelioma; giant-cell tumour of bone; glioblastoma; epidermoid carcinoma; colloid carcinoma; myxoma; embryonic mixed tumours of the kidney; neuroblastoma; lymphadenoma; Paget carcinoma; glomangioma; lymphoma, etc. The list is long and life is short, but if, in consequence, we cut down our text, we hope to make provision for ample illustration and for the continuation of the good work. In this matter of restriction of text, we have decided on four headings only—definition, description, discussion, and directional literature. That leaves out an important section, differential diagnosis; it may to some extent be found under "discussion."

In a discussion of tumour growth, much depends on the view taken of malignancy in general. Broadly speaking, our view is that there is no specific cancer cell but rather a cell of normal character and origin, altered by reason of environmental conditions. The causal factors underlying such pathological environment are, in our present view, non-specific.

I. Lymphoepithelioma.

Definition.—Lymphoepithelioma (Regaud and Schmincke) is a radiosensitive tumour in which lymphoid tissue is intimately associated with an immature squamous or epidermoid type of malignant epithelium.

Description.—The tissue of origin of this epithelioma is (1) the faucial, lingual and pharyngeal tonsils (Figs. 1, 2); (2) the naso-pharyngeal mucosa, and (3) more rarely, the thymus, which is an organ derived from the third and fourth pharyngeal pouches of the embryonal entodermal tract. The malignant tumour material available for examination from these regions in our collections includes tonsils 72 cases, pharynx (including naso- and hypopharynx) 117, thymus 8, together with a large number of tumours of varied origin in cervical lymph nodes.

The lymphoepithelioma forms a carcinoma whose component cells are of rather immature but apparently definitely pavement type (Figs. 3, 4, 7). No well-developed "pearl" formation and no cornification are manifest in these tumours, while the supporting tissue is peculiar in being essentially lymphoid in character (Figs. 3 to 7), with an argyrophile

scaffolding. This supporting tissue is in close relationship to the epithelial element and lymphocytes may penetrate between the mosaic of epithelial cells. The tumour itself is highly cellular and is composed of large, ill-defined cells with abundant granular cytoplasm and a vesicular nucleus ; this nucleus is large, usually pale but well-defined, with one or more nucleoli. Mitoses are easily found. The proliferating tumour cells form solid alveolar strands, bands and masses (Fig. 4). The delimitation of cells from stroma is, however, not everywhere sharp ; indeed, the penetration of the tumour cell mosaic by lymphocytes is one of the chief features of this peculiar tumour type. The metastases of this tumour in lymph nodes, which are composed of lymphoid tissue, naturally exhibit this lymphoepithelial architecture. Metastases of a similar character in lung, liver and bone have been described and must present a striking picture ; we have had no examples of these for examination. When, as is often the case, the primary growth is small, the first indication of malignancy may be the appearance of a tumour in the neck (Figs. 13 to 15) and difficulty then arises in the diagnosis between lympho-epithelioma, reticulum-cell sarcoma, branchiogenic carcinoma and secondary deposits or extensions from other malignant tissues.

Discussion.—Although the tonsil and pharynx are usually accepted as sites of origin for a lymphoepithelioma, it remains a fact that most epithelial tumours of these regions are squamous-cell carcinomas and their stroma may be either fibrous or lymphoid in character (Figs. 5, 6). It seems, therefore, questionable whether there is justification for making separation of a peculiar type of epithelioma, the " lympho-epithelioma," still less for assuming " a peculiar physiological relation between epithelial and lymphoid components " or their " symbiosis." Lymphocytic aggregations in the mucosa and submucosa are characteristic of the embryonal entodermal tract and persist to some extent in the adult, *e.g.* in the intestine and appendix, as well as in the tonsils and pharynx. This lymphoid tissue differs from that of the lymph node in having no afferent lymphatics and no distinctly constituted sinuses (Figs. 1, 2). When an epithelioma arises in such a structure or penetrates such a tissue, it seems only natural that the lymphoid tissue should still maintain its original relation to the epithelium. In tissues other than lymphoid, incidental

or secondary lymphoid aggregations about an invading metastatic tumour are not uncommon and there are primary tumours other than lymphoepithelioma, such as seminoma and pinealoma, which are characterised by some degree of lymphoid aggregation. It is also understandable that considerable variation should occur in the appearance of epitheliomas, especially those arising from crypt epithelium, which may be of more immature character than surface epithelium. In the case of the lymphoepithelioma the name once given to it by Ewing of transitional-cell carcinoma seems quite appropriate. We ourselves prefer, in all these cases of variation in epitheliomas, which are not definitely squamous or basal-cell in type, to call them epidermoid carcinomas, by which is meant tumours resembling carcinomas of the epidermis. The lymphoepithelioma is therefore an epidermoid carcinoma. Where the genesis of the tumour and its epidermoid cell character are obvious (Figs. 5, 6), there is little difficulty in histological diagnosis. If neither feature is present, the question arises whether the malignant growth is actually epithelioma or sarcoma. We have found few cases of lymphoepithelioma, as described and illustrated in the literature, in which the genesis of the epithelial element is traced from surface or crypt epithelium. Many illustrations likewise of lymphosarcomas in the regions concerned might equally well stand for lymphoepithelioma. It should be emphasised that the formation of an epithelioid cell is characteristic of many reticulum-cell sarcomas and also of some types of reticuloendotheliosis (Figs. 9, 10).

There are several points to consider in the difficult diagnosis of the lymphoepithelioma: (1) the general architecture of the normal (Figs. 1, 2), hyperplastic (Figs. 9, 10) and malignant tissue (Figs. 3, 4); (2) the character of the malignant cell; (3) the demonstration of a primary focus, when tumour growth in a lymph node is diagnosed as lymphoepithelioma; and (4) the clinical features, radio-sensitivity, age, etc. Neither architecture (mosaic, alveolar or syncytium-like) nor cytology is sufficiently distinctive, as is evident from the illustrations. A primary focus may not be apparent and cervical adenopathy may be the only obvious lesion. Radio-sensitivity is associated with rapid growth and immature cell type, whether carcinomatous or sarcomatous, and therefore does not necessarily differentiate between these tumours, since the lympho-

epithelioma, as originally described, shows malignant epithelium of immature character. If the cervical tumour retrogresses with irradiation and still no primary focus becomes evident, it seems to us difficult to maintain primary epithelial origin in that case, unless it be vestigial branchiogenic. Age is an important factor in diagnosis. Carcinomas are not unknown at very early ages, but then they arise from embryonal tissues. When, however, tissues from three cases in rapid succession were presented to us of tumours, which histologically might well have been examples of lymphoepithelioma, occurring in nasopharynx (" adenoids "), tonsil and cervical lymph nodes in children of 8, 10 and 11 years, we felt compelled to question the likelihood of their being epithelial at all (Figs. 11 to 18). That would immediately throw us on to the diagnosis, in these cases, of reticulum-cell sarcoma. This growth and the " thymoma " we shall consider later in this series of debatable tumours.

The statement has indeed been made that the reticulum of the thymus and even of the tonsil is really epithelial reticulum, but this view, if justified, would scarcely be sufficient to save the situation against the contrary diagnosis of primary, mesenchymal reticulum-cell sarcoma. Our study of normal tonsillar and pharyngeal tissue has given no support to the conception of epithelial reticulum; this point, however, requires more embryological study for its elucidation. Another suggestion, which gives derivation of lymphoepithelioma from the " endothelium " of lymph spaces, relegates the tumour in our opinion to the sarcoma group. Such views, we believe, further strengthen our opinion that many so-called lymphoepitheliomas are actually reticulum-cell sarcomas, that is, lymphosarcomas.

Conclusion.—We have described and illustrated the lymphoepithelioma, but consider that the tumour thus described really covers two types of malignant growth, the epidermoid or transitional-cell carcinoma and the reticulum-cell or lymphosarcoma.

We are grateful to the Carnegie Trust for the Universities of Scotland for their generous help towards the cost of illustration.

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(*To be continued.*)

Debatable Tumours : Lymphoepithelioma

NOTES, CLINICAL AND HISTOLOGICAL, ON ILLLUSTRATIONS.

FIG. 1.—*Tonsil.* Child aged 5. Showing : (*a*) surface epithelium ; (*b*) crypt epithelium ; (*c*) diffuse lymphoid tissue ; (*d*) germinal centres. Lymphoid tissue in tonsillar, pharyngeal and intestinal mucosal areas has neither the afferent lymphatics nor the sinuses of a lymph node. ×10.

FIG. 2.—*Tonsil.* The same as in Fig. 1 with the same lettering. ×400.

FIG. 3.—"*Lymphoepithelioma.*" Showing an epidermoid carcinoma of a lymph node in a man aged 49 ; a painless slow swelling of 12 months' duration : (*a*) pavement epithelium ; (*b*) lymphoid stroma ; (*c*) lymphocytes within the epithelial mosaic. 6777/1928. ×350.

FIG. 4.—"*Lymphoepithelioma.*" The same tissue as in Fig. 3. Showing : general architecture ; (*a*) solid alveolar masses of malignant epithelium ; (*b*) lymphoid stroma. The delimitation of tumour from stroma is sharp, although some lymphocytes have penetrated the epithelium. 6777/1928. ×90.

FIG. 5.—*Tonsil.* An epidermoid carcinoma in a man aged 62. Tumour at base of tonsil, with catarrh of throat of some weeks' duration. Showing (*a*) distinctive squamous character of invading malignant epithelium with many mitoses ; (*b*) stroma infiltrated with lymphocytes and plasma cells. 7086/1935. ×500.

FIG. 6.—*Tonsil.* An epidermoid carcinoma in a man aged 63. Originally diagnosed on biopsy as squamous-cell carcinoma : irradiated abroad : 10 months later re-examined and found to be "still a ragged ulcer with proliferating edges." Showing : (*a*) infiltrating malignant epithelium ; (*b*) stroma with lymphocytes and plasma cells ; (*c*) surface epithelium. 7069/1935 and 185/1936. ×150.

FIG. 7.—"*Lymphoepithelioma.*" Swelling of tonsil, six weeks' duration, in a man aged 52. Tonsil friable, vascular and ulcerated. Showing : an epidermoid carcinoma ; (*a*) mosaic of malignant epithelial cells with vesicular nuclei ; (*b*) mitosis ; (*c*) lymphoid stroma. 9604/1930. ×500.

FIG. 8.—*Tonsil.* A lymphosarcoma in a woman aged 60, invading and destroying crypt epithelium. Showing : (*a*) remains of keratinising crypt epithelium ; (*b*) cells of lymphosarcoma. 937/1930. ×350.

FIG. 9.—*Lymph node.* Showing : (*a*) sinuses filled with proliferated reticulum cells, producing solid alveolar strand architecture ; (*b*) lymphocytic tissue. This appearance of reticuloendotheliosis is common in lymph nodes as a pathological reaction. ×60.

FIG. 10.—*Lymph node.* The same as in Fig. 9. Showing : (*a*) the solid alveolar character of proliferated sinus reticulum cells and (*b*) the apparent sharp delimitation from the adjacent lymphocytes. ×200. In Figs. 9 and 10 there is no malignancy but the proliferated cells produce an architecture not unlike that of lymphoepithelioma. *Cf.* Figs. 4 and 5.

FIG. 11.—*Nasopharynx* ("adenoids"). Diagnosis "lymphoepithelioma" in child aged 8. Clinically, typical symptoms of sore throat and mouth-breathing ; suggested tuberculous condition. Showing : (*a*) tumour-cell mosaic with solid alveolar character ; (*b*) mitosis ; (*c*) lymphocytic tissue ; (*d*) isolated lymphocytes within the tumour-cell mosaic. The tumour tissue, in sections, had no connection with the surface nasopharyngeal epithelium. 100/1936. ×350.

FIG. 12.—*Nasopharynx* ("adenoids"). Same tissue as in Fig. 11. Showing : (*a*) more elongated nuclei. ×350.

FIG. 13.—*Lymph node.* Diagnosis "lymphoepithelioma." Boy aged 11. Swelling in neck, clinically tuberculous. At operation, dissection impossible. Six months later very large bilateral cervical lymph nodes suggestive of

Hodgkin's disease. Biopsy and X-ray therapy, with disappearance of tumour. No primary epithelial tumour is evident after 16 months in this case, which we regard as lymphosarcoma. Showing: (*a*) reticulate architecture of tumour growth; (*b*) intervening lymphocytes. 153/1936. ×110.

FIG. 14.—*Lymph node.* Same tissue as Fig. 13. Showing architecture and cytology. ×350.

FIG. 15.—*Lymph node.* Same tissue as Figs. 13 and 14. Showing an area with (*a*) diffuse tumour growth reaching to (*b*) the capsule of the lymph node. ×350.

FIG. 16.—*Tonsil.* Diagnosis "lymphoepithelioma." Boy aged 10, with tonsillar growth and enlarged lymph nodes at angle of jaw. Radium mass unit therapy. Still well after 19 months. Showing: (*a*) ill-defined solid alveolar masses; (*b*) accompanying stroma with very few lymphocytes. ×50. To be compared with the other two cases, aged 8 and 11 (Figs. 11 to 15). All three cases are probably lymphosarcoma.

FIG. 17.—*Tonsil.* Same case as Fig. 16. Showing: (*a*) a tumour area of solid alveolar type; some of the cells are of spindle shape; (*b*) a fibrous stroma with few lymphocytes. 186/1936. ×350.

FIG. 18.—*Tonsil.* Diagnosis "lymphoepithelioma"; see further notes of same case, boy aged 10, under Figs. 16 and 17. Showing cell characters: (*a*) vesicular nucleus; (*b*) prominent single nucleolus or chromatin nodes; (*c*) abundant cytoplasm. 186/1936. ×1800.

FIG. 19.—*Lymphosarcoma.* Showing the arrangement of reticulum fibres in relation to tumour cells: (*a*) tumour cells in alveolar masses with, in some parts, no intercellular reticulum. 1235/1937. ×350.

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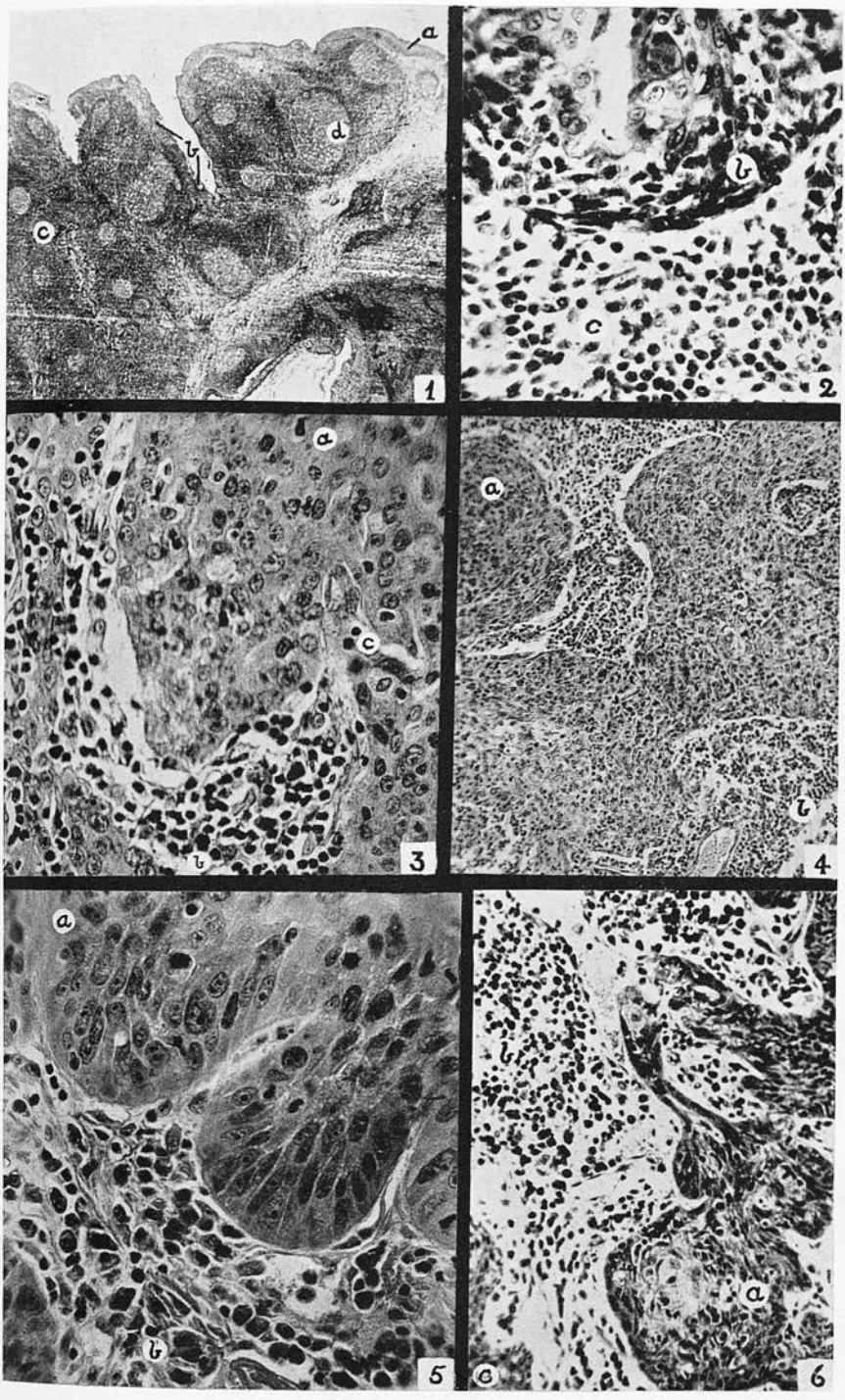
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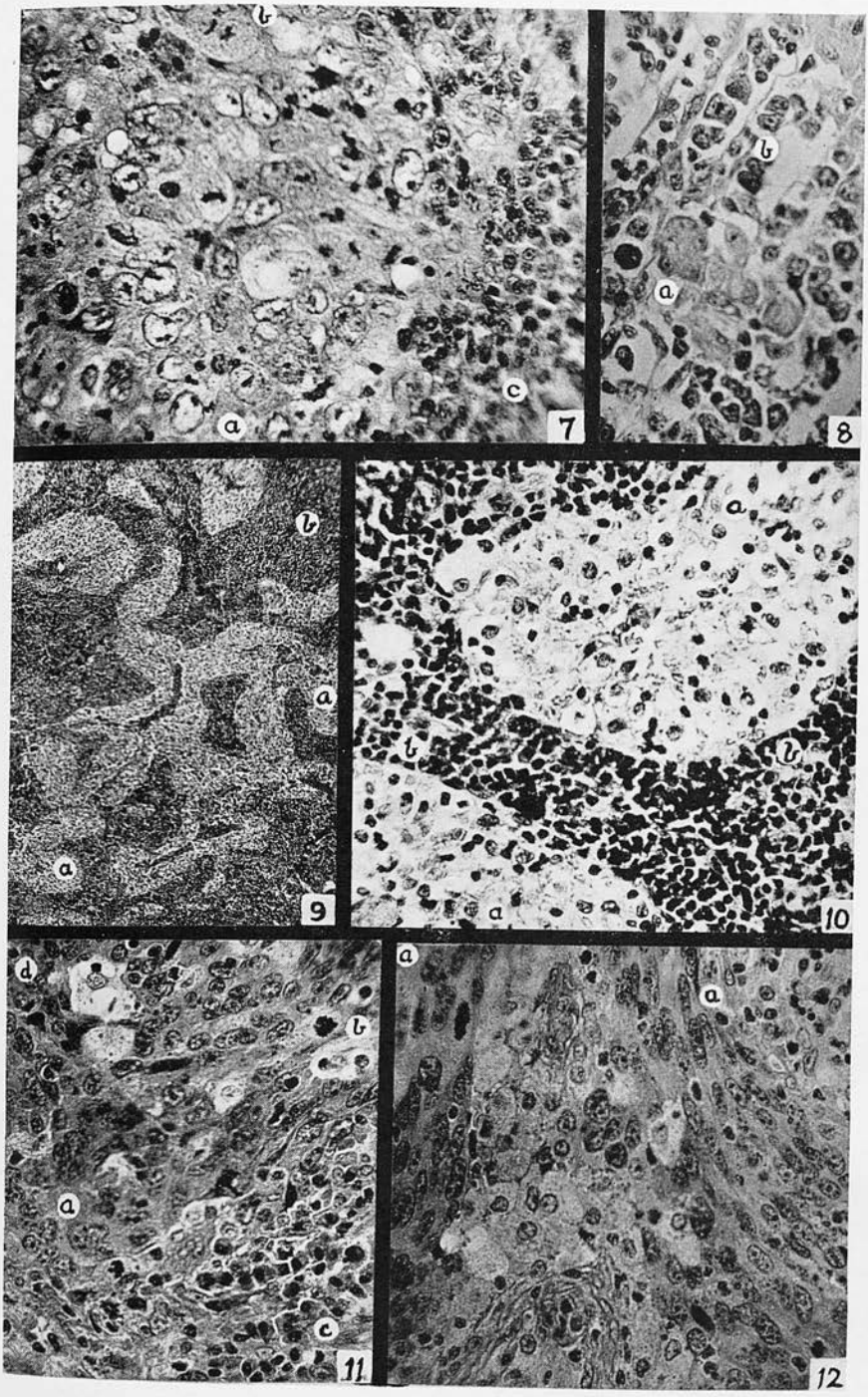
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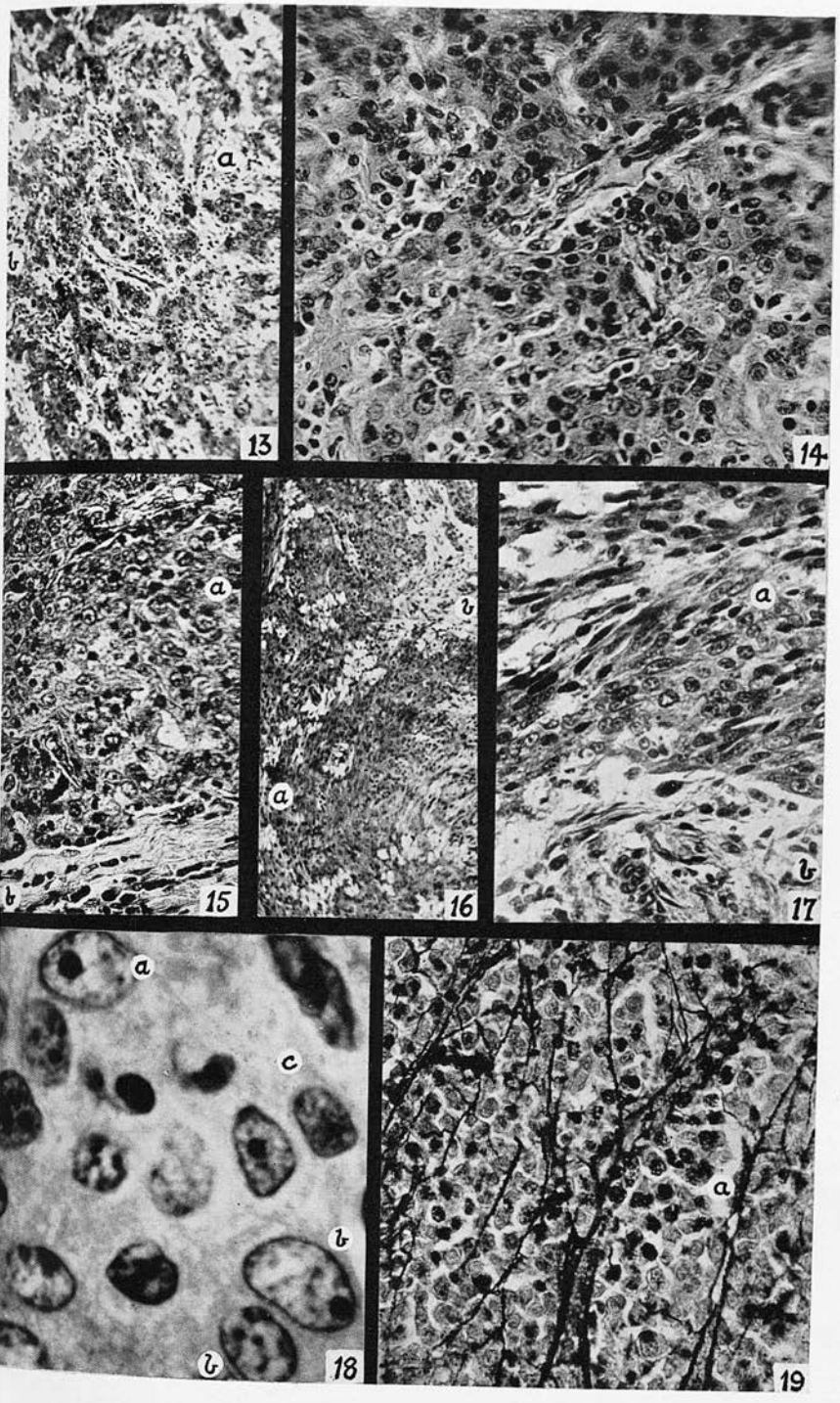
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NO. 16.

DEBATABLE TUMOURS IN HUMAN AND ANIMAL PATHOLOGY.

II. Lymphosarcoma.

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DEBATABLE TUMOURS.

II. Lymphosarcoma.

By E. K. DAWSON, J. R. M. INNES and W. F. HARVEY.

(*From the Research Laboratory of the Royal College of Physicians, Edinburgh, the Cancer Control Organisation of Edinburgh and S.E. Scotland and the Institute of Animal Pathology, University of Cambridge.*)

Definition.—Lymphosarcoma or reticulum-cell sarcoma is a radio-sensitive tumour of lymphoid tissue, arising from the embryonal stem-cells of that tissue.

Description.—In the fully-developed tumour the microscopic architecture of the lymphoid tissue is lost (Fig. 9). It is changed from one of normal differentiation to germinal centres, pulp cords and sinuses into one of more uniform and uniformly dispersed cells, which are always larger than the small lymphocyte. The presence of mitoses is a characteristic and invariable feature (Fig. 10). The occurrence of this tumour in lymph nodes, such as those of neck, mediastinum and retroperitoneal tissue, is fairly common. Its appearance, however, must also be carefully considered, both as primary and metastatic tumour, in other situations where lymphoid tissue, its residues, or the lymphoid parent cell is found, such as tonsil, naso-pharynx, thymus, gastro-intestinal tract and, more rarely, thyroid, spleen, bone-marrow, lung, liver and kidney. Extension to neighbouring lymph nodes is the common and usually characteristic feature. The locally-invasive tendency and, probably, multicentric origin of the growth are also distinguishing features. Invasion takes place into muscle or other surrounding tissues; metastatic deposits, however, in other organs do occur and apparently result from blood transportation and embolism of tumour cells. The usual separation into large and small cell types is unnecessary, if the origin of the tumour from lymphoid stem-cells is accepted. A purely small lymphocytic cell proliferation does not, in our opinion, represent lymphosarcoma.

The component cells of lymphosarcoma are polyhedral, ovoid or spheroidal; they may possess little, moderate or abundant cytoplasm and a somewhat vesicular nucleus, which contains more chromatin than the parent reticulum cells. The essentially reticulate or stellate character of the primitive cell is largely lost when the rapid proliferation of tumour formation takes place. Giant cells are rare and are large

or even monster cells with bulky hyperchromatic nuclei rather than bi- or multinucleated forms. Central nucleoli (Figs. 1, 4), such as are common in the squamous-cell carcinomas, may occur but are not so common an appearance as that of two or three large, separate, chromatin nodes. Reticulum, as one would expect in lymphoid tissue, can be demonstrated in the form of argyrophile fibrils, which may be intercellular or independent of cell arrangement (Fig. 8). Leukæmic manifestations in the peripheral blood are usually absent.

The malignant tumour material available for our examination, with the diagnosis of lymphosarcoma, totals 192 cases, of which 164 were human. For the human cases, where the age was indicated, the distribution is as follows :—

5	15	25	35	45	55	65	75	85	years
9	9	11	18	10	20	15	8	1	cases

Contrast this with the age distribution of our collection of squamous-cell carcinomas of the lip :—

5	15	25	35	45	55	65	75	85	years
0	0	1	5	12	28	42	19	5	cases

25 = 20-29 years.

Discussion.—Our outlook on the subject of lymphosarcoma necessitates a brief statement of our conception of the architecture and cytology of lymphoid tissue. This may be illustrated from the lymph node. We regard this as consisting of a fibrous tissue capsule with its trabecular prolongations and an internal scaffolding of anastomosing reticulate cells with their associated reticulum fibrils. The essentially lymphocytic tissue, consisting of peripheral nodules (germinal centres) and anastomosing pulp cords running through the medulla to the hilum, is developed from the reticulate cells and occupies the reticulate meshwork. Sinuses are those parts of this structure in which the reticulate meshwork is comparatively free of lymphocytes : they include the peripheral sinus and its internal offshoots which, at the hilum of the node, are continuous with the efferent lymphatics (Fig. A).

The reticulum fibrils, which become collagen fibres in the capsule and trabeculæ, may be considered as young, or fine, single collagen fibres (Fig. 8). These fibrils are deeply argyrophile and stain black, whereas collagen stains brown. The association of reticulum cell and reticulum fibril seems to us in no way different from that of fibroblast and collagen,

whether one considers the latter a secretion product of the cell itself or a fibrous transformation of cytoplasm or a fibrillar separation from the primitive mesodermic myxoid matrix. Reticulum cell and lymphopoietic lymphoblast are, we consider, the same cell, or at least only slightly differentiated from the stem-cell. The lymphoblast is " determined " for lymphopoiesis and produces finally the small lymphocyte ; the reticulum cell has two main functions, (1) as a scaffolding and lining cell, and (2) phagocytic macrophage production (Fig. B).

It seems to us that undifferentiated mesenchyme must be almost universally existent in the loose areolar, myeloid,

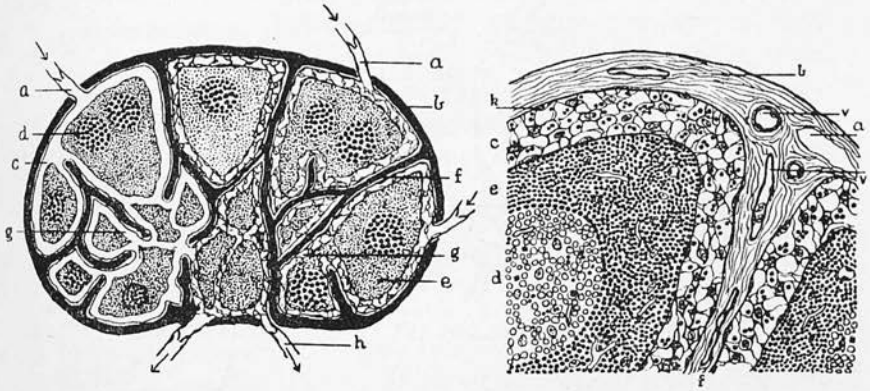

FIG. A.—*Lymph Node.* Low and high power (diagrammatic). Showing : (*a*) afferent lymphatics ; (*b*) fibrous capsule ; (*c*) peripheral sinus ; (*d*) germinal centre ; (*e*) lymphocytic tissue ; (*f*) fibrous trabeculæ ; (*g*) medullary sinus ; (*h*) efferent lymphatics ; (*k*) sinus (littoral) endothelium.

sinusoid and lymphoid tissue of the adult body. We would prefer to denominate this tissue and its cells *mesenchymal* rather than *reticuloendothelial.* These progenitor cells, though showing a " determination " to the production of specific tissue in certain localities, *e.g.* the lymphoblast of the germinal centre, may still be regarded as mesenchymal tissue persisting in the adult. This view simplifies the distinction, for lymphoid tissue, between lymphoblast, reticulum cell, endothelium, macrophage and lymphocyte.

Lymphosarcoma is a malignant tumour growth of the progenitor cells of lymphoid tissue. In the evolution of the tumour we should therefore expect, during the period of rapid cell growth, to find the gradual elimination of the more differentiated cell types. It is thus only at a late stage that the characteristic picture of large cells, uniform in size and

distribution is observed. The uniform production of the differentiated small lymphocyte, " lymphoma," is, as already mentioned, not a malignant growth, though frequently referred to as " small round-cell sarcoma " and we have eliminated it from the strictly lymphosarcoma group. Local aggregations of cells in organs, associated with a definite leukæmia whether lymphogenous or myelogenous, present considerable difficulty. The component cells may be small lymphocytes or may resemble the lymphoblast and myeloblast. Histological

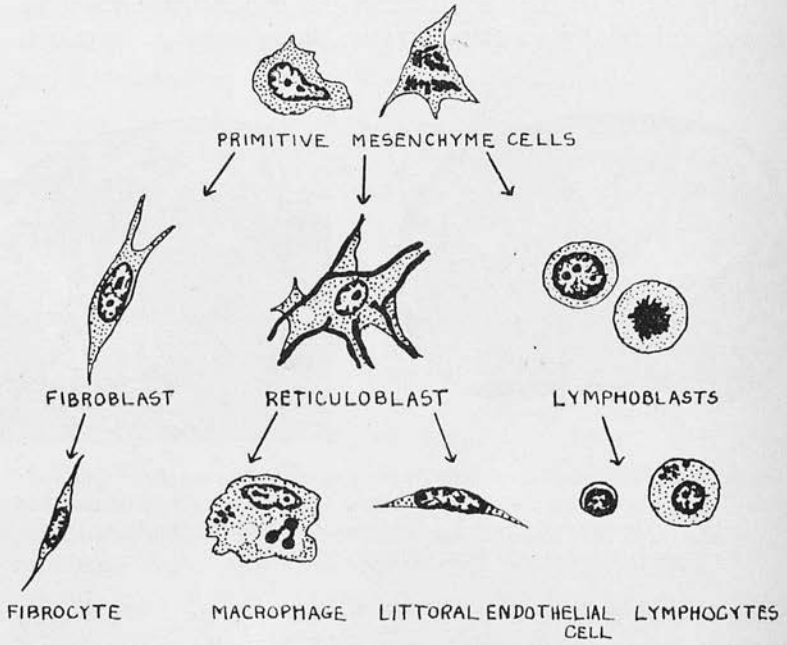

FIG. B.—Genesis of cells of lymphoid tissue.

diagnosis of lymphosarcoma then demands, in our opinion, specific information regarding the blood picture. It may be, of course, that leukæmias are to be regarded as sarcomas.

The component cells of lymphosarcoma are always, at least, larger than the small lymphocyte. Hyperplasia of inflammatory origin (Fig. 27), productive of the larger cell type, *i.e.* of the reticulum cell, may closely simulate or even, in the opinion of high authority (Ewing, p. 342), pass over into lymphosarcoma. It is understandable from the term " epithelioid " applied to this large cell type how, on occasion, it may be difficult to differentiate the lymphosarcoma from an epidermoid carcinoma, as was discussed and illustrated

in our previous paper on " lymphoepithelioma." The diagnosis of lymphosarcoma from Hodgkin's lymphadenoma also constantly raises difficulty. Mitoses are a feature of lymphosarcoma as contrasted with the Hodgkin lymphoid tumour ; giant cells, rare in lymphosarcoma, are more or less specific for Hodgkin's disease (Fig. 14) and so may be the presence of eosinophile leucocytes. Invasion of the node capsule gives little help as a diagnostic feature, as we have not found it in early cases of either disease (Figs. 9, 13). Once the capsule has given way, the advance of lymphosarcoma into the surrounding tissues rapidly obliterates all boundaries and involves all structures. This is not the case with Hodgkin's lymphadenoma, however great the enlargement, or with the leukæmias. As we identify, genetically, the endothelium and reticulum cell of the germinal centres, sinuses and pulp cords, we consider it unnecessary to separate the lymphosarcomas from the lymphoid endotheliomas. Endotheliomas of lymphoid tissues are lymphosarcomas, and endotheliomas proper are more accurately classed as angioblastomas.

The age distribution of the lymphosarcomas, a comparatively early one, is a definitely useful link in the diagnostic chain, especially when carcinoma is the alternative, as was emphasised in our treatment of the lymphoepithelioma. The rather irregular age distribution which we give of our lymphosarcoma series, is probably an indication of difficulty of diagnosis. The shift to the left is definite, but it may be emphasised that lymphosarcoma occurs at any age.

Some of the other " debatable tumours," such as mycosis fungoides (Figs. 21, 22) and Ewing's tumour (Fig. 26), we also regard as probably reticulum cell sarcomas. Interesting examples have been published, in which a variety of appearances were manifest in the same individual, such as a combination of lymphosarcoma, leukæmia, mycosis fungoides and Hodgkin's lymphadenoma.

Conclusion.—We consider that the lymphosarcoma of smaller or larger cell type arises from an undifferentiated or little differentiated mesenchymal cell which constitutes in adult life the stem-cell of lymphoid tissue and has, *in situ*, the productive potentialities of reticuloblast, fibroblast and lymphoblast.

We are grateful to the Carnegie Trust for the Universities of Scotland for their generous help towards the cost of illustration.

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Debatable Tumours : Lymphosarcoma

NOTES, CLINICAL AND HISTOLOGICAL, ON ILLUSTRATIONS.

FIG. 1.—*Lymphosarcoma.* Cervical lymph node. Male aged 52. Seven months previously noticed small swelling left side of neck. Gradually increased in size to form a large projecting tumour beneath the sternomastoid. Displacement of tonsil and pharyngeal wall. No primary tumour in throat. Biopsy and radiotherapy. Apparent local recurrence 2 months later. Showing: (*a*) a uniform large reticulum cell type; (*b*) cells with single nucleoli and multiple chromatin nodes; (*c*) mitoses; (*d*) sparse small lymphocytes. *Cf.* same tissue, Figs. 9 and 10. Stroma sparse and not obvious with this H. and E. staining. ×500.

FIG. 2.—*Hodgkin's lymphadenoma.* Lymph node at root of neck. Woman aged 30. Clinically suggested tuberculosis but diagnosed simple inflammatory. Six months later nodule developed under scar and was then diagnosed Hodgkin's lymphadenoma. Showing: (*a*) large cells of reticulum type; (*b*) monster cell types; (*c*) small lymphocytes; (*d*) abundant stroma. This diagnosis was difficult and was based on the cytology, the marked hyaline fibrosis in other areas and the paucity of mitoses. H. and E. 1971/37. ×500.

FIG. 3.—*Lymphosarcoma.* Lymph node in neck. Primary tumour in the palatoglossal fold. Showing: (*a*) a fairly uniform large reticulum cell type; (*b*) a few enlarged and hyperchromatic nuclei; (*c*) mitosis; (*d*) small lymphocytes. H. and E. 4888/32. ×500.

FIG. 4.—*Lymphosarcoma.* Tonsil. Female aged 50. Showing: (*a*) a uniform large reticulum cell type; (*b*) cells with single nucleoli and multiple chromatin nodes; (*c*) mitosis; (*d*) small lymphocytes. H. and E. 7450/33. ×500.

FIG. 5.—*Lymphosarcoma.* Kidney tumour. Aged cat. Large white tumour mass attached to mesenteric border of lower end of ileum. Mesenteric nodes and intestine fused into one solid lump. Smaller nodules in kidney. Showing: (*a*) a uniform, rather small reticulum cell type; (*b*) hyperchromatic nuclei; (*c*) mitosis; (*d*) kidney tubule. H. and E. 949/31. ×500.

FIG. 6.—*Lymphosarcoma.* Lymph node. This illustrates the lymphosarcoma of small round cell type. Showing: (*a*) very uniform small cells of reticulum type; (*b*) some hyperchromatic nuclei; (*c*) mitosis; (*d*) small lymphocytes. These are smaller than the tumour cells. H. and E. 4581/31. ×500.

FIG. 7.—*Lymphosarcoma.* Lymph node. Female aged 56. Painless, slow swelling in the neck for 5 months. Showing: (*a*) large, fairly uniform tumour cells of reticulum type with abundant cytoplasm; (*b*) small lymphocytes H. and E. 3389/34. ×500.

FIG. 8.—*Lymphosarcoma.* Same tissue as Fig. 7. Showing: (*a*) ramifying argyrophile fibrils of reticulum; (*b*) intercellular fibril arrangement. Silver impregnation method (Wilder). ×500.

FIG. 9.—*Lymphosarcoma.* Same tissue as Fig. 1. Showing: loss of lymph nodal architecture and still no invasion of capsule: (*a*) capsule; (*b*) tumour cells. H. and E. ×100.

FIG. 10.—*Lymphosarcoma.* Same tissue as Figs. 1 and 9. Showing similar cytological features as Fig. 1: (*a*) a fairly uniform large reticulum cell type; (*b*) mitosis; (*c*) numerous small lymphocytes. H. and E. ×500.

FIG. 11.—*Lymphosarcoma.* Soft tissues of forearm. Male aged 75. Three years previously noticed swelling over upper end of forearm. This was excised. Twenty-one months later, swelling in region of biceps, with gradual increase in size. Swelling irregular, soft; no connection with bone. Dissection of tumour and radiotherapy. Alive and well 3 years after removal of first tumour.

This is an unusual site for lymphosarcoma; it is diagnosed as such on the cytological picture and clinical features. Showing an unusual picture of anastomosing tumour strands which suggest sinus or cord architecture. H. and E. ×100.

FIG. 12.—*Lymphosarcoma.* Same tissue as Fig. 11. Showing tumour of small round cell type very similar in cytology to Fig. 6. H. and E. ×500.

FIG. 13.—*Hodgkin's lymphadenoma.* Lymph node. Male aged 22. Painless swelling in neck of long duration with recent enlargement. Showing: (*a*) loss of lymph nodal architecture; (*b*) monster cells, obvious even at this magnification; (*c*) non-invasion of capsule. H. and E. 8389/36. ×100.

FIG. 14.—*Hodgkin's lymphadenoma.* Same tissue as Fig. 13. A very typical picture of Hodgkin's disease. Showing: (*a*) numerous monster cells of Reed-Sternberg type with one or more nuclei and abundant cytoplasm;

(*b*) numerous small lymphocytes; (*c*) abundant stroma. The diagnostic features were the characteristic monster cells and the absence of mitoses. H. and E. ×500.

FIG. 15.—*Lymphosarcoma.* Ileocæcal region of intestine. Male aged 16. History of 5 days' severe abdominal pain with vomiting. No palpable tumour. Tumour area resected. Alive and well 7 months later. Showing: (*a*) glands of mucosa; (*b*) tumour origin in lymphoid tissue with invasion of mucosal stroma. H. and E. 623/36. ×100.

FIG. 16.—*Lymphosarcoma.* Same tissue as Fig. 15. Showing: (*a*) fairly uniform large reticulum cell type; (*b*) considerable hyperchromatism and mitosis. H. and E. ×500.

FIG. 17.—*Lymphosarcoma.* Eyelid. Boy aged 12. Wassermann reaction strongly positive. No improvement in tumour with salvarsan therapy and pot. iodide. Tumour removed and diagnosed as lymphosarcoma. Boy alive and well 3 years later. Showing the condition before operation. Case published by V. M. Métivier, *Brit. Journ. Ophth.*, 1937.

FIG. 18.—*Lymphosarcoma.* Same case as Fig. 17. Condition 5½ months after operation.

FIG. 19.—*Lymphosarcoma.* Same case as Figs. 17 and 18. Sagittal section of whole tumour area with eyeball. Note location in both eyelids, (*a*) upper, (*b*) lower, and no contact with eyeball. H. and E. 5011/35. Natural size.

FIG. 20.—*Lymphosarcoma.* Same case as Figs. 17 to 19. Showing: (*a*) uniform large round-cell type; (*b*) abundant cytoplasm; (*c*) mitosis; (*d*) small lymphocytes. H. and E. ×500.

FIG. 21.—*Mycosis fungoides.* Man aged 79. Skin of chin. Erythematous patches on trunk and legs 12 months previously. Diagnosed clinically as mycosis fungoides. The patches disappeared with X-ray therapy after other remedies had failed. The chin tumour was freely movable: submental lymph nodes secondarily involved with later ulcerations. Further spread to tonsillar node on opposite side. X-ray therapy now ineffective. Death in 6 months. Showing: (*a*) cellular aggregation in the cutis; (*b*) overlying epidermis. H. and E. 7343/35. ×65.

FIG. 22.—*Mycosis fungoides.* Same tissues as Fig. 21. Showing: (*a*) fairly uniform large round cells; (*b*) large hyperchromatic nucleus and doubtful mitoses. H. and E. ×500.

FIG. 23.—*Leukæmia cutis.* Skin of right arm. No further data. Showing: (*a*) diffuse infiltration of cutis by leucocytes; (*b*) sweat glands of cutis in the tumour area; (*c*) collagenous fibrous tissue. H. and E. 4811/31. ×65.

FIG. 24.—*Lymphosarcoma.* Tumour in liver. Female aged 39. Primary probably in retroperitoneal lymph nodes. Mesenteric lymph nodes also involved. Showing: (*a*) edge of tumour nodule; (*b*) compressed liver cells. H. and E. 2463/31. ×65.

FIG. 25.—*Lymphosarcoma.* Multiple tumours in lung. Primary tumour in mediastinal lymph nodes. Showing: (*a*) edge of tumour nodule; (*b*) pulmonary alveoli. H. and E. 8464/33. ×65.

FIG. 26.—"*Ewing's tumour.*" Distal ends of tibia and fibula. Boy aged 11. Treated for osteomyelitis. Tumour growth had destroyed bone-shaft and invaded surrounding muscle. Amputation. Died some years later. Showing: (*a*) strand formation of tumour growth; (*b*) remains of bone; (*c*) marked rosette formation; very obvious under higher magnification. H. and E. 1755/34. ×65.

FIG. 27.—*Reticulum cell hyperplasia.* Lymph node. No history. An inflammatory condition, almost certainly tuberculous. Showing: (*a*) masses of proliferated reticulum cells derived from germinal centres and sinuses; (*b*) lymphoid tissue; (*c*) fibrous trabeculæ. H. and E. 4581/31. ×40.

FIG. 28.—*Lymphosarcoma.* Cervical lymph node. Girl aged 17 with marked anæmia and mass of large lymph nodes in the neck. No sign of other primary tumour. Showing: (*a*) solid masses of reticulum cells with some preservation of nodal architecture; (*b*) lymphocytic tissue with fibrovascular trabeculæ. H. and E. 6621/28. ×50.

FIG. 29.—*Leukæmic cell aggregation.* Liver. Alsatian dog. Female aged 6 years. Epistaxis. Gross intraocular hæmorrhage. Leucocyte count, 232,000 per c.cm. Generalised enlargement of all lymph nodes of body. Leukæmic cell aggregations in liver and lungs; splenomegaly. Showing: (*a*) a uniform small but lymphoblastic cell type; (*b*) well-defined periphery; (*c*) liver tissue; (*d*) hepatic artery; (*e*) bile-duct.

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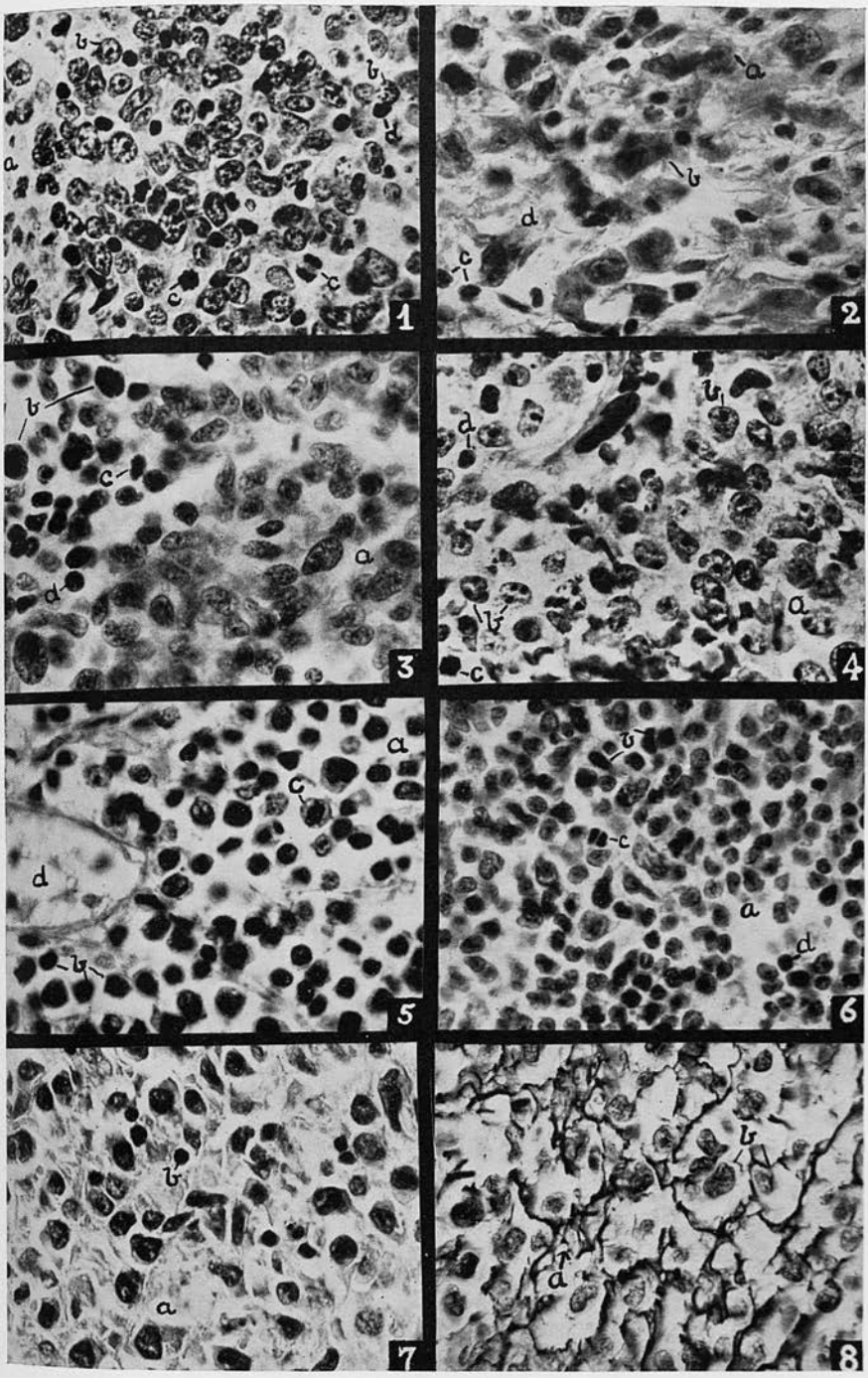
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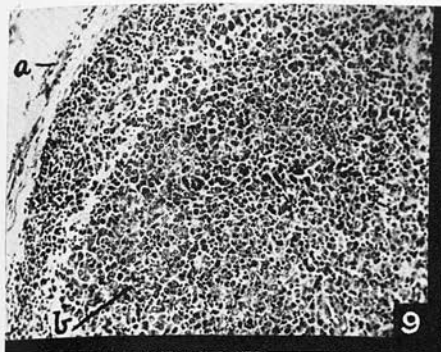
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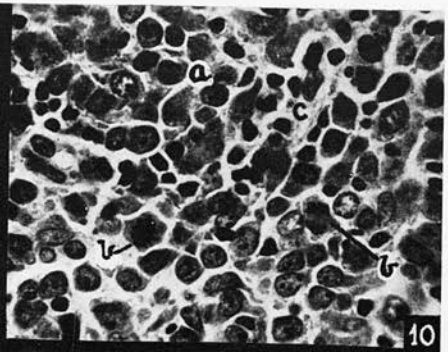
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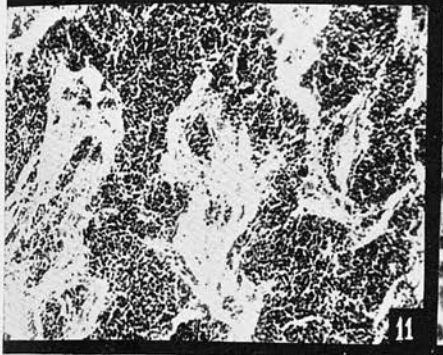
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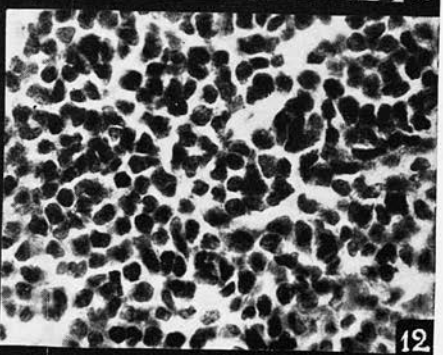
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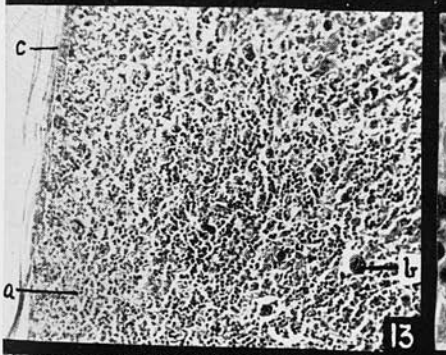
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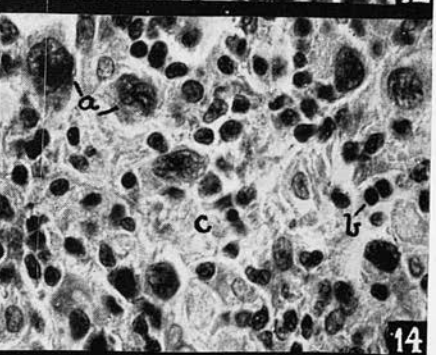
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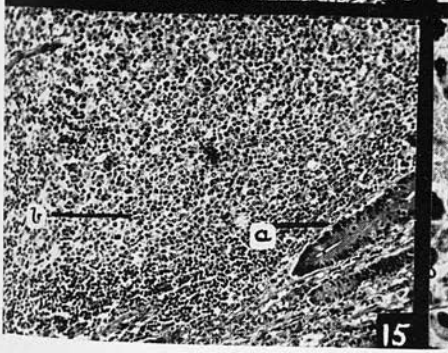
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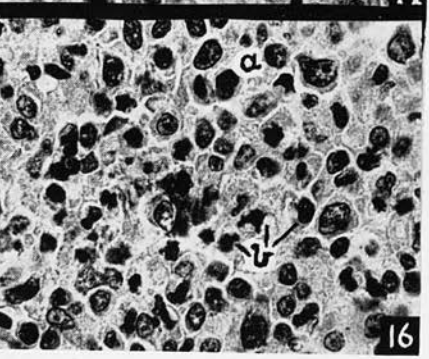
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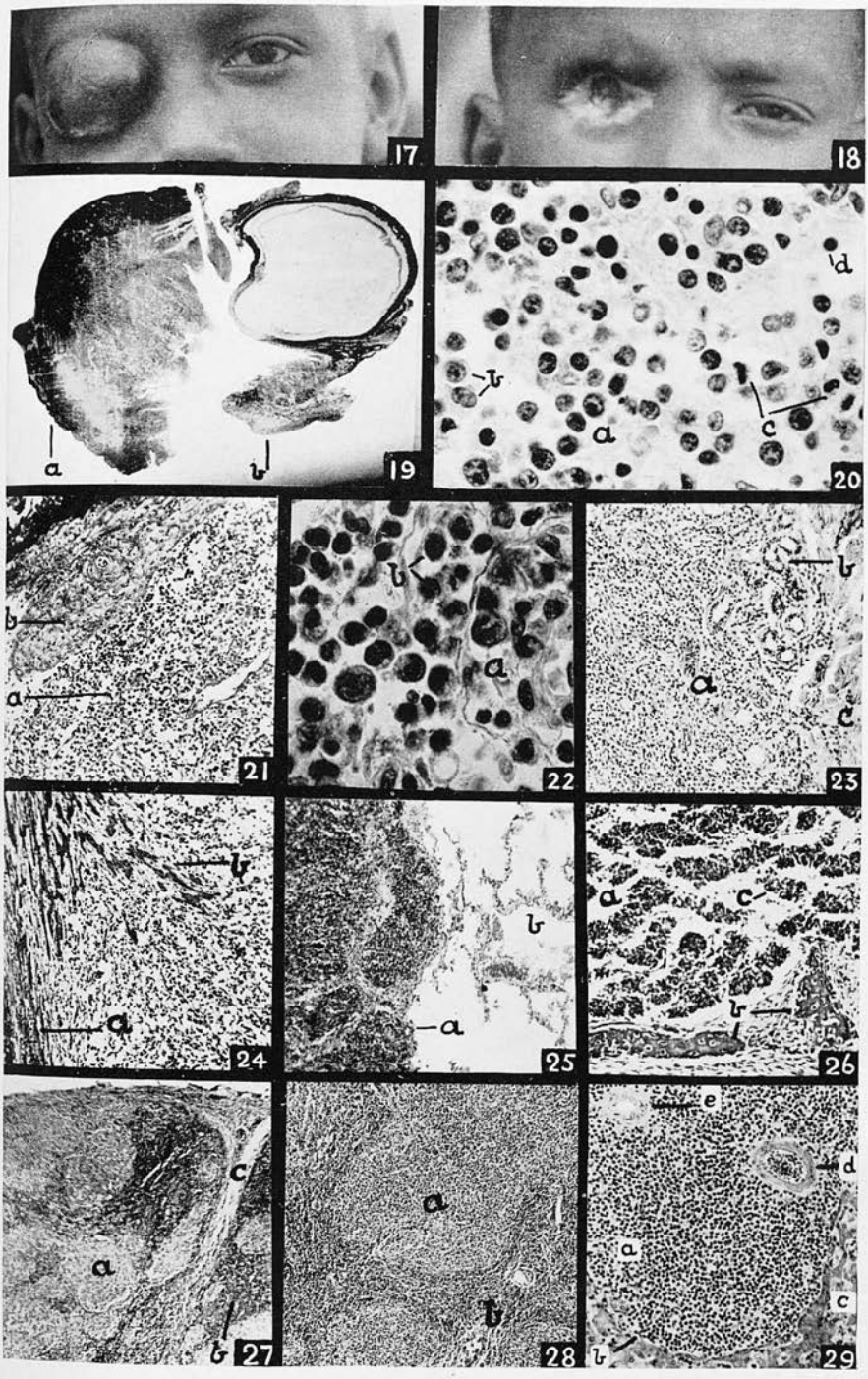
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DEBATABLE TUMOURS IN HUMAN AND ANIMAL PATHOLOGY.

III. Seminoma.

Debatable Tumours in Human and Animal Pathology

III. Seminoma

BY

J. R. M. INNES, W. F. HARVEY and E. K. DAWSON

(*From the Institute of Animal Pathology, University of Cambridge, the Research Laboratory of the Royal College of Physicians, Edinburgh, and the Cancer Control Organisation of Edinburgh and South-East Scotland.*)

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DEBATABLE TUMOURS IN HUMAN AND ANIMAL PATHOLOGY.*

III. Seminoma.

By J. R. M. INNES, W. F. HARVEY and E. K. DAWSON.

(*From the Institute of Animal Pathology, University of Cambridge, the Research Laboratory of the Royal College of Physicians, Edinburgh, and the Cancer Control Organisation of Edinburgh and South-East Scotland.*)

Definition.—Seminomas are radio-sensitive epithelial tumours derived from precursor cells of the spermatogonium of the testis.

Description.—Seminoma is the commonest tumour of the testis and in its typical form is composed of medium-size spheroidal (Fig. 17) or polyhedral (Fig. 9) epithelial cells with hyperchromatic nucleus. The nuclei are of vesicular epithelial type. The cells are said to be rich in glycogen. In human material, differentiation of the tumour cells into the organoid structure of seminiferous tubules is seldom, if ever, to be made out. The tumour, indeed, shows the condition of so-called anaplasia, that is to say, the immature cells from which it originates either divide too rapidly to differentiate to any degree or fail to differentiate altogether. Thus the general microscopic appearance of the tumour is one of uniform cells disposed in sheets or bands, in which mitotic figures may be numerous. A fine argyrophile fibrillary stroma is always present separating large and small groups of cells (Fig. 19); a true intercellular arrangement is rarely seen. These fine reticulum fibrils merge with the thick collagenous bands which are responsible for the lobulated appearance, often evident macroscopically (Figs. 3 and 4). In dogs, when these tumours are observed at a very early stage of development, their genetic relationship to the seminiferous tubules may be apparent (Fig. 7). The rate of growth may be slow or rapid and necrosis (Fig. 23) and hæmorrhage (Fig. 4) are common. One of the salient features of this tumour is the presence of small lymphocytes embedded as small or large groups among the epithelial cells (Figs. 14 and 20). It resembles in this respect the so-called lympho-epitheliomas and the pinealomas. This lymphocytic arrangement in the seminomas may be stroma reaction, but appears

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to be part of the tumour formation and differs in appearance from that which is often associated with the invasion of supporting tissues by epithelial growths. In some cases, however, lymphocytes are sparse (Fig. 8).

The site of origin of the tumour is said by some observers to be the *rete testis*, that is, the *duct* structure of the organ, and the tumour is considered to invade the testicle from this position. On the other hand, it is also stated that it originates from the seminiferous tubules and that the *mediastinum testis* and *rete testis* are involved secondarily. Examination of our own material has led us to prefer the latter view. The mode of growth of the seminoma is essentially expansile and the tunica albuginea long resists invasion and rupture. Even with great enlargement the general testicular contour is retained (Figs. 3 and 4). Any unaffected testicular parenchyma shows as a rim to the tumour area (Fig. 2). Metastases of the seminoma follow the lymph paths and are found first intra-abdominally in the para-iliac and para-aortic lymph nodes. The inguinal lymph nodes are affected only when the tumour passes outside the boundary of the tunica. The blood vessels of the tumour may, however, show penetration.

An age distribution of the seminomas presented by Chevassu places their occurrence in the fourth decade, contrasting thus with the teratomas, which make their appearance in the third decade. Tumour material available for microscopical examination in our collections includes 90 seminomas and 24 teratomas, of which 65 seminomas were human and 25 animal. These figures do not include our " hypernephromatoid " tumours. No teratomas were found in animals.

A reference may be made here to the hormonal manifestations of testicular tumours and the test (Aschheim-Zondek) used for their diagnosis and prognosis. The reaction is said to be greater in the seminoma than in the teratoma, higher still in the embryonal adenocarcinoma and highest in the chorionepithelioma.

Discussion.—Most of the discussion must relate to whether seminomas are to be regarded as teratomas of such one-sided development that they represent only one of the blastodermic embryonal tissues, or as tumours arising from undifferentiated spermatogenic tissue. There seems now little necessity to argue whether seminomas are sarcomas or carcinomas, as their epithelial character is apparently generally admitted, even though the embryonal tissue be accepted as mesoderm;

nor does it seem necessary to consider the possibility of origin of the typical seminoma from the interstitial cells of the testis. Thus we are left to decide whether seminomas are really teratomas. Serial sections of some of our own seminoma material have failed to reveal any cell elements suggestive of bi- or tri-dermal structures. We ourselves, however, are prepared to maintain that this separation of the two types is very near to being a distinction without a difference, because we maintain that all tumours are developed out of those undifferentiated embryonal cells which remain as such into adult life. These undifferentiated cells are, in our opinion, also the source of regular tissue cell replacement. We might suggest, in this connection, that the cult of the basalioma and the endothelioma, each in its time an important tenet, was an admission of this simple fact. All the usual malignant tumours of any site are thus embryonal carcinomas or sarcomas, if they continue in their proliferation to maintain their original derivative cell form. It is therefore not at all difficult to accept the seminoma, in the sense of a tumour derived from the undifferentiated epithelium of the seminiferous tubules, as an " embryonal carcinoma." We find further support for our views on the distinction between seminoma and teratoma in the remark : " When observers begin to derive their tumours from embryonal tubule cells, it is obvious that the two theories of origin become identical " (Ewing, 1928, p. 841). This pregnant sentence contains a suggestion which should be acceptable to the contestants on either side. The development of malignant testicular tumours as a whole may conceivably take place from foundation cells of different embryonic age, cells which we allocate as blastomeric, trophoblastic, epiblastic, mesoblastic, hypoblastic or combinations of these. The result may be a tri-, bi- or monodermal tumour. We do not therefore identify the component cell of the seminoma with the spermatogonium. We uphold rather the doctrine of its derivation from an earlier cell. The spermatogonium is so far differentiated as to be " determined " to the production of spermatozoa, just as the lymphoblast, for example, is to the production of the small lymphocyte. Both the spermatogonium and the lymphoblast require an earlier existent stem cell as progenitor, which, in the case of the testis, gives rise to the seminoma. The doctrine of the continued existence into adult life of primitive stem cells may even provide us with the explanation of the teratomas, which are usually tridermal,

or at least bidermal. The early segregation of blastomeres in the segmentation of the ovum, for the future gonads, is a thesis which is very widely accepted, as is also the possible presence of somatic blastomeres in ectopic situations. The blastomere would possess a totipotency as compared with the pluripotency of a blastodermic cell or the still more restricted potency of a cell such as the stem cell of the spermatogonium, with well-defined " determined " character. " Potencies . . . are often much more widely distributed and retained than normal development and structure lead us to suppose . . . " (Nicholson, 1929, p. 384). Undeveloped dormant germinal epithelium may well be the cell or tissue of origin of the teratoma, containing the various structures (Figs. 30-34) which are familiar to us in the teratomas of the ovary and testicle. The origin, therefore, of tumours of the testicle, taken as being from embryonal cells, may be blastomeric, Wolffian, trophoblastic, or blastodermic, with corresponding variation in the type of tumour development. The earlier the embryonal age of the tumour stem cell the greater its range of differentiated tissue formation. Acceptance of this mode of histogenesis explains the occurrence in the testicle of two allied tumour types, the teratoma and the seminoma. The former is a tumour usually of many tissues or organoid structures; the latter is a carcinoma which shows most of the features of the anaplastic carcinoma of glandular type observed elsewhere. Some of the teratomas may show seminoma tissue as one of their component structures (Fig. 29).

These views seem to us sufficient to explain the seminoma and to reconcile the view that teratomas and seminomas are different tumours with the view that the seminoma is a one-sided teratoma. In conclusion, it may be frankly stated that the tumour with some adenoid architecture (Fig. 11) and the large clear cell type of tumour with ragged or vacuolated cytoplasm (Figs. 24 and 25), sometimes diagnosed hypernephroma, sometimes interstitial cell tumour, create difficulty in diagnosis. We relegate all adenoid and neuro-epithelial types to the teratomas and leave the large clear cell, hypernephromatoid type at present *sub judice*.

We are grateful to the Carnegie Trust for the Universities of Scotland for their generous help towards the cost of illustration.

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NOTES, CLINICAL AND HISTOLOGICAL, ON ILLUSTRATIONS.

FIG. 1.—*Seminoma.* Spaniel, aged. Very early stage, small single tumour, embedded in the testis proper. Microscopically, this showed typical seminoma with apparent genesis from seminiferous tubules. *Cf.* Figs. 5, 6 and 7. Natural size. 5130/22.

FIG. 2.—*Seminoma.* Fox terrier, aged 5 years. Tumour occupying almost all the testis; compressed remains of testis seen at one pole. Epididymis not involved. Microscopically typical seminoma (Figs. 14 and 15). *Circ.* half natural size. 5143/22.

FIGS. 3 and 4.—*Seminoma.* Dog aged 12 years. Advanced tumour replacing the entire testis which was much enlarged but preserved normal testicular contour; bosselated external surface (Fig. 3). Cut surface (Fig. 4) fleshy, lobulated, with one large hæmorrhage. Epididymis not involved. Microscopically, typical seminoma (Figs. 19, 20 and 21). *Circ.* half natural size. 5149/22.

FIG. 5.—*Seminoma.* Bulldog, aged. Multiple tumour areas varying greatly in size. Multilocular cystic area at one pole (*a*). Autopsy case; no metastases in regional lymph nodes or lung. Natural size. 5166/22. A development of seminomas in the dog and horse has been observed in association with advanced age.

FIG. 6.—*Seminoma.* Dog, aged. Microscopic section, natural size, same case as Figs. 5 and 7, showing the same features rendered more distinct by sectioning and staining.

FIG. 7.—*Seminoma.* Dog, aged. Same case as Figs. 5 and 6. Showing apparent genetic relationship of tumour to tubules; (*a*) normal tubules; (*b*) tumour nodule; (*c*) degenerate tubule in centre of tumour growth; (*d*) early tumour cell proliferation in a tubule. ×80.

FIG. 8.—*Seminoma.* Dog aged 3 years. Showing sheets of tumour cells, sparse stroma and few lymphocytes. Alive and well five years after removal (18.6.32-2.4.37). ×150. 5128/22. The comparatively favourable prognosis in dogs, even with only simple orchidectomy, is in contrast to the outcome of seminomas in human beings.

FIG. 9.—*Seminoma.* The same tissue as in Fig. 8. Component cells large and polyhedral, forming a loose mosaic; vesicular nucleus with nucleolus. A few isolated lymphocytes. Mitoses (*a*). ×350.

FIG. 10.—Tissue from single malignant tumour in aged dog. Large hæmorrhagic cystic growth, weight 450 gm., obliterating all normal testicular tissue. Suggestive of type of tumour regarded as hypernephroma; massive lipoid deposition in tumour cells. This dog was destroyed 15 months after operation; large mass of tumour, probably metastatic, in a lymph node posterior to the kidney. ×150. 5179/22.

FIG. 11.—Same tissue as in Fig. 10. Showing: (*a*) large clear cells with, in some parts, semblance of glandular character; (*b*) post-degenerative cystic spaces. It has been suggested that the ragged or vacuolated appearance of the cytoplasm is due to the presence of glycogen. Our own hypernephromatoid tumours have shown abundant lipoid cell content. ×350.

FIG. 12.—*Seminoma.* Man, aged 35. Showing invasion without rupture of tunica albuginea. Note atrophic seminiferous tubules (*a*) in tumour area. ×150. H. 7076/35.

FIG. 13.—*Seminoma.* Same tissue as in Fig. 12. Showing juxtaposition of invading tumour cells (*a*) and, as contrast, a band of interstitial cells (*b*). ×350.

FIG. 14.—*Seminoma.* Dog aged 5 years. Tumour illustrated in Fig. 2. Typical seminoma, showing sheets of large polyhedral cells with hyperchromatic nuclei; a large aggregation of lymphocytes (*a*); scanty fine stroma. ×150. 5143/22.

FIG. 15.—*Seminoma.* Same tissue as in Fig. 14. Showing tumour cells (*a*) and lymphocytes (*b*). ×350.

FIG. 16.—*Seminoma.* Aberdeen terrier aged 14 years. Autopsy case. Large tumour of right testis; early metastases in para-aortic, iliac and lumbar lymph nodes. Showing sheets of uniform tumour cells with small aggregations of lymphocytes (*a*). ×150. 5140/22.

FIG. 17.—*Seminoma.* Same case as in Fig. 16. Showing: (*a*) large round tumour cells, with excentric nucleus and abundant cytoplasm; (*b*) multi-, and (*c*) bi-nucleated forms. ×350.

FIG. 18.—*Seminoma.* Same case as in Figs. 16 and 17. Metastasis in lymph node, showing lymphoid tissue and sheets of tumour cells similar to those in the primary growth. ×150.

FIG. 19.—*Seminoma.* Dog aged 12 years. Naked-eye view shown in Figs. 3 and 4. Showing the delicate argyrophile reticulum separating larger and smaller groups of tumour cells. Wilder's silver impregnation stain. ×150. 5149/22.

FIG. 20.—*Seminoma.* Same case as in Fig. 19 and Figs. 3 and 4. Showing tumour structure and lymphocytes (*a*). ×150.

FIG. 21.—*Seminoma.* Same case as in Figs. 19 and 20 and Figs. 3 and 4. Showing architecture and cytology. ×350.

FIG. 22.—*Seminoma.* Man aged 52. Showing bands of tumour cells (*a*) in a fibrous stroma (*b*). ×150. H. 61/30.

FIG. 23.—*Seminoma.* Man aged 37. Showing areas of early necrosis (*a*) and subsequent liquefaction (*b*). ×150. H. 7077/35.

FIG. 24.—A so-called *testicular hypernephroma* in a horse. Showing the large pale foamy cell tissue characteristic of this tumour. This tumour is regarded by some observers as of interstitial cell (Leydig cell) origin. It has little resemblance to the typical seminoma. ×150. CG 4.

FIG. 25.—So-called *testicular hypernephroma* in a horse. Same tissue as in Fig. 24. ×350.

FIG. 26.—*Undescended testicle.* Found at operation in an adult aged 35, presumed to be a woman. Showing: (*a*) atrophic seminiferous tubules, with (*b*) extreme hyperplasia of the interstitial cell element. The Sertoli cell (*c*) of the tubule is rather characteristic of atrophic and senile conditions. ×150. H 3745/34.

FIG. 27.—*Undescended testicle.* Same tissue as in Fig. 26. Showing: (*a*) the character of the interstitial cells and (*b*) part of an atrophic tubule. Some tumours of the testis in animals arise from these cells and certain observers regard the so-called testicular hypernephromas as interstitial cell tumours. ×350.

FIG. 28.—*Seminoma.* Man aged 34. Extensive metastases in para-iliac and para-aortic lymph nodes from tumour in right testis. Showing loose sheet of uniform tumour cells and isolated lymphocytes. ×350. Autopsy No. 196/33. Path. Instit. Munich Univ. (by courtesy of Professor Max Borst). 5133/22.

FIG. 29.—*Teratoma.* Man aged 20. Showing: (*a*) seminoma tissue; (*b*) intestinal glandular element lined with columnar epithelium. The interest of this case lay in the presence of a seminoma within a teratoma. ×100. H 1711/37.

FIGS. 30-34.—*Teratoma testis.* Man aged 33. Showing a variety of structures, the presence of any of which serves to establish the diagnosis of teratoma as distinct from seminoma.

Fig. 30. A neuro-epithelial canal with internal limiting membrane and lining of primitive spongioblasts. Fig. 31. Ependyma-lined neural cavity and choroid plexus papillary formation. Fig. 32. Hyaline cartilage (*a*), and adjacent pigmented retinal epithelium lining neural cavities. Fig. 33. An organoid glandular formation. Fig. 34. A squamous epithelial cell nest without skin appendages. All at the same magnification. ×200. H 9679/36.

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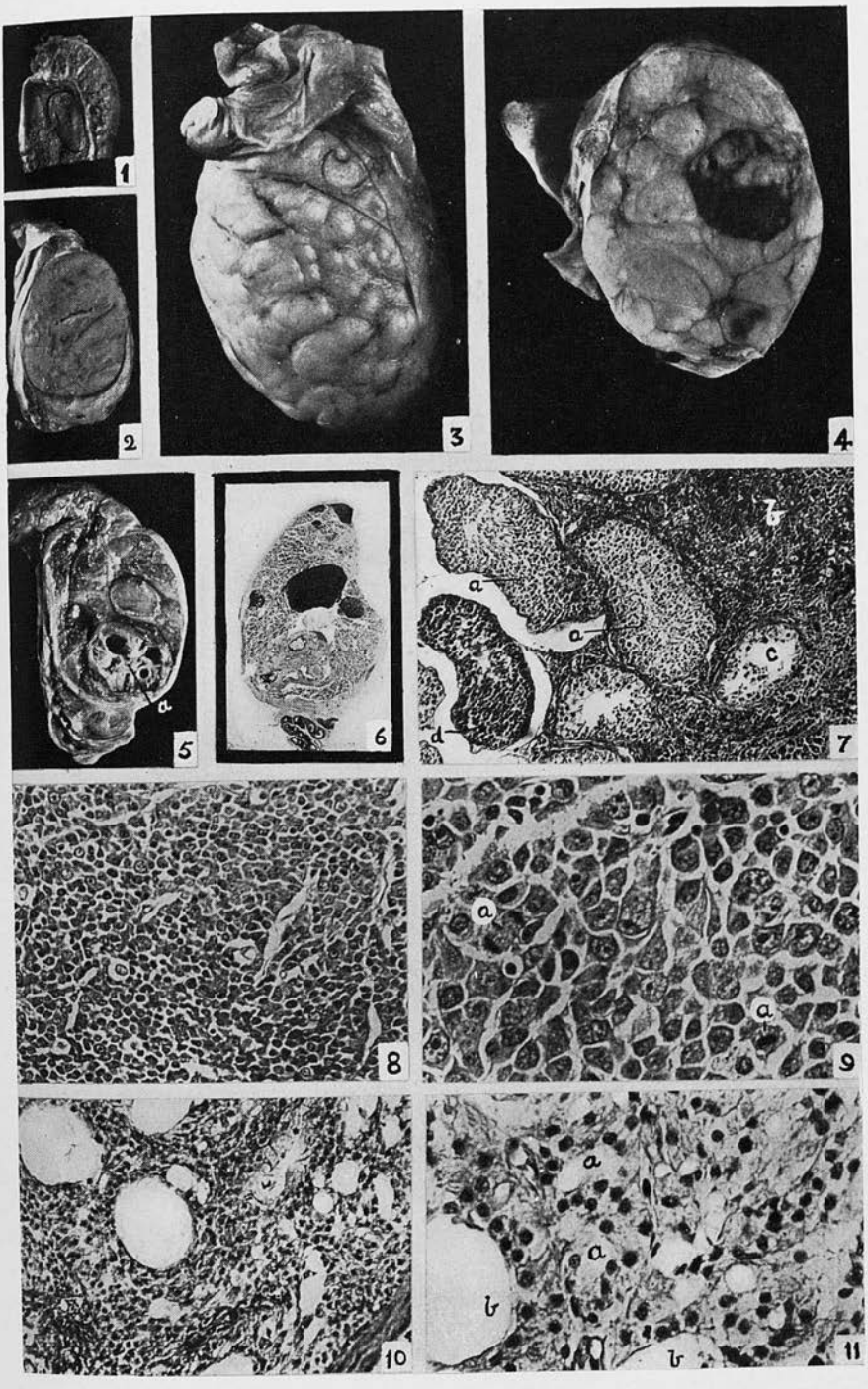
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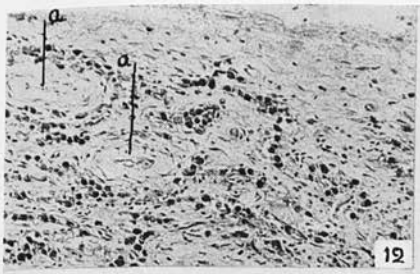

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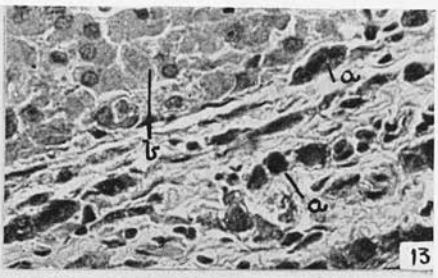

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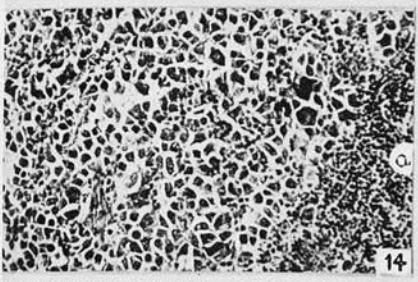

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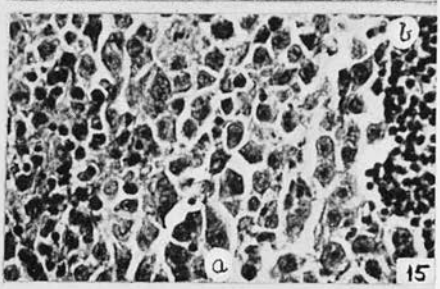

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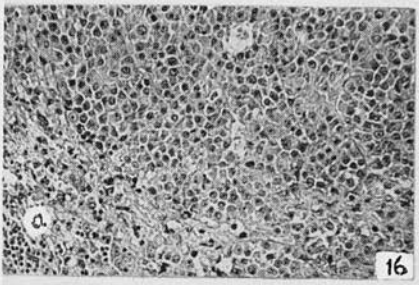

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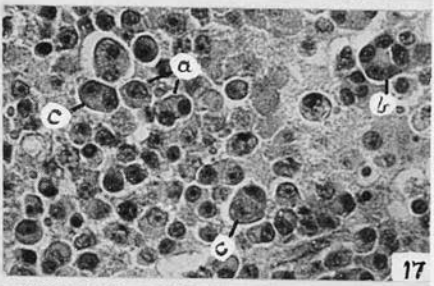

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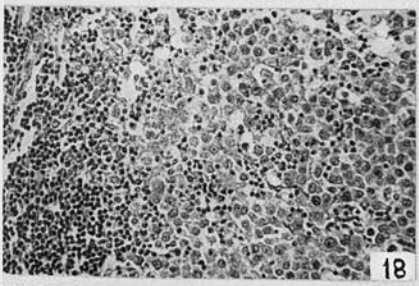
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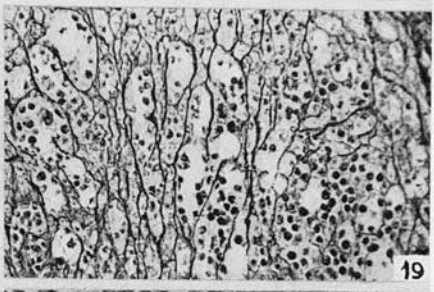
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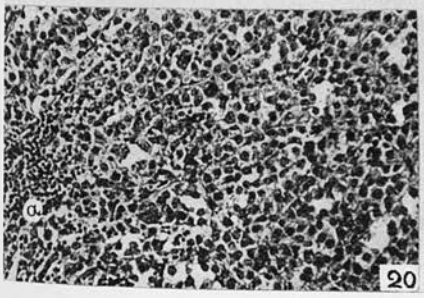

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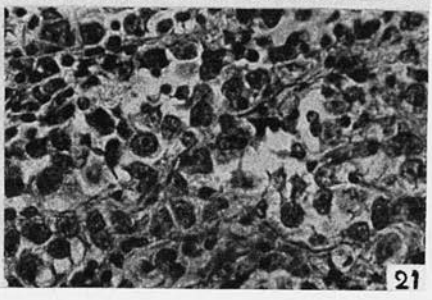
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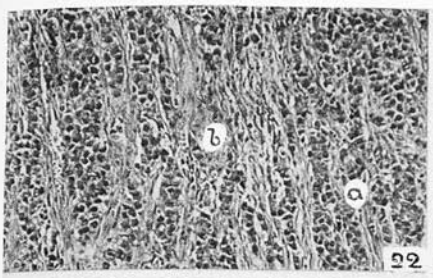

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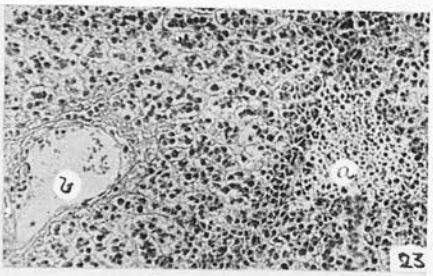

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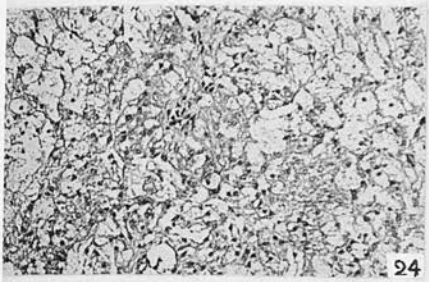
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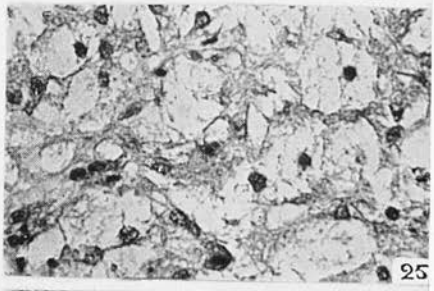
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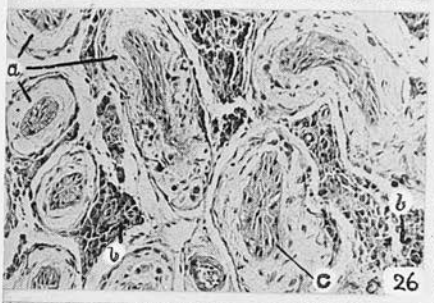

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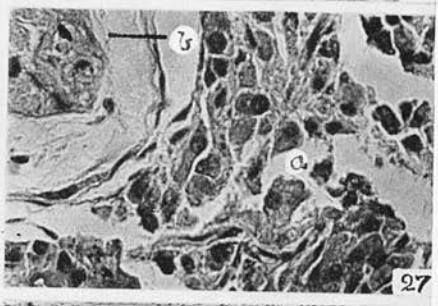

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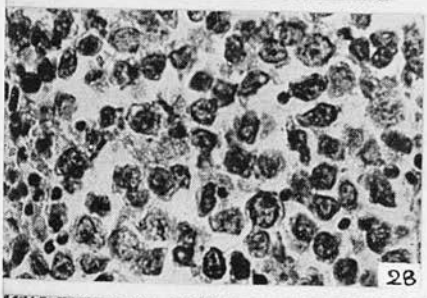
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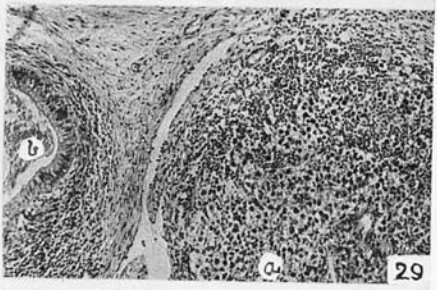

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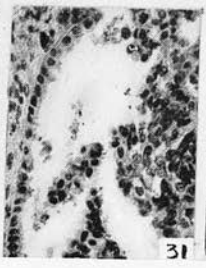
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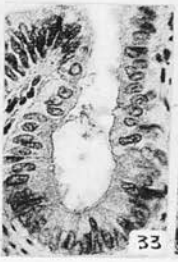
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NO. 1

DEBATABLE TUMOURS IN HUMAN AND ANIMAL PATHOLOGY.

IV. "Mixed Tumours" of Salivary Glands.

Debatable Tumours in Human and Animal Pathology

IV. "Mixed Tumours" of Salivary Glands

BY

W. F. HARVEY, E. K. DAWSON and J. R. M. INNES

(*From the Research Laboratory of the Royal College of Physicians, Edinburgh, the Cancer Control Organisation of Edinburgh and South-East Scotland and the Institute of Animal Pathology, University of Cambridge.*)

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DEBATABLE TUMOURS IN HUMAN AND ANIMAL PATHOLOGY.

IV. "Mixed Tumours" of Salivary Glands.*

By W. F. HARVEY, E. K. DAWSON and J. R. M. INNES.

(*From the Research Laboratory of the Royal College of Physicians, Edinburgh, the Cancer Control Organisation of Edinburgh and South-East Scotland and the Institute of Animal Pathology, University of Cambridge.*)

Definition.—These tumours are adenomas of the serous and seromucous salivary or lachrymal glands, of undifferentiated and gland-lobular types, which are very prone to a species of mucoid autolytic self-destructive transformation of their component tissues and are seldom truly malignant.

Description.—The epithelial component which is the characteristic element of the tumour is essentially of glandular type. The cells are mainly undifferentiated in character, but may develop some degree of differentiation along either glandular (Figs. 7, 12, 15) or epidermoid lines (Fig. 49), or both. The glanduliform elements, when present in the tumour, may consist of acini with one layer of cubical or columnar epithelium, or of ducts with two layers of epithelium, or of spaces lined with numerous layers of uniform cells. Some of the cell-complexes may be of basal-cell type (Fig. 18) and may be cystic (Figs. 10, 22), with mucoid, colloid or hyaline content or no content at all. In another direction and probably affording an instance of metaplasia of undifferentiated cell elements, the tumour cells exhibit epidermoid characters, not only as basal cells but more strikingly as squamous cells (Fig. 50) with intercellular bridges, keratohyalin granulation and "pearl" formations. In the main, however, these tumours consist rather of undifferentiated polyhedral or spindle-shaped cells arranged in parenchymatous masses, irregular anastomosing strands or islands of closely-packed uniform basophile cells, without indication of keratinisation. The nucleus is hyperchromatic and a nucleolus may or may not be evident. The scanty cytoplasm is faintly acidophile. Mitoses are scarce, except when the tumour is truly malignant (Figs. 51-54). The very varied cellular picture presented by

* Submitted for publication, 20.12.37.

this tumour led, in the past, to certain features being picked out which determined its nomenclature as endotheliomatous, cylindromatous, chondromatous, basaliomatous, carcinomatous and even sarcomatous. The endothelial and sarcomatous designations have lapsed with the more or less general acceptance of the epithelial elements as the essential component of the tumour. The tendency of the epithelium, and of the stroma also, to show myxoid, hyaline and chondroid transformation gives the tumour its special character and led to its being regarded as a " mixed " tumour of bi-dermal composition. These transformation or " inclusion " areas have been taken to be true cartilage. Such cartilage has, however, little resemblance to fœtal cartilage and is without a perichondrium. It may now, however, be justifiably contended that it is not cartilage but pseudo-cartilage (Figs. 37, 39) or a myxo-chondroid substance developed from epithelium and stroma by a transformation which we must regard as a degeneration, possibly combined with a manifestation of the secretory function of the tumour cells. The substance developed is of the nature of mucin, a conjugate protein, or one of its allies, pseudomucin, " mucoid," or chondro-protein. Epithelial cells which have not quite disappeared into the acellular, fibrillar or homogeneous mucoid material tend to be isolated in a retraction space which gives them all the appearance of cartilage cells within their capsules (Figs. 38, 40), especially as the margins of the containing spaces stain somewhat deeply. The supporting tissue of the tumour may be of ordinary fibrous connective tissue type and may contain groups of fat cells (Fig. 45), but is usually myxoid, whether by imbibition of the epithelial mucinous product or by an autogenous metamorphosis. In this process, epithelial and connective tissues merge one into the other and may lose almost all indication of their usual delimitation borders, the epithelial cells being disseminated singly or in groups in the mucoid matrix. Staining reactions of this matrix vary, but are usually metachromatic. It must be contended here, however, that the stains thionin blue, toluidin blue and mucicarmin, which are used to pick out mucin, are probably only partially specific. We find, too, that hyaline staining may also be a marked feature of these tumours; they have indeed been called muco-hyaline carcinomas. It seems probable that hyaline and mucoid changes are only phases of the same process and that the two types of stroma may merge into one another.

The tumours are mainly benign, of long duration and of slow and even intermittent growth, but may, especially when internal pressure has caused rupture of the condensed fibrous capsular tissue, be locally invasive. In some of our cases the tumours were present for twenty and thirty years. Rupture of the capsule may be coincident with a history of rapid increase in size. The tumours occur mostly in association with the parotid gland, less commonly with the submaxillary and rarely with the sublingual, but they are to be found in various parts of the orofacial region and arise presumably from the serous or mucous glands which are comprised under the denomination salivary. Occasionally they are found in the lip, palate, nose, bronchi, and in the orbit, arising from the lachrymal gland. The figures given by McFarland for the large salivary glands, as parotid 93·6 per cent., submaxillary 6·1 per cent., and sublingual 0·3 per cent., probably represent an unselected distribution of tumour occurrence for the glands concerned. The tumour material available for our examination, with the diagnosis of " mixed tumour," totals 316 cases ; the locality was given or evident in 275 of these cases and the distribution was as follows—parotid gland 230, submaxillary 21, sublingual 2, lip 9, palate 6, nose 1, and lachrymal 6. Ages at operation ranged from 15 to 89 years. In addition to these human cases, 5 tumours occurred in dogs.

The tendency to recur after operation may be ascribed to difficulty in complete removal and the surgeon's reluctance to damage the facial nerve or its branches in the parotid cases. With each recurrence, the same or a more undifferentiated type of tumour growth appears, and various structures, including lymph nodes, may be involved. The occurrence of tubules of salivary gland type in lymph nodes or in collections of lymphoid tissue has been frequently noted and is not necessarily evidence of metastasis. Malignancy may, however, develop in a " mixed tumour " and show either glandular or, more frequently, epidermoid characters.

Discussion.—As regards the nature of these " mixed tumours," discussion by pathologists has swung from a connective tissue to a purely epithelial and, for a time at least, to an endothelial composition. They have been regarded as tumours of either glandular or branchiogenic origin. One criticism of the view that the tumours are essentially adenomas is that the tumour appears very constantly walled off from normal gland by a dense fibrous tissue (Fig. 14). Our

preparations, however, give indications that, in some cases, there is continuity between gland and tumour (Figs. 27, 28), and it is said that this continuity can be quite frequently made out somewhere if serial sections are cut. A glance at our illustrations shows, in a number of instances, the well-known resemblance of the tumours of the parotid to the basal-cell carcinomas of the skin. Here it may be well to recall that these skin tumours, just as the parotid tumours, have their adenoid and adenoid cystic, as well as their epidermoid types. When, therefore, the basal-cell carcinoma, the " cylindroma " and the " benign cystic epithelioma " occur in the parotid, we propose to call them salivary gland adenomas. The term " cylindroma," with its own special connotation, was applied originally to tumour material examined in the fresh condition. It is a misleading term for the actual microscopical appearances, which are nevertheless characteristic (see Figs. 17, 18, 22, 34, 36). When salivary gland tumours are malignant, even when, as is very common, they show epidermoid character, they are salivary gland adenocarcinomas.

The favourite belief hitherto regarding salivary gland tumours seems to have been that they were " enclavomas " or branchiomas in the Cohnheim sense—bidermal fibro-epithelial tumours. While the close relationship in which the parotid and submaxillary glands are placed to the first and second branchial arches in embryological development would favour this conception of a teratoid constitution, it would not easily account for the " mixed tumours " of lip, palate, nose, etc. It seems, however, only necessary to return to the original Cohnheim conception of a *residual* embryonal foundation (" Anlage "), representing the original embryonal production of cells in excess of immediate local structural requirements, to find all that is needed in explanation of these tumours and make them gland-lobular instead of bidermal. We should not, in our conception of tumour development anywhere, lay stress on the *excessive* production of the cells of a primordium, for it requires only a slight modification of the theory to extend the conception of tumour production to a normally persistent rather than a residual embryonal cell. The cell concerned becomes then the seed, stem, or replacement cell of all normal adult tissues, while benign and malignant tumours may be pictured as arising only in these embryonal cells and never in adult differentiated cells. Such a theory still finds room for the conception of a residual paraglandular

debris of cells and cell groups, as is invoked for the origin of the adamantinoma. The branchiogenic or fœtal fissure origin of salivary gland tumours was almost a necessity as an explanation of the cartilage supposed to be characteristically present in them. Uncertainty also regarding the exact nature and importance of the component tissues of the " enclavoma " necessitated the use of compound descriptive titles such as fibromyxochondro-adenoma or carcinoma. Removal of the stumbling block of association of cartilage with epithelium in these " mixed " salivary gland tumours by denying it to be true cartilage and regarding it as degenerative transformation of epithelium and stroma sweeps away much of the difficulty in considering them as salivary gland epithelial tumours with lobular stroma. In this we return to what was an early tenet, one which has always been vigorously held, what we may call the gland-lobular view. We have thus to do with an adenoma, similar to the gland-lobular adenoma of the breast. The latter tumour commonly makes its appearance before the pathologist with a preponderance of the fibrous element of the gland lobule and receives the name of " fibro-adenoma," but not, as a rule, " mixed tumour " of the breast. Moreover, myxoid change is very frequent in it and there may even be development of cartilage or bone, especially in the tumours found in the dog's mamma (Figs. 47, 48). Cartilage and bone in such cases are usually regarded as metaplasia in mesodermic tissue. Any inclination to resort to an explanation by way of ectodermic epithelial production of cartilage, such as apparently seems to happen in some lowly animals, may, we think, be discouraged.

The mucoid change in salivary adenomas is very striking. It may be evident in mesodermic, ectodermic and entodermic tissue. The " colloid " carcinoma, the myxoma and the " mixed " tumours may all represent something of a similar disintegrative change, a suicidal autolytic process. It occurs to us that the transformation may have some analogy or even identity with the suicidal colony of bacteria, which disappears by autolysis or " bacteriophagy," in a viscous mucinous mass.

To sum up : We regard the " mixed tumour " of salivary glands as arising in embryonal, glandular, replacement epithelium, whether sequestered or non-sequestered. Proliferation slowly takes place in this epithelium and its stroma for the same reason as in any benign tumour. The proliferation of the stem cells may be anaplastic, metaplastic or differentiated

to an organoid glandular character. A special proneness to autodestruction of parenchyma and stroma is a feature of the tumour and this may have some relation to the type of secretion proper to the gland affected. Clinical malignancy is extremely rare in the sense of metastases to lymph nodes or distant parts. On the other hand, recurrence is common, with involvement of neighbouring tissues. Where mastication and deglutition are interfered with by huge growths, death may result from malnutrition or from sepsis.

Conclusion.—The mixed tumour of salivary glands is an adenoma (sialoma) and should be so designated in all its forms, even if epidermoid in type, unless it is malignant. It is specially subject to extensive degenerative change in its component cells.

We are grateful to the Carnegie Trust for the Universities of Scotland for generous help towards the cost of illustration. We desire to acknowledge the great help given us by Mr David Aitken of the R.C.P. Laboratory in preparing the photomicrographs.

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NOTES, CLINICAL AND HISTOLOGICAL, ON ILLUSTRATIONS.

The plates illustrate the extremely varied types of the salivary gland tumours. In some cases, owing to exigencies of space, only a low power view has been possible. The great variety of type and locality of these tumours justifies, we consider, the numerous photographs.

FIG. 1.—*Normal salivary gland.* Parotid. Showing ducts and acini embedded in fatty tissue. C.G. 47. ×40.

FIG. 2.—*Normal salivary gland.* Same tissue as in Fig. 1. Showing duct (*a*), and acini (*b*), of serous type. C.G. 47. ×150.

FIG. 3.—*Normal salivary gland.* Same tissue as in Fig. 1, stained by Wilder's reticulum impregnation method. Showing acini and ducts defined by argyrophile fibrils and collagen. C.G. 47. ×150.

FIG. 4.—*Salivary gland tumour.* Parotid. Showing typical " mixed " tumour with glandular (see Fig. 7) and chondroid areas, loose capsule (*a*) and normal gland (*b*). C.G. 62. ×40.

FIG. 5.—*Salivary gland tumour.* Parotid. Sent with diagnosis of chondrosarcoma. A benign adenoma showing a network of epithelial strands with some degenerating cartilage-like cells (*a*) in a mucoid matrix. C.G. 32. ×150.

FIG. 6.—*Salivary gland tumour.* Submaxillary. Showing epithelial strands, groups and single cells in mucoid matrix with chondroid character. 2211·9. I.A.P. (From Professor Borst, Munich.)

FIG. 7.—*Salivary gland tumour.* Parotid. Same tissue as Fig. 4, showing glandular architecture of typically two-layer duct type, with granular content and a tendency to mucoid degeneration of the scanty supporting tissue. C.G. 62. ×100.

FIG. 8.—*Salivary gland tumour.* Submaxillary. Female, aged 47. Slow enlargement for 6 months. Showing cytology of two-layer glandular formations. See same tissue, Fig. 33. 7131/35. ×250.

FIG. 9.—*Salivary gland tumour.* Parotid. Showing tumour structure with glandular elements sharply demarcated from degenerated mucoid areas. C.G. 10. ×40.

FIG. 10.—*Salivary gland tumour.* Parotid. Showing cystic glandular formations with intervening structure of mixed epithelial and stroma elements. C.G. 39. ×40.

FIG. 11.—*Salivary gland tumour.* Parotid. Showing diffuse parenchymatous proliferation with tendency to glandular (rosette-like) formations, which are obvious in Fig. 12. C.G. 36. ×100.

FIG. 12.—*Salivary gland tumour.* Parotid. Another area of the same tumour as in Fig. 11. Showing primitive glandular formations of rosette-like type. C.G. 36. ×100.

FIG. 13.—*Salivary gland tumour.* Parotid. "Tumour of mandible." Female, aged 20. Showing a fibro-adenomatous picture reminiscent of a breast tumour. The parenchymatous areas and the degenerated mucoid stroma in other parts of this tissue determined the laboratory diagnosis. 5185/28. ×40.

FIG. 14.—*Salivary gland tumour.* Parotid. "Lymph gland from neck; swelling present for some years." Female, aged 39. Showing an appearance again reminiscent of the intracanalicular fibro-adenoma of the breast. The stroma shows mucochondroid change. A fibrous capsule (*a*) separates tumour from normal parotid gland (*b*). 6348/35. ×40.

FIG. 15.—*Salivary gland tumour.* Upper lip. "Encapsuled solid tumour which shelled out easily." Female, aged 17. Showing typical intracanalicular fibro-adenoma, as found in the breast. Other areas in the section were chondroid in character. 6553/35. ×40.

FIG. 16.—*Salivary gland tumour.* Lip. "Cyst of lip." Female, aged 57. Showing tumour of pure adenomatous type with a mucoid stroma. It was enclosed in a dense fibrous capsule. 2233/9. I.A.P. ×40.

FIG. 17.—*Salivary gland tumour.* Sublingual. "Solid tumour, size of walnut, in floor of mouth under mucous membrane; shelled out." Male, aged 89. Showing pseudolobular structure, consisting of parenchymatous masses honeycombed with spaces of degenerative character—one of the types often referred to as cylindroma. 617/30. ×40.

FIG. 18.—*Salivary gland tumour.* Parotid. Showing more solid parenchymatous cell-complexes with loose vascular stroma. Another "cylindroma." C.G. 35. ×40.

FIG. 19.—*Salivary gland tumour.* Parotid. An unusual type, consisting of numerous cysts filled with papillary tumour. Showing wall of a cyst (*a*), and papilliform tumour (*b*). 2202/9. I.A.P. ×40.

FIG. 20.—*Salivary gland tumour.* Parotid. Sent with diagnosis of malignant endothelioma. Showing benign glandular tumour, of the type illustrated in the older literature as endotheliomatous with vascular spaces. C.G. 16. ×100.

FIG. 21.—*Salivary gland tumour.* Parotid. A very rare type, unique in our own experience, showing perithelial arrangement of tumour cells and intervening necrosis—evident perivascular cell survival. C.G. 52. ×25.

FIG. 22.—*Salivary gland tumour.* Lachrymal gland. Tumour of orbit. Male, aged 32. Showing "cylindroma" type (*cf.* Figs. 17 and 18) with pseudo-glandular spaces containing eosinophile coagulum—a structure reminiscent of the "epithelioma adenoides cysticum." The tumour showed little demarcation in places from normal lachrymal gland. 850/4. I.A.P. ×40.

FIG. 23.—*Salivary gland tumour.* Dog, aged. Parotid. Large lobulated growth freely movable. Showing well-marked gland lobular architecture; stroma hyaline within the lobules, loose and vascular around them. 583/36. I.A.P. ×40.

FIG. 24.—*Salivary gland tumour.* Dog, aged 2½ years. " Tumour from jaw." Showing generalised lobular structure with interlobular collagenous septa. 397/37. I.A.P. ×40.

FIG. 25.—*Salivary gland tumour.* Submaxillary. Showing " mixed " tumour (*a*), fibrous capsule (*b*), and normal seromucous glandular tissue. C.G. 19. ×40.

FIG. 26.—*Salivary gland tumour.* Lachrymal gland. Showing tumour (*a*) without capsule but well separated from normal gland (*b*). 8479/33. ×40.

FIG. 27.—*Salivary gland tumour.* Parotid. " Tumour present 6 years, lately more rapid growth." Showing normal tissue (*a*) passing into tumour (*b*). 4777/35. ×40.

FIG. 28.—*Salivary gland tumour.* Parotid. Same tissue as in Fig. 27. Showing continuity between normal gland tissue (*a*) and tumour tissue (*b*), without the usual separation by a capsule. 4777/35. ×150.

FIG. 29.—*Salivary gland tumour.* Parotid. Sent as " adenocarcinoma of parotid with sarcomatous formation of the stroma." Showing benign tumour with duct (*a*), well-formed lobular structure (*b*), and a more diffuse parenchymatous area (*c*). C.G. 15. ×40.

FIG. 30.—*Salivary gland tumour.* Parotid. " Small swelling 6 years' duration ; gradual increase for last 2 years." Female, aged 45. Showing extensive mucoid, " suicidal " degeneration reminiscent of " colloid " carcinoma. 1204/30. ×40.

FIG. 31.—*Salivary gland tumour.* Parotid. Malignant tumour (see Fig. 54) showing branch of facial nerve (*a*) embedded in tumour tissue with adjacent normal gland tissue (*b*). 1177/26. ×25.

FIG. 32.—*Salivary gland tumour.* Parotid. Showing mucoid adenoma type of tumour in lobular masses (*a*), normal gland (*b*), and lymphoid tissue (*c*). Lymphoid tissue is not uncommon in the parotid and actual nodes are abundant in fœtal tissue. C.G. 47. ×40.

FIG. 33.—*Salivary gland tumour.* Submaxillary. Same tissue as in Fig. 8. Showing a tendency of tumour cells to spindle and fascicular character which led earlier observers to diagnose sarcoma of the parotid. This field shows transition from glandular to fascicular arrangement. 7131/35. ×250.

FIG. 34.—*Salivary gland tumour.* Submaxillary. " Tumour from neck infiltrating glands." Male, aged 61. Showing solid alveolar basal-cell masses, " cylindroma " type, some in the midst of submaxillary gland tissue (*a*) and some outside (*b*). Lymphoid aggregations in the tumour area (*c*). Clinically and histologically, tumour was present in lymph nodes, suggesting invasion. In many of these tumours, the clinical and histological criteria of malignancy do not coincide. 7445/29. ×40.

FIG. 35.—*Salivary gland tumour.* Parotid. " Tumour from cheek." Showing typical " mixed " tumour, subcutaneous in position, with parenchymatous (*a*) and chondroid (*b*) areas, adjacent hair shaft (*c*), and sebaceous glands (*d*). It is not a skin tumour. C.G. 27. ×25.

FIG. 36.—*Salivary gland tumour.* Parotid. " Small tumour of many years duration ; recent localised increase in size." Female, aged 56. Showing another " cylindroma " type (*cf.* Fig. 34), with tumour masses of basal-cell character among (*a*) and adjacent to (*b*) normal gland lobules embedded in fat. 6774/32. ×40.

FIG. 37.—*Salivary gland tumour.* Parotid. " Swelling 8 years' duration with central breaking down of tumour." Female, aged 55. Showing typically chondroid degenerative area in a " mixed " tumour with a small adjacent parenchymatous area. 3789/34. ×40.

FIG. 38.—*Salivary gland tumour.* Parotid. Same tissue as in Fig. 37. Showing (*a*) an epithelial cell aggregate from the chondroid area embedded in a mucohyaline matrix ; (*b*) another such aggregate in a state of more advanced degeneration with several pyknotic nuclei ; (*c*), (*d*), still smaller cell aggregates enclosed in capsule-like spaces, now reminiscent of cartilage cells, in extremely homogeneous matrix. 3789/34. ×250.

FIG. 39.—*Salivary gland tumour.* Parotid. "Subcutaneous tumour in front of ear; present several years. Tending to increase in size; not attached to skin or deeper structures." Female, aged 31. Showing the tissue which of all our preparations most resembled true cartilage. It had the consistency of cartilage, as may be judged from the knife markings in the section. The "cartilage cells," however, showed transition stages to large cystic spaces. 4569/31. ×40.

FIG. 40.—*Salivary gland tumour.* Parotid. Same tissue as in Fig. 39. Showing (*a*) and (*b*), stages of transition from encapsuled "cartilage" cells to empty cystic spaces. 4569/31. ×250, and (*c*) for comparison, normal epiphyseal cartilage, guinea-pig. ×100.

FIG. 41.—*Salivary gland tumour.* Parotid. Showing pure adenoma of hyperplastic gland lobular type, without mucohyaline or chondroid degeneration. The duct is surrounded by what superficially appears to be reticular stroma but is definitely acinar (see Fig. 42). C.G. 12. ×100.

FIG. 42.—*Salivary gland tumour.* Parotid. Same tissue as in Fig. 41. Stained by Wilder's reticulum impregnation method. Showing argyrophile fibrils outlining shrunken acini—an indication of epithelial gland structure. C.G. 12. ×100.

FIG. 43.—*Salivary gland tumour.* Parotid. Showing a feltwork of reticulum fibrils passing to and disappearing in a chondroid area of degeneration. C.G. 10. ×40.

FIG. 44.—*Salivary gland tumour.* Parotid. Same tissue as in Fig. 43, stained by Verhoeff's method for elastic fibres. These fibres are coarser than the argyrophile fibres of Fig. 43 and cannot be traced so far into the chondroid area.

FIG. 45.—*Salivary gland tumour.* "Tumour of mouth." Showing typical "mixed" tumour structure with degenerating chondroid areas and fatty tissue. C.G. 45. ×40.

FIG. 46.—*Salivary gland tumour.* Upper lip. "Tumour of 30 years' duration." Female, aged 54. Showing benign tumour area (*a*), regarded, from microscopical examination, as of salivary gland origin; a well-marked focus of metaplastic lamellar bone formation (*b*) with osteoblasts and myeloid tissue. 2219/9. I.A.P. ×40.

FIGS. 47 and 48.—*Mammary gland tumour.* Dog, aged 6 years. "Small bone-hard encapsuled tumour beneath the nipple." This is an example of a common finding in the fibro-adenoma of the dog's breast, in which all stages of endochondral bone formation are evident: (*a*) normal mammary tissue; (*b*) fibro-adenoma with capsule; (*c*) cartilage; (*d*) calcified cartilage; (*e*) lamellar bone with osteoblasts and a marginal osteoid band. 5252/32. I.A.P. ×40.

FIG. 49.—*Salivary gland tumour.* Parotid. "Hard localised swelling; said to be recent." Female, aged 59. Showing epidermoid metaplasia in a benign tumour. 83/36. ×250.

FIG. 50.—*Salivary gland tumour.* Parotid. Showing epidermoid metaplasia with prickle-cell formation. C.G. 26. ×650.

FIG. 51. *Salivary gland tumour.* Palate. "Encapsuled tumour from palate; duration 9 months." Showing benign (*a*) and malignant (*b*) areas. The malignant area showed hyperchromatism and numerous mitoses. 2690/37. ×100.

FIG. 52.—*Salivary gland tumour.* Parotid. Showing malignant epidermoid anastomosing strands with intervening stroma. Other areas showed sheets of epidermoid cells with numerous mitoses. C.G. 34. ×100.

FIG. 53.—*Salivary gland tumour.* Parotid. Male, aged 65. "Gland from side of neck; hard fixed glands in neck. No primary source found. Tumour adherent and inoperable." Examination showed considerable unaffected parotid tissue (*a*) and tumour (*b*) of malignant epidermoid squamous character. The genetic relationship of tumour to parotid gland is not shown here. 871/36. ×40.

FIG. 54.—*Salivary gland tumour.* Parotid. Same tissue as in Fig. 31. Showing metastatic deposit (*a*) in lymph node and tubercle follicles (*b*). In this case, the parotid gland and lymph nodes were affected with both carcinoma and tuberculosis. 1177/26. ×25.

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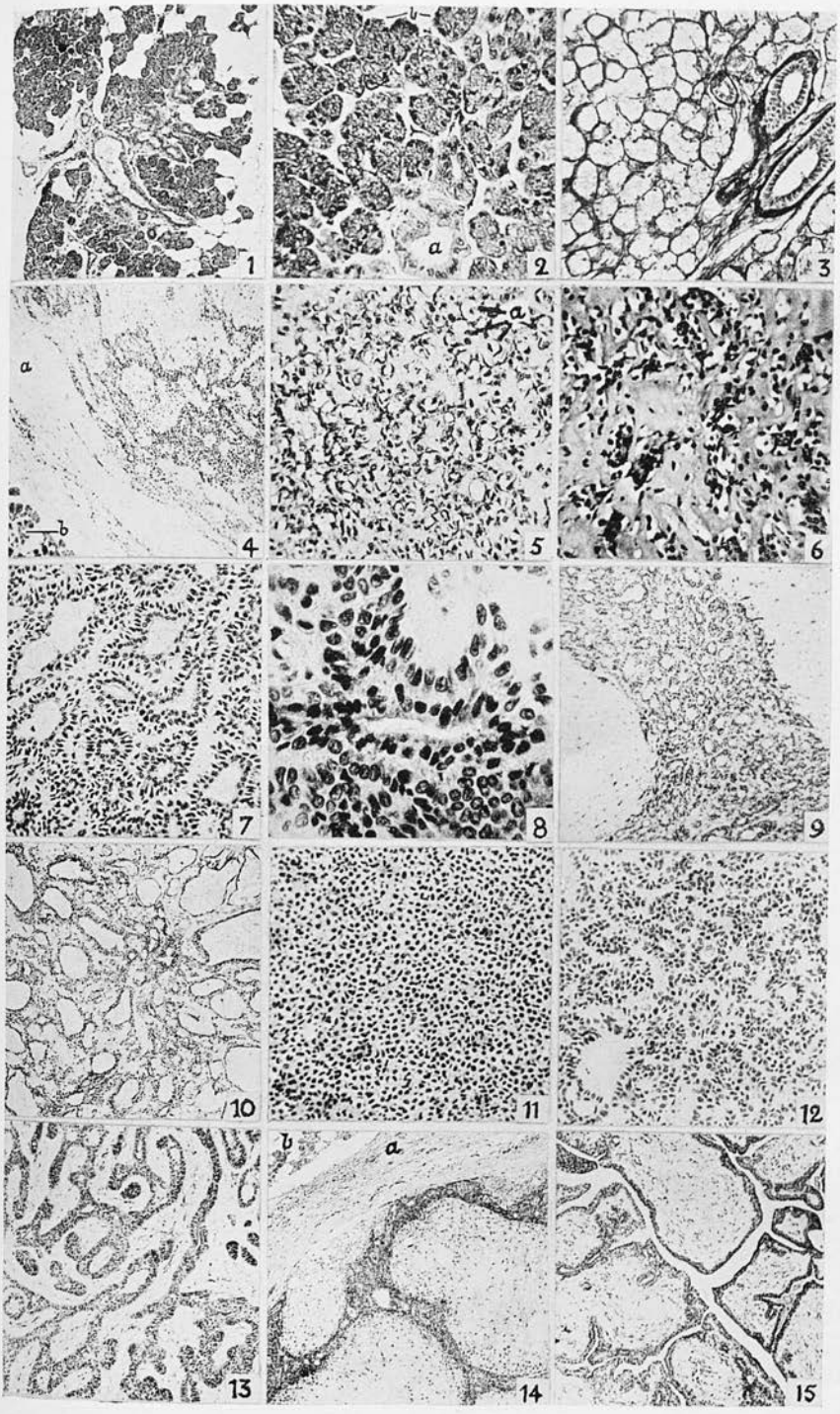
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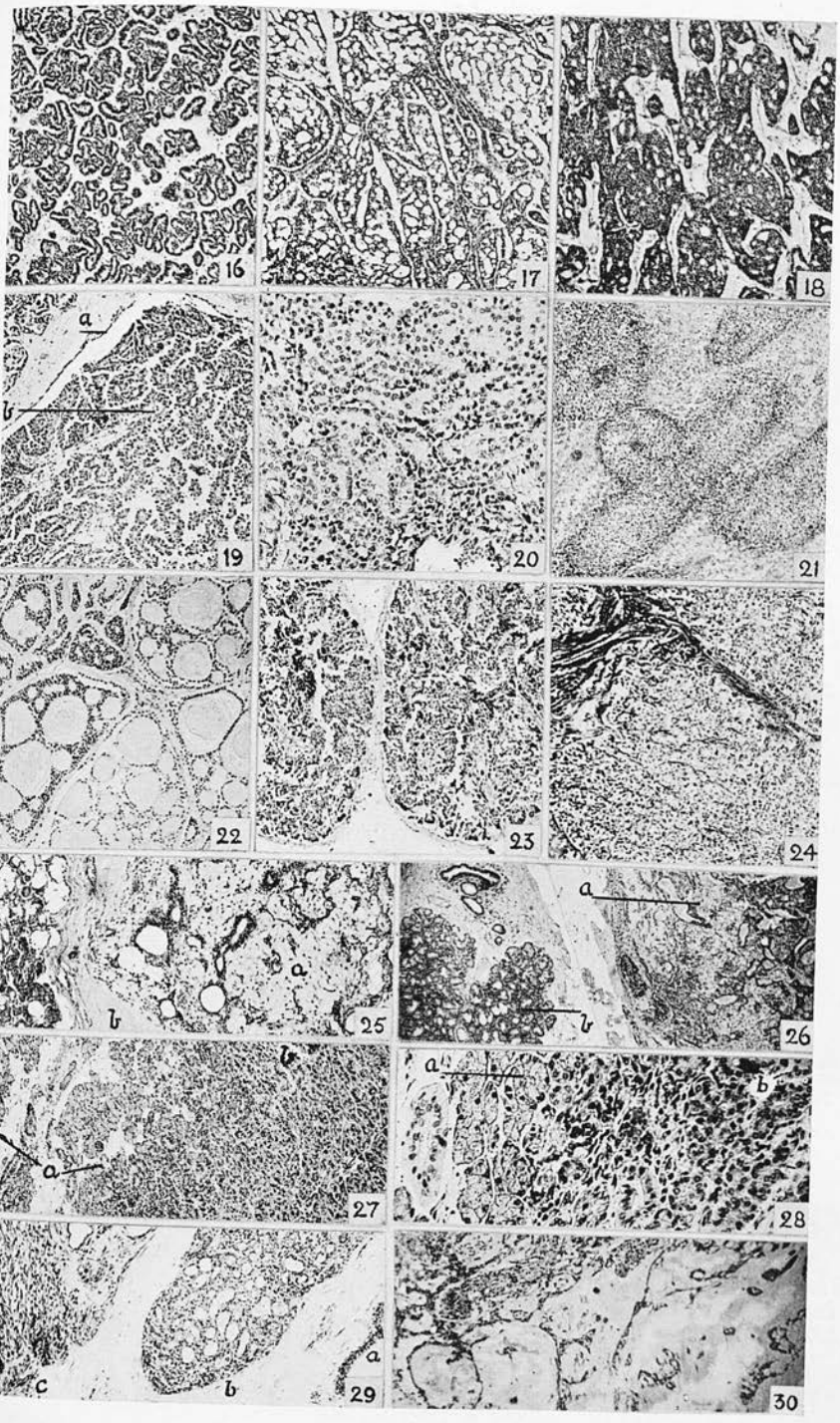
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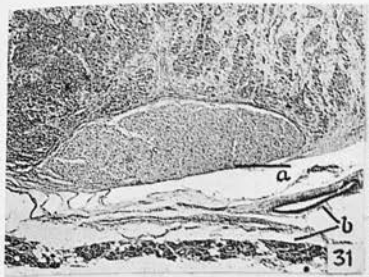
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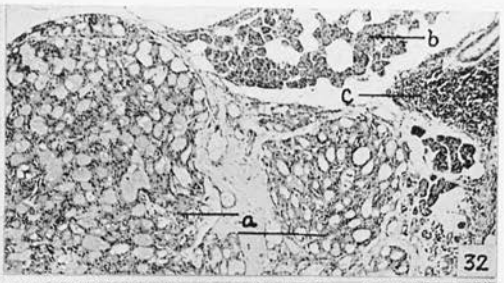
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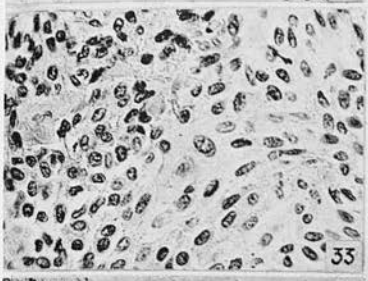
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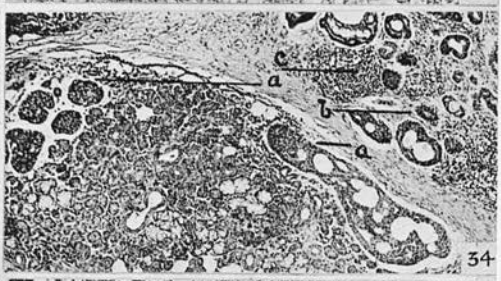
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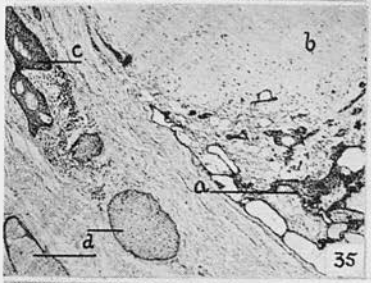
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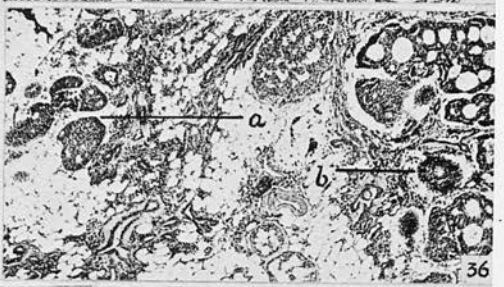
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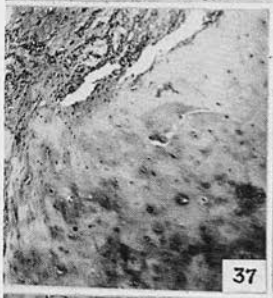
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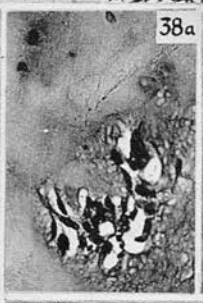
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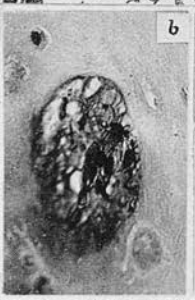
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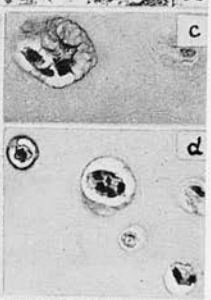
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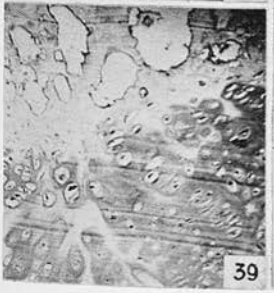
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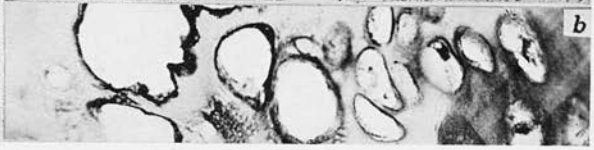
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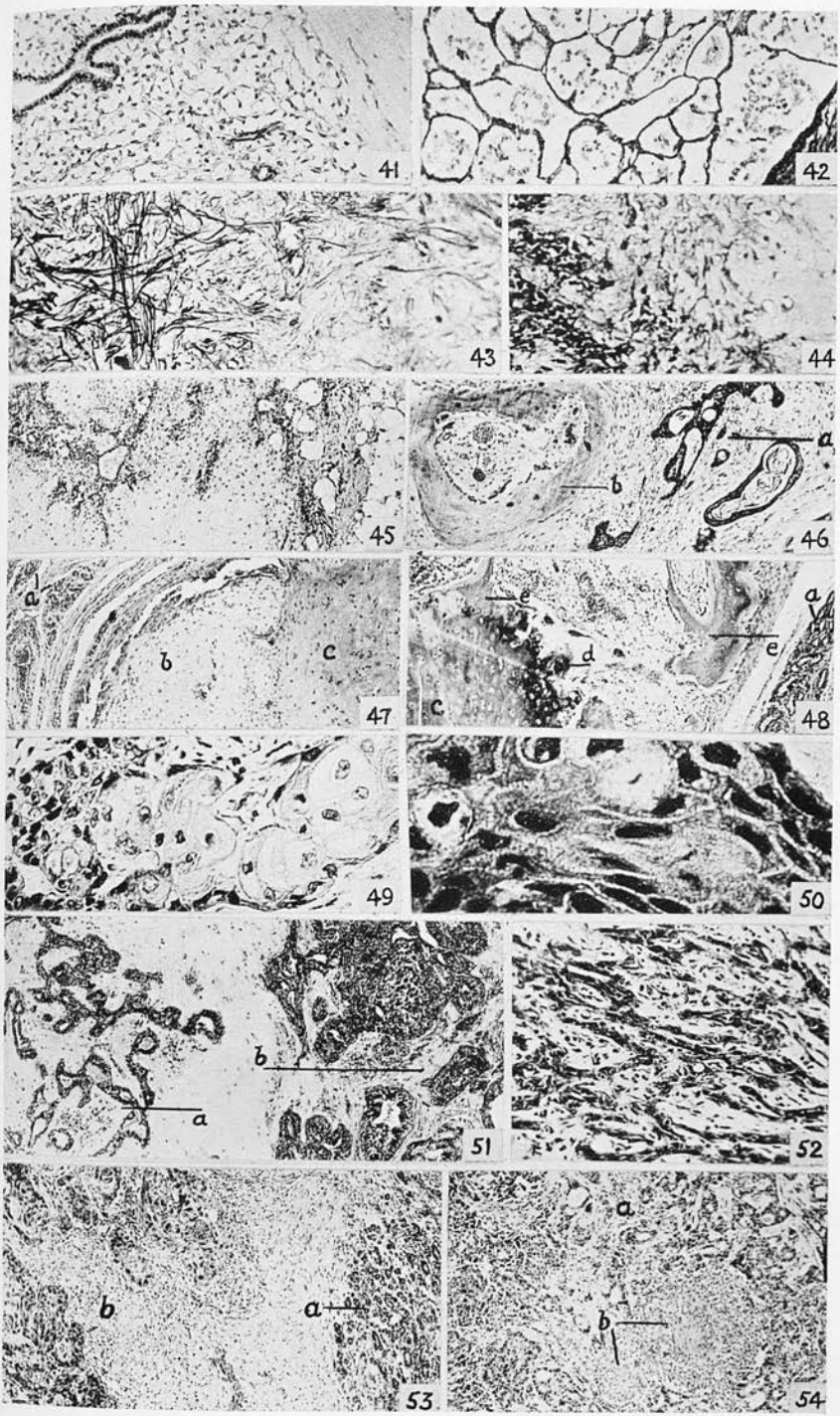
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DEBATABLE TUMOURS IN HUMAN AND ANIMAL PATHOLOGY.

V. Giant cell Tumour of Bone.

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DEBATABLE TUMOURS IN HUMAN AND ANIMAL PATHOLOGY.

V. Giant-Cell Tumour of Bone.*

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Definition.—The giant-cell tumour of bone is a highly cellular, essentially osteogenic tissue growth, characterised, in its typically active form, by the presence of numerous discrete multinucleated giant cells in the midst of much more numerous polyhedral or fusiform cells. The tumour may recur, but so rarely develops malignancy with metastases that it may be regarded as practically benign.

Description.—Various names have been given to this tumour, such as myeloid tumour (Paget), osteomembranous myelogenous sarcoma (Virchow), tumeur à myéloplaxes (Nélaton), myeloma, osteoclastoma, localised osteitis fibrosa, hæmorrhagic osteomyelitis, giant-cell sarcoma, giant-cell tumour of epulis type, giant-cell granuloma, giant-cell pseudo-tumour, indicating that we have here one of the most debatable tumours in oncology. The element of trauma bulks very largely in the causation of this growth and these peculiar brownish-red tumours may be said to be constantly associated with hæmorrhage, which is followed by a characteristic neoplastic reaction on the part of the osteogenic cellular tissues and vessels. This reaction seems to be of a progressive nature, although usually showing some regressive or reparative character in the shape of collagenous fibrocytic tissue, cysts, bone formation and disappearing multinucleated cells. In the fully-developed active growth we find a close cellular admixture of two distinct cell types, with a scanty homogeneous or loosely fibrillar intercellular substance. The main tissue is composed of small cells with oval nucleus and

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tendency to fusiform character. The second cell-type of the tumour tissue is the more striking, although probably the more differentiated and therefore the less important of the two. It is the multinucleated giant cell which is scattered over the tumour area, sometimes regularly and discretely (Fig. 5), sometimes in patches only (Fig. 11). These cells give the impression of being remarkably benign plasmodia of osteoclast cell type ; they show numerous well-developed uniform nuclei, frequently with a defined nucleolus. The abundant, finely-granular, slightly eosinophile cytoplasm has short blunt processes which, in ordinary sections, become curtailed and lost in the matrix. The nuclei of the giant cells appear to be identical with those of the small cells forming the main mass of the tumour. Mitoses are rare, being found with difficulty in the small cells and rarely, if ever, in the giant cells. The blood supply of the growth is abundant and, in some cases, appears largely sinusoidal or even cavernous, with a thin endothelial wall, closely bordered by tumour cells. Massive or intercellular hæmorrhage is common and may be recent or old ; it is indicated, in many of these tumours, by the presence of phagocytic cells containing blood pigment. The true giant cells, however, do not contain hæmosiderin. It is common for the multinucleated giant cells to project into apparent capillary blood spaces (Figs. 2 and 3) or even to be free in their lumen. This is an important point, for it is a matter for discussion whether the giant cell is an endothelial cell, representing vascular endothelial budding, or a multinucleated incompletely-divided mesenchymal cell, or, as we believe, a giant bone-cell produced by fusion of small primitive osteogenic tissue cells, the function of which is osteoclastic. A close examination of our sections has suggested that there is much more new or potential bone present in these growths than is usually described. The sections are often cut without decalcification and this may account for the omission of significant reference to the osteo-formative character of the chief component cells. We have noted intercellular material and small irregular areas of non-calcified homogeneous or granular eosinophile tissue with or without fibrillar character scattered all over some sections (Fig. 27) ; sometimes this material lies in a retraction space which is lined by a cell layer of what we regard as osteoblasts. This substance may or may not contain bone cells ; we take it to be uncalcified bone or matrix at the pre-collagen or

pre-ossein stage. This point is an important one for the thesis of derivation of the tumour from osteogenic mesenchymal blastema.

Tumour cells and nuclei, when typical, are of very uniform size and shape, but there are cases with distinct irregularity and hyperchromatism, such as raise the suggestion of cellular hyperactivity and malignancy. This impression of malignancy is strengthened by the presence of mitoses in the matrix tumour cells ; such appearances are, however, not necessarily indicative of a bad prognosis.

Distinctions are not commonly emphasised between foreign-body giant cells and osteoclasts, but a distinction is usually drawn between the osteoclast, to which type we allocate the multinucleated tumour cell in question here, and the giant cell of malignant bone tumours. The latter is irregularly contoured, with hyperchromatic nuclei of irregular size and shape. This distinction is mainly correct but is not without exception, as multinucleated giant cells of osteoclast type may be found in osteogenic sarcoma. Typical giant cells of the giant-cell tumour of bone do not contain foreign inclusions, but this does not exclude them from belonging to the category of foreign-body giant cell. Perhaps they are " determined " to be more absorptive than phagocytic. Some giant-cell tumours may contain small patches of " foamy " cells (Fig. 25), but " xanthomatosis " is not at all a common feature of this tumour in bone, although it occurs frequently in the similar tumours of joints and tendon sheaths. There is, however, the condition of " essential xanthomatosis " in which very cellular bone reactions are outstanding. This seems to be a generalised metabolic disorder, but it is permissible to think of the " foamy " lipoid-containing cells as a development *in situ* of osteogenic or perhaps myeloid tissue in the direction of the embryonic fat cell rather than as the vagrant phagocyte.

An active typical giant-cell tumour of bone is a bone-resorptive growth but, as already indicated, osteoid new bone is met with in this tumour. Nor is the new bone simply the new bone of the reacting periosteum forming a crepitant capsule to the expanding tumour, as bone is not infrequently found in the midst of the growth. This new bone formation may be a regressive healing phenomenon comparable with fibrosis or a functional activity, but we have reason to remark on its presence. Tumours may be diagnosed as sarcoma

largely because of the presence of bone formation, especially in jaw tumours, and this was our own experience. Knowledge, however, that the bearer of such a lesion was still alive five and more years later led, in two instances, to a reversal of our original diagnosis in favour of a benign type of giant-cell tumour (Figs. 17 and 18). We must admit the idea of regression and repair in these tumours as factors of some importance and the rare cases of spontaneous cure therefore raise little difficulty of acceptance. Regression means fibrosis, bone formation instead of bone absorption, and disappearance of giant cells, a histological picture which may give rise to errors in diagnosis. Again, most of the classical descriptions of giant-cell tumours appeared before the discovery of the parathyroid hormone and the effects of hyperparathyroidism, vitamin D and intake of calcium upon calcium and phosphorus metabolism. We consider the giant-cell tumours of generalised osteitis fibrosa are connected with the parathyroid and vitamin dyscrasias evident in resorption of bone and osteoporosis, but only indirectly because the resultant bone lesions are favourable to the production of the giant-cell tumour by trauma. The tumour in that case may be no longer single and is, indeed, usually multiple. Bone cyst formation, with or without obvious hæmorrhage, is probably a late residual manifestation of trauma and tumour. Solitary giant-cell tumours occurring in the jaw after such a trauma as tooth extraction or in the epiphysis of a long bone are, when typical, resorptive of bone. They remove bone up to the periosteum, which reacts to produce a thin capsular shell of new bone. This new bone is absorbed and the periosteal capsule is further distended, with further production of new bone. It is this continuous absorption which gives the special diagnostic character of honey-comb ("soap-bubble") porosity to the radiographic picture of these growths (Fig. 4). In untreated tumours, the resistance of the periosteum to rupture by this continued expansion is remarkable and by its long duration practically makes them benign and non-metastatic. Articular cartilage is even more resistant to their advance than periosteum.

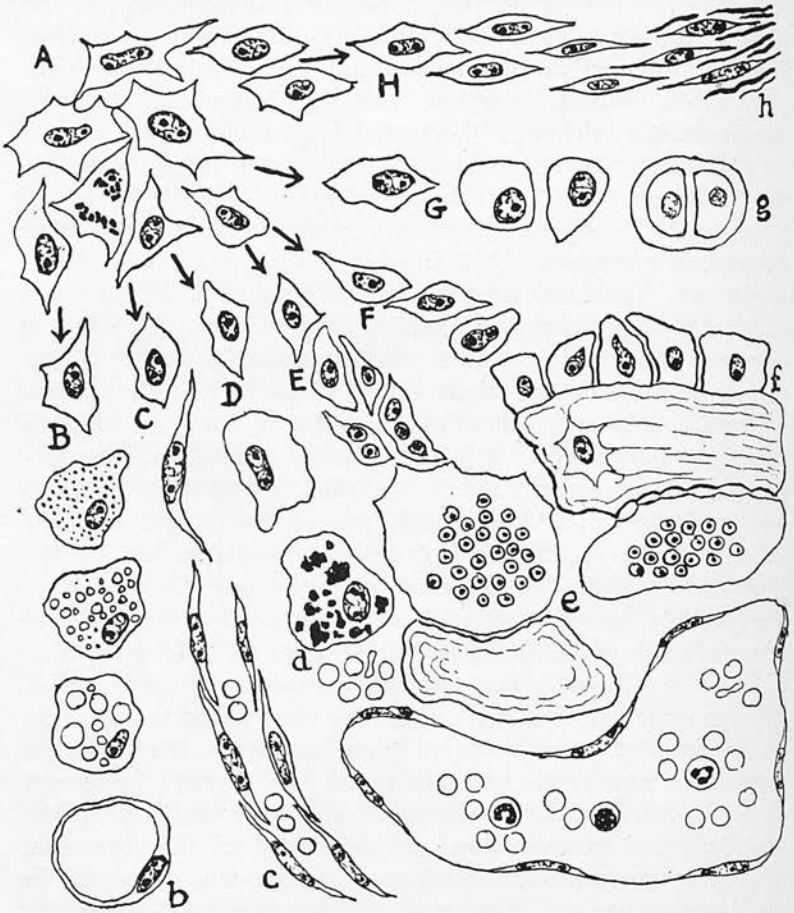
The tumour material from bone available for our examination totals 117 cases, of which 115 were human.

Discussion.—There are many views to be harmonised in a discussion of the nature, development and end-results of the giant-cell tumour of bone. Is it a granulation tissue or an

" endothelial sponge " or a true neoplasm ? Is it composed of primarily osteogenic tissue, myelogenous tissue or vascular tissue ? Is it wholly benign or is it sometimes primarily malignant, or does malignancy arise by transformation in the benign tumour ? Is it a localised condition only or may it have a systemic causation such as would link it up with generalised osteitis fibrosa, osteitis deformans (Paget), osteomalacia, rickets, " essential xanthomatosis," etc. ? Similar questions are relevant to giant-cell tumours of the tendon sheaths and synovial membranes. The diversity of views seems to be best unified by regarding the more primitive component cell types of the tumour as fibroblast, reticuloblast, angioblast, lipoblast, chondroblast, osteoblast and osteoclast respectively, proliferating continuously, though in varying degree, and taking origin in a mesenchymal cellular primordium. The prototype cell is then the primitive pluripotential cell, with determination to a type of growth represented by fibropoiesis, angiopoiesis, lipopoiesis, chondropoiesis, osteopoiesis and osteoclasis. Determination to hæmopoiesis, which is of course one of the potencies of the primitive cell, is excluded from our consideration of the giant-cell tumour. In the final grade of cell determination, pluripotency becomes reduced to unipotency. Determination of matrix substance has been shown to go with determination of its cells. There is undoubtedly a vascular element in this tumour entity, which has given rise to discussion as to its origin from a vascular foundation and even made of the prominent multinucleated giant cell a littoral, overgrown vascular and endothelial element. We prefer to look on the vascularity of these tumours as incidental, of the sinusoidal and cavernous type, and analogous to that which prevails in the bone marrow. This type of vascularity is essentially arteriovenous as distinct from that in which there is the intervention of a definitely capillary system.

There are many giant-cell tumours in which osteoid bone formation is quite prominent. The explanation which we give of this phenomenon is that the essential tissue of the tumour is osteogenic cellular primordium, although decalcification is the prominent feature of the active growth. The general conception of the cellular morphology of the tumour may best be rendered by a diagrammatic sketch which goes back to the pluripotential progenitor cell and sets out all the cells

concerned in the giant-cell tumour. The only "determination" not depicted in the sketch is the myeloblastic, for the giant-cell tumour is not myeloid or myelomatous, even though



Showing various stages of the "determination" of the primitive mesenchyme cells from which recognisable giant-cell tumour elements are derived. Primitive mesenchyme, A; lipoblast, B, to lipocyte, *b*; angioblast, C, to angiocyte (littoral endothelium), *c*; reticuloblast, D, to reticulocyte (macrophage), *d*; "pre-osteoclast," E, to osteoclast, *e*, absorbing bone; osteoblast, F, to osteocyte, *f*, forming bone; chondroblast, G, to chondrocyte, *g*; fibroblast, H, to fibrocyte, *h*, with collagen fibres. Hæmocytoblast to myeloid blood cells not indicated.

we do conceive of both bone and bone-marrow arising from the same primordium.

This figure is explanatory of our view that the giant-cell

tumour of bone is a slow-growing, mainly osteogenic tissue. The tumour itself emerges as a neoplastic osteodystrophy to which the terms bone-absorbing, bone-forming, hæmorrhagic, lipoid and fibrous have all been applied. A phase of the tumour may very possibly initiate the ordinary osteogenic sarcoma but, if so, the phase is rarely observed microscopically. *This view implies, however, that both giant-cell tumour of bone and osteogenic sarcoma are tumours of osteogenic tissue.*

We must, in a discussion of the giant-cell tumour, give special heed to the contention that it is only a simple inflammatory reaction to trauma, to hæmorrhage, in fact a "granuloma." This view might imply that the lesion is no tumour at all but only cellular granulation tissue in a bone environment. We do not think that such an explanation offers insuperable difficulties of some acceptance. If we are prepared to uphold that the growth known as granulation tissue in the broadest sense is on a par with tumour growth, then the point almost becomes a distinction without a difference. Granulation tissue ("granuloma") is of course benign and in due course recessive. It may be regarded as a tissue culture of cells in a suitable medium and as having developed by "induction" through response to trauma. The "granuloma" is thus entitled to its termination "oma," as being the simplest type of neoplasia. Reaction tissue of any sort is, in this sense, neoplastic ; a hæmorrhage undergoing organisation, a fibrosing or ossifying "hæmatoma," is also a tumour type of tissue. This decision whether the giant-cell tumour is granulation tissue or neoplasm is, therefore, waived by taking both of these states as forms or phases of new growth. There is probably persistent in the adult an abundance of embryonal mesenchymal and epithelial tissues, with all the original capabilities of differentiation and it is this "residue" or "rest" which is concerned with the production of granulation tissue, reparative tissue and tumour tissue. We owe this conception, in part at least, to Cohnheim and to Ribbert. In bone, such "residue" tissue persists to some degree in all parts of all bones, but this persistence is most marked in the epiphyseal and metaphyseal regions, a fact which may explain the more frequent occurrence of solitary giant-cell tumour in adolescents and young adults in this situation. The views which make giant-cell tumour of bone a simple reaction and a true tumour respectively are thus reconciled, but the contention which regards recurrence

or metastasis as due to a change of cellular character, explained by therapeutic interference or by an original error of diagnosis, still requires our consideration. We regard the giant-cell tumour as osteogenic, highly-reactive tissue, of benign character. Interference or other disturbing conditions, however, may be factors in the development of greater cellularity and neoplastic activity, with ultimate malignancy and metastasis. The implication of ultimate acquirement of malignant character by a benign tumour applies to many tissues and has been touched on in our previous studies of lymphosarcoma and salivary gland adenoma. Not only is this implication also a feature of the " benign " giant-cell tumour of bone, tendon sheath and joint, but there is the further suggestion made that neither the " granuloma " nor the metabolic and hormonal dystrophies are capable of rigid exclusion from the category of neoplasms. There seems little necessity to resort to an originally wrong diagnosis in explanation of the occurrence of typical giant-cell tumour metastasis, if it be admitted that metastasis is probably such a late development that its possibility is usually excluded by early and adequate removal of the growth. The statement that giant-cell tumour of bone has never been seen in metastatic deposits in the lung is interesting in connection with a tumour in our series, in which such a growth did occur (Figs. 40-45).

Lastly, there is the relationship of giant-cell tumour to osteitis fibrosa. The solitary tumour is not to be regarded, we think, as " localised osteitis fibrosa." It has been shown that in cases of solitary tumour there is no hypercalcæmia and no rise of the calcium-phosphorus ratio, such as occurs in the generalised osteitis fibrosa of hyperparathyroidism, nor is there any sign of osteoporosis in bone elsewhere than in the locality of the lesion itself. We would rather maintain that the systemic parathyroid condition which brings about the depletion of calcium from its storage deposit in the bones renders those bones more liable to trauma and thus to multiple reactions of the type present in giant-cell tumour—that is, to the occurrence of hæmorrhage and the formation of multiple giant-cell tumours of bone.

It seems to us that no better example could be found for the discussion of all that is involved in the concepts " neoplasm " and malignancy than the giant-cell tumour. There is abundant evidence also in the discussion of this

tumour type for the importance of the co-operation of the histologist with the surgeon and the radiologist. The haunting fear of harm due to biopsy should, we think, be firmly countered. It is essential to have adequate material for examination from both centre and periphery of the new growth.

We have purposely eliminated frequent reference in the text to the illustrations, as the legends are intended to amplify description and discussion.

Conclusion.—Giant-cell tumour of bone is a neoplastic growth, probably originating with trauma and hæmorrhage and developing as an osteogenic tissue reaction and proliferation, which may slowly progress and ultimately regress, but which, in rare cases, acquires the characters of an osteogenic sarcoma. It may be classed as a type of new growth intermediate between purely reactive tissue and true blastoma.

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(*To be continued.*)

Debatable Tumours : Giant-Cell Tumour of Bone

NOTES, CLINICAL AND HISTOLOGICAL, ON ILLUSTRATIONS.

FIG. 1.—*Giant-cell tumour of bone.* Head of humerus. Male, aged 17. "Increasing difficulty with abduction of shoulder, 6 months; fall on shoulder with much pain and discovery of tumour. Excision of upper part of humerus, with fibula graft. Alive and well 8 years." Showing tumour occupying epiphyseal and metaphyseal regions. Disappearance of epiphyseal cartilage; articular cartilage and subjacent bone intact. Note definition of tumour with characteristic club-shaped appearance. Small cysts present; larger ones at other levels. Beginning down growth into marrow of shaft. Large section of decalcified bone. 9716/30. ×⅔.

FIG. 2.—*Giant-cell tumour of bone.* Head of humerus. Same case as in Fig. 1. Showing discrete multinucleated giant cells of osteoclast type in a cellular matrix. 9716/30. ×100.

FIG. 3.—*Giant-cell tumour of bone.* Head of humerus. Same case as in Figs. 1 and 2. Showing multinucleated giant cells projecting into apparent spaces usually regarded as vascular, but which we interpret as probably structureless, non-calcified osteomucin (*a*), with bone cells of osteoclast and osteoblast type attached. A definite capillary (*b*) is also shown. 9716/30. ×250.

FIG. 4.—*Giant-cell tumour of bone.* Radiograph of tumour in head of humerus shown in Figs. 1, 2 and 3. Showing typical club-shaped enlargement and "soap-bubble" appearance.

FIG. 5.—*Giant-cell tumour of bone.* Tibia. Male, aged 45. "Tumour of tibia protruding as rounded swelling between lower ends of tibia and fibula. Pain 8 months. Reddish-purple on surface and slightly nodular. No cysts evident. Suggested either giant-cell tumour or sarcoma." Showing discrete distribution of numerous multinucleated giant cells, some vacuolated, in a uniform matrix of uninucleated cells. 8972/36. ×70.

FIG. 6.—*Giant-cell tumour of bone.* Same tissue as in Fig. 5. Showing large plasmodial multinucleated giant cells, with oval, uniform, discrete nuclei. Some of these cells show cytoplasmic vacuoles, probably degenerative with retraction. The small polymorphic matrix cells we regard as the true tumour cells. No mitoses evident. A reticulum stain (Wilder) shows abundant argyrophile fibrils between the matrix cells of the tumour and forming a capsule-like framework around the giant cells. 8972/36. ×250.

FIG. 7.—*Giant-cell tumour of bone.* Jaw. Dog. Showing a group of separate multinucleated giant cells of osteoclast type surrounded by tumour cells of fusiform character. 6841/29. I.A.P. ×150.

FIG. 8.—*Giant-cell tumour of bone.* Mandible. Goat, aged 3 years. "Mandibular tumour, osteitis fibrosa?" Showing what may be a regressing giant-cell tumour in one of its sites of election. 6846/29. I.A.P. ×150

FIG. 9.—*Giant-cell tumour of bone.* Jaw. Male. Showing the formation of multinucleated giant cells by fusion of uninucleated primordial cells. 6842/29. I.A.P. ×250.

FIG. 10.—*Giant-cell tumour of bone.* Spine. Female, aged 13. "Occasional stabbing pain at foot of spine for 11 months. General health good. X-ray report benign giant-cell tumour." Showing disintegration and disappearance of multinucleated giant cells in a regressing tumour. F.U.D. 20. ×250.

FIG. 11.—*Giant-cell tumour of bone.* Mandible. Female, aged 12. "Bony tumour of lower jaw; swelling and pain 6 months. Sarcoma? dentigerous cyst? On scraping, a good bony wall was found almost all round tumour." Selected to show patchy distribution of giant-cell areas in the tumour tissue. 4160/35. ×40.

FIG. 12.—*Giant-cell tumour of bone.* Jaw. Showing very fibrous character and few, small, disappearing giant cells. C.G. 18. ×90.

FIG. 13.—*Giant-cell tumour of bone.* Mandible. Female, aged 43. "Small tumour from alveolus in edentulous mouth. Noticed 6 months." Showing rather small multinucleated giant cells in a fusiform-cell, fasciculated matrix. Production of new bone with surrounding osteoblasts and enclosed bone cells (*a*). These appearances suggest a regressive (reparative) phase of the tumour. The traumatic factor of removal both of deciduous and permanent teeth is often mentioned in connection with the origin of these jaw tumours. 3367/34. ×150.

FIG. 14.—*Giant-cell tumour of bone.* Mandible. Showing elongated spindle tumour cells and an area of fibrous "woven" bone. This tumour showed so few and insignificant multinucleated giant cells that it may stand for the type which is examined at the stage when they have disappeared in the process of regression. 9907/33. ×150.

FIG. 15.—*Giant-cell tumour of tendon sheath.* Finger. Female, aged 55. "Duration 1 year. Fibroma?" Showing multinucleated giant cells, irregular in size and shape, in a cellular matrix. These tendon sheath tumours are often highly "xanthomatous" (lipoid reticulosis) in contrast to the bone tumours. Some authors would make the giant-cell tumours of tendon a derivative from sesamoid bone. 6271/35. ×150.

FIG. 16.—*Generalised osteitis fibrosa.* Female, aged 56. "Swelling upper part of tibia for some months. Biopsy, reported as 'giant-cell sarcoma.' Diagnosed radiographically osteitis fibrosa because of multiple lesions. Blood calcium 14 mgm. per 100 c.c. Parathyroid tumour removed with improvement in health and fall of blood calcium to normal." Showing typical multinucleated giant-cell tumour area adjacent to well-formed bone with osteoblasts (*a*). 8997/33. ×80.

FIG. 17.—*Giant-cell tumour of bone.* Maxilla. Female, aged 4. "History of removal of small stone from nose 2 years before swelling developed in maxilla, bulging of canine fossa and floor of nose and lachrymation. Clinical diagnosis malignant." Original pathological diagnosis of osteogenic sarcoma revised on re-examination 12 years later, when patient reported alive and well. No further trouble in tumour area 7 years later still. Showing much active bone formation, few multinucleated giant-cells and fibrous stroma. F.U.D. 1125. ×100.

FIG. 18.—*Giant-cell tumour of bone.* Maxilla. Female, aged 11. "Tumour of maxillary antrum with swelling of cheek for at least 2 months. No invasion of palate or nose. Malignant? Odontoma?" Original diagnosis of osteogenic sarcoma revised on re-examination 6 years later, when patient reported alive and well. Patient still well 9 years after removal. Showing numerous islets of imperfect bone, some surrounded by a definite layer of osteoblasts (*a*). The tumour tissue was highly cellular but had few mitoses. The hæmosiderin so frequently found in these tumours is present in large amount (*b*). 8004/29. ×250.

FIG. 19.—*Giant-cell tumour of bone.* Fibula. Female, aged 22. "Tumour from just below head of fibula. No symptoms. Tumour discovered accidentally while being X-rayed for foreign body." Paraffin section of head of fibula, stained H. and E. Showing tumour in epiphyseal and metaphyseal area. Replacement of bone and epiphyseal cartilage (union not until 25 years) by tumour with cyst formation and thinning of compact bone externally. 6612/32. ×3/4.

FIG. 20.—*Giant-cell tumour of bone.* Fibula. Same case as in Fig. 19. Showing tumour composed of fibrous and lipoid-cell ("xanthomatous") areas replacing epiphyseal cartilage, bone and marrow. Other areas also showed sparse disintegrating multinucleated giant cells of osteoclast type. This picture affords support for the view that giant-cell tumour is an osteogenic tissue reaction to trauma and may undergo spontaneous regression with development of scar tissue, bone cysts and "foam" cells on their way to become fat cells of marrow. 6612/32. ×80.

FIG. 21.—*Giant-cell tumour of bone.* Same tissue as in Figs. 19 and 20. Showing fibrous and lipoid ("xanthomatous") areas and bone (*a*). 6612/32. ×250.

FIG. 22.—*Gaucher's disease of bone.* The interest of this condition, a constitutional familial metabolic lipoid dyscrasia, lies in its involvement of bone, its "xanthomatous" appearance, the occasional presence of multinucleated giant cells and the occurrence of hæmosiderin. All these features may be found in the giant-cell tumour and provide evidence, with other features, in favour of a granulomatous osteogenic tissue reaction. Showing closely-packed lipoid-containing "foam" cells replacing bone-marrow up to the compact bone. 8986/33. ×150. Section donated by Prof. Pick, Berlin.

FIG. 23.—*Niemann-Pick's disease.* A congenital metabolic disease which has much in common with Gaucher's disease. Showing the "foam" cells stained with osmic acid. 8986/33. ×150. Section donated by Prof. Pick, Berlin.

FIG. 24.—*Hand-Schüller-Christian disease of bone.* "Forming scapular tumour in a child." A congenital familial metabolic disorder, another of the "essential xanthomatoses." Showing a very mixed cell picture with giant cells with ingested polymorphs, numerous eosinophils and "foam" cells, which are said, in this disease, to contain neither phosphatide nor kerasin, but almost entirely cholesterol. 8375/33. ×130.

FIG. 25.—*Giant-cell tumour of bone.* Femur. Male, aged 26. "Nine months' pain lateral side of knee joint, with restriction of movement; bony outline normal. Radiographically and histologically giant-cell tumour." Showing an area of fat-containing ("xanthomatous") cells, an unusual occurrence in the bone tumours as contrasted with the tendon sheath and synovial tumours. F.U.D. 18. ×150.

FIG. 26.—*Myositis ossificans.* The case was a typical one. This area shows a fragment of well-formed fibrous bone on left and, on right, muscle bundles in a fibroblastic stroma. 4580/31. ×90.

FIG. 27.—*Giant-cell tumour of bone.* Jaw. Showing scattered multinucleated giant cells in a fibrocellular tissue which contains throughout an ill-defined matrix of fibrous and granular bone. C.G. 17. ×100.

FIG. 28.—*Giant-cell types.* (*a*) From giant-cell tumour. Showing multinucleated cell with discrete uniform oval nuclei, nucleoli and a vacuole. No obvious cytoplasmic processes and no cell inclusions. ×500. (*b*) From tubercle follicle. Showing multinucleated cell and elongated peripherally situated oval nuclei with long axis directed towards centre and nucleoli. The cytoplasm is faintly eosinophile and has the granular character of caseation necrosis. Deviations from the classical "Langhans' cell" are frequent in tuberculous lesions. ×500. (*c*) From a case of Hand-Schüller-Christian disease. Showing multinucleated cell and phagocytosis of polymorphs; cell nuclei obscured. Illustrating the type of giant cell which is manifestly phagocytic. ×500. (*d*) From cellular bone-marrow. Showing a megakaryocyte with multilobed (not multinucleated) basket nucleus. ×500. (*e*) From bone tumour. Showing multinucleated malignant giant cell with few but large hyperchromatic nuclei. ×500.

FIG. 29.—*Foreign-body giant cells.* (*a*) Injection of Kieselgur. Reaction in subcutaneous tissue represented by many giant cells containing diatoms. 4897/38. ×150. (*b*) Injection of calcium phosphate, showing small giant cells and reaction in subcutaneous tissue. 4897/38. ×150. (*c*) Injection of hæmoglobin, showing giant cell with crescent-shaped hæmoglobin. 4897/38. ×150. (*d*) Cholesterol deposit. Multinucleated giant cells surrounding cholesterol crystals in wall of gall-bladder. (*e*) Injection of paraffin. Injected after laparotomy to prevent adhesions; specimen removed 3 years later. Showing giant cells around paraffin in omentum. 7242/32. ×150.

FIG. 30.—*Experimental fracture.* Femur of rabbit, 17 days. Showing callus formation around the fracture. Such callus frequently exhibits many of the cell elements of primitive osteogenic tissue and has a resemblance to the benign forms of giant-cell tumour. ×8. Material lent by Mr R. I. Stirling, F.R.C.S.Ed.

FIG. 31.—*Experimental fracture.* Same tissue as in Fig. 30. Showing the cartilaginous and fibrous elements of bone tissue reaction in proximity to muscle. ×100.

FIG. 32.—*Osteogenic sarcoma.* Showing highly cellular endosteal tumour composed of polyhedral cells with large nuclei and scanty cytoplasm; many mitoses (*a*). 997/37. ×150.

FIG. 33.—*Giant-cell tumour of bone.* Metastasis in rib. Male, aged 65. Primary tumour in femur after trauma. Death in 1 year with metastases in lungs, diaphragm and ribs. Showing polymorphic malignant large matrix cells, hyperchromatism, mitoses and a multinucleated cell of malignant type. 28/266. I.A.P. ×350. (By courtesy of Dr R. Williamson, Pathology Department, University of Cambridge.)

FIG. 34.—*Giant-cell tumour in muscle.* Male, aged 32. "Tumour from within vastus medialis; 3 months' increase in size. No history of trauma obtainable. No connection with femur or periosteum. Reported elsewhere as myeloma." Showing a typical giant cell tumour area with multinucleated cells bordering on a large hæmorrhage (*a*); adjacent bone formation (*b*). 4345/35. ×40.

FIG. 35.—*Giant-cell tumour in muscle.* Same case as in Fig. 34. Showing benign (*a*) and malignant (*b*) types of giant cell and bone formation (*c*). 4345/35. ×350.

FIG. 36.—*Giant-cell tumour in breast.* Female, aged 43. Large hard tumour in a fatty breast, clinically malignant; radical removal. Patient alive 3 years 4 months later. Showing defined cystic tumour in fatty tissue without attachment to deep muscle or remains of *corpus mammae*. Many of the tumour cysts were filled with blood, giving the characteristic appearance of the giant-cell tumour in bone. Wash-drawing of sectioned tissue. 3938/35. ×$\frac{1}{8}$.

FIG. 37.—*Giant-cell tumour in breast.* Same case as in Fig. 36. Showing giant-cell tumour area bordering on a hæmorrhage. 3938/35. ×80.

FIG. 38.—*Giant-cell tumour in breast.* Same case as in Figs. 36 and 37. Showing multinucleated giant cells in a fibrous matrix and newly-formed bone surrounded by osteoblasts. 3938/35. ×100.

FIG. 39.—*Giant-cell tumour in breast.* Same case as in Figs. 36, 37 and 38. Showing benign (*a*) and malignant (*b*) types of multinucleated giant cells. 3938/35. ×250.

FIG. 40.—*Giant-cell tumour of bone.* Female. "At age 29, exostosis removed from upper part of humerus shaft. Fifteen years later, swelling reappeared at site of removal, which at first grew slowly and then rapidly. The tumour when examined at age 50 was fixed to bone but not to skin." The first biopsy tissue (Fig. 41) showed what was regarded as typical giant-cell tumour of bone, although the situation was unusual. A second biopsy showed considerable cell polymorphism. A third biopsy (Fig. 42) still showed numerous typical multinucleated giant cells, but the growth at this stage must be regarded as sarcomatous. Seven months later, death at age 51 (total duration 22 years), with extensive pulmonary metastases in both lungs (Fig. 43) but none in other bones, brain, liver or kidneys. Instances such as this metastasis from true giant-cell tumour are described in the literature (Leitch, Muir, Finch and Gleave, Dyke, Orr, etc.) and must be admitted without necessary resort to a judgment of "wrong initial diagnosis." They are greatly disturbing because of their unexpectedness and fatal issue. The illustration shows tumour (*a*) at age 50. 4544/35. ×$\frac{1}{4}$.

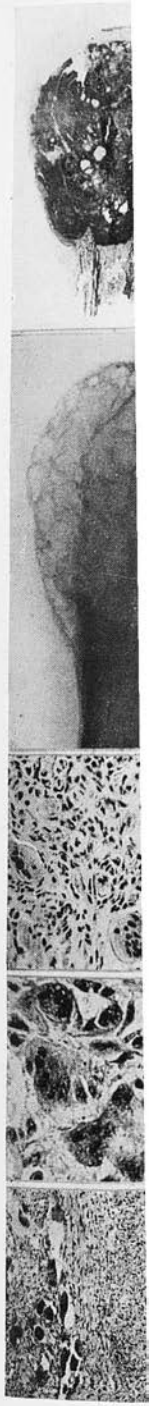
FIG. 41.—*Giant-cell tumour of bone.* Humerus. Case shown in Fig. 40. First biopsy tissue showing typical giant-cell tumour area with rather fibrous matrix. 4517/35. ×250.

FIG. 42.—*Giant-cell tumour of bone.* Humerus. Case shown in Fig. 40. Third biopsy tissue showing marked polymorphism of matrix tumour cells, some malignant multinucleated giant cells (*a*) and scattered non-malignant giant cells. There were numerous mitoses present and the tissue at this stage must be regarded as sarcomatous. 4544/35. ×250.

FIG. 43.—*Giant-cell tumour of bone.* Metastasis in left lung. Same case as in Figs. 40, 41 and 42. Showing a massive well-defined single tumour area extending obliquely from the base almost to the apex. 5022/35. ×$\frac{1}{4}$.

FIG. 44.—*Giant-cell tumour of bone.* Lung metastasis. Same case as in Figs. 40, 41, 42 and 43. Showing lung parenchyma (*a*); tumour tissue with border of giant cells (*b*). 5022/35. ×40.

FIG. 45.—*Giant-cell tumour of bone.* Lung metastasis. Same case as in Figs. 40, 41, 42, 43 and 44. Showing typical multinucleated giant cells, active tumour matrix cells of fusiform character, few mitoses and much osteoid bone formation. 5022/35. ×150.



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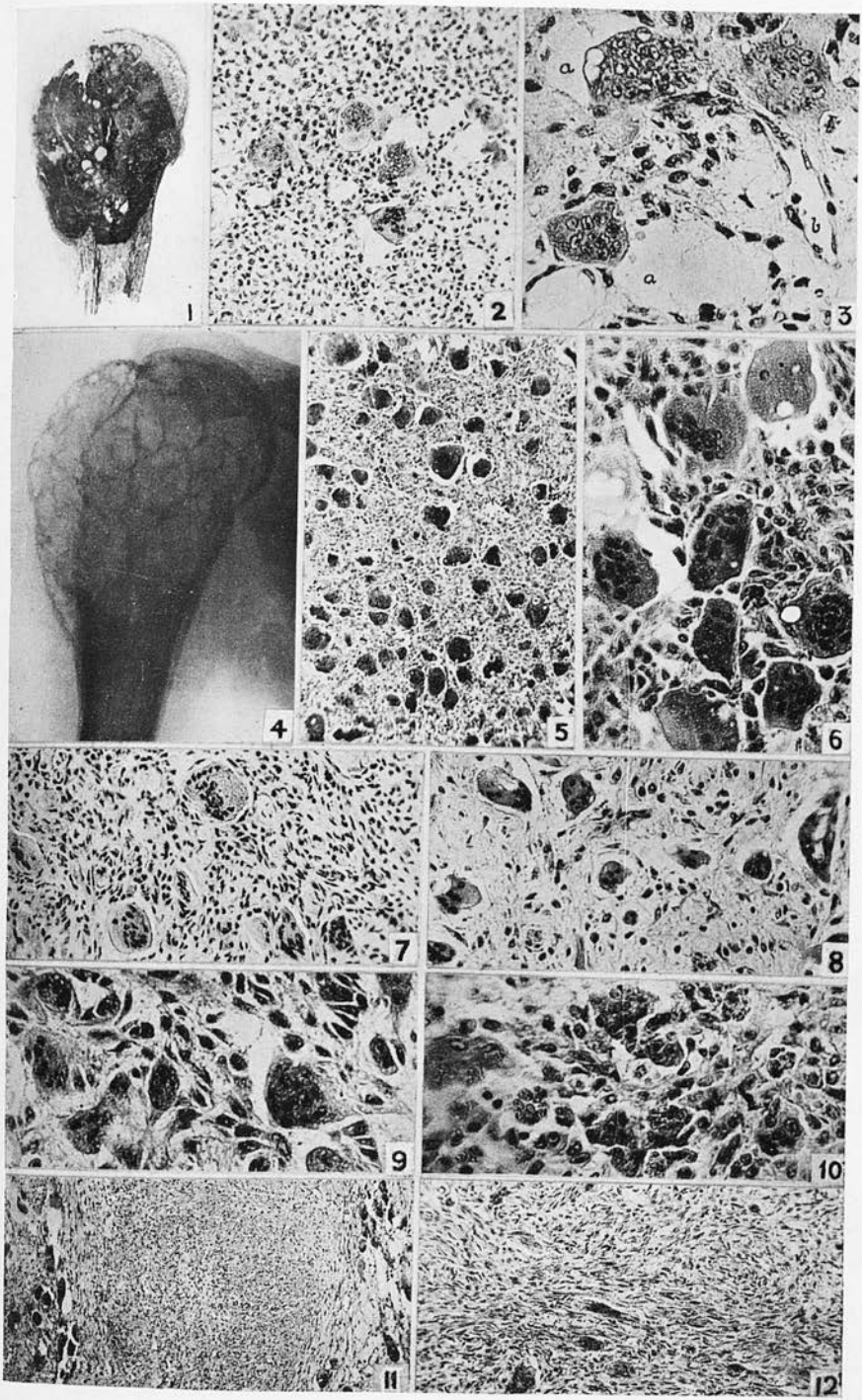

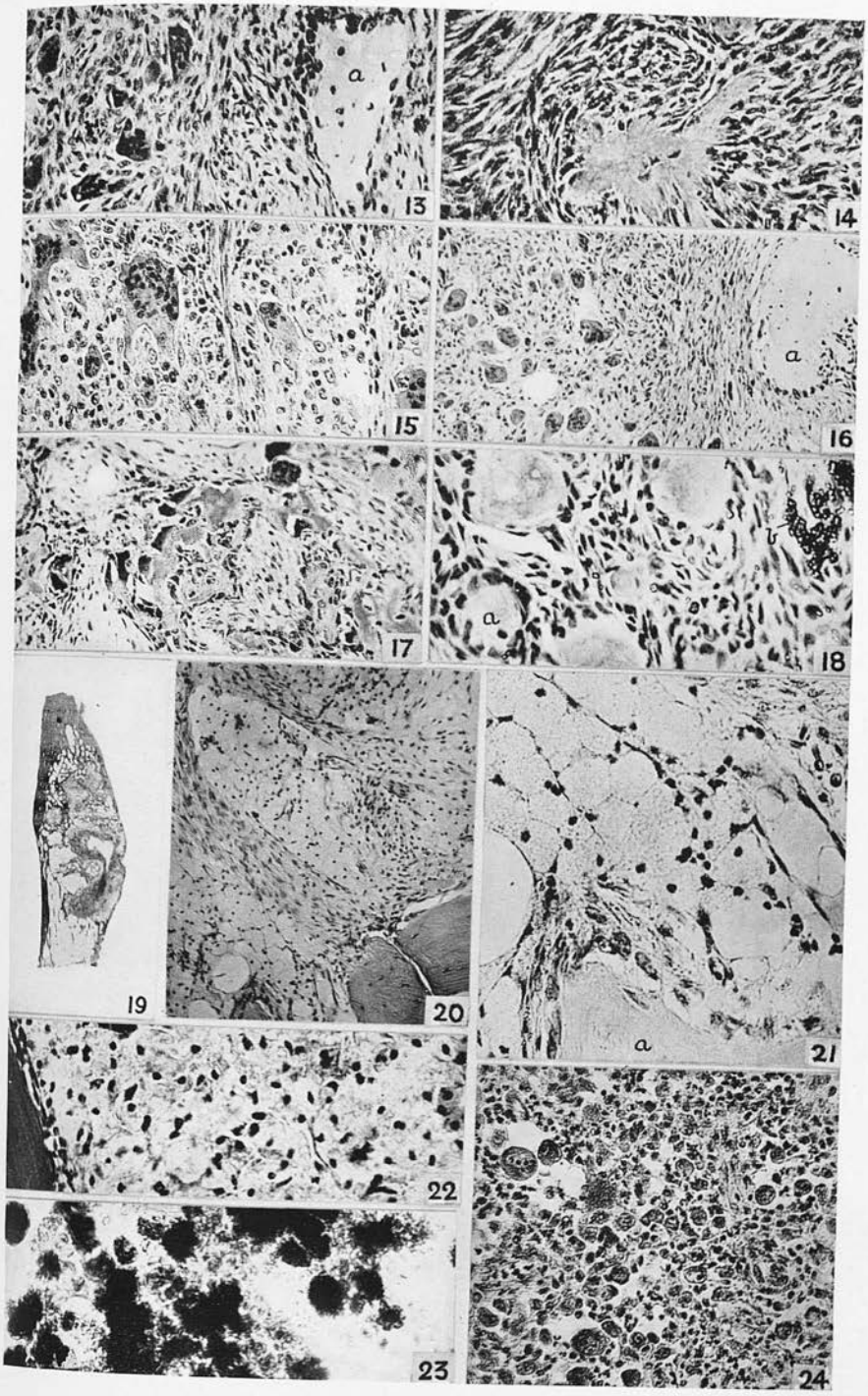
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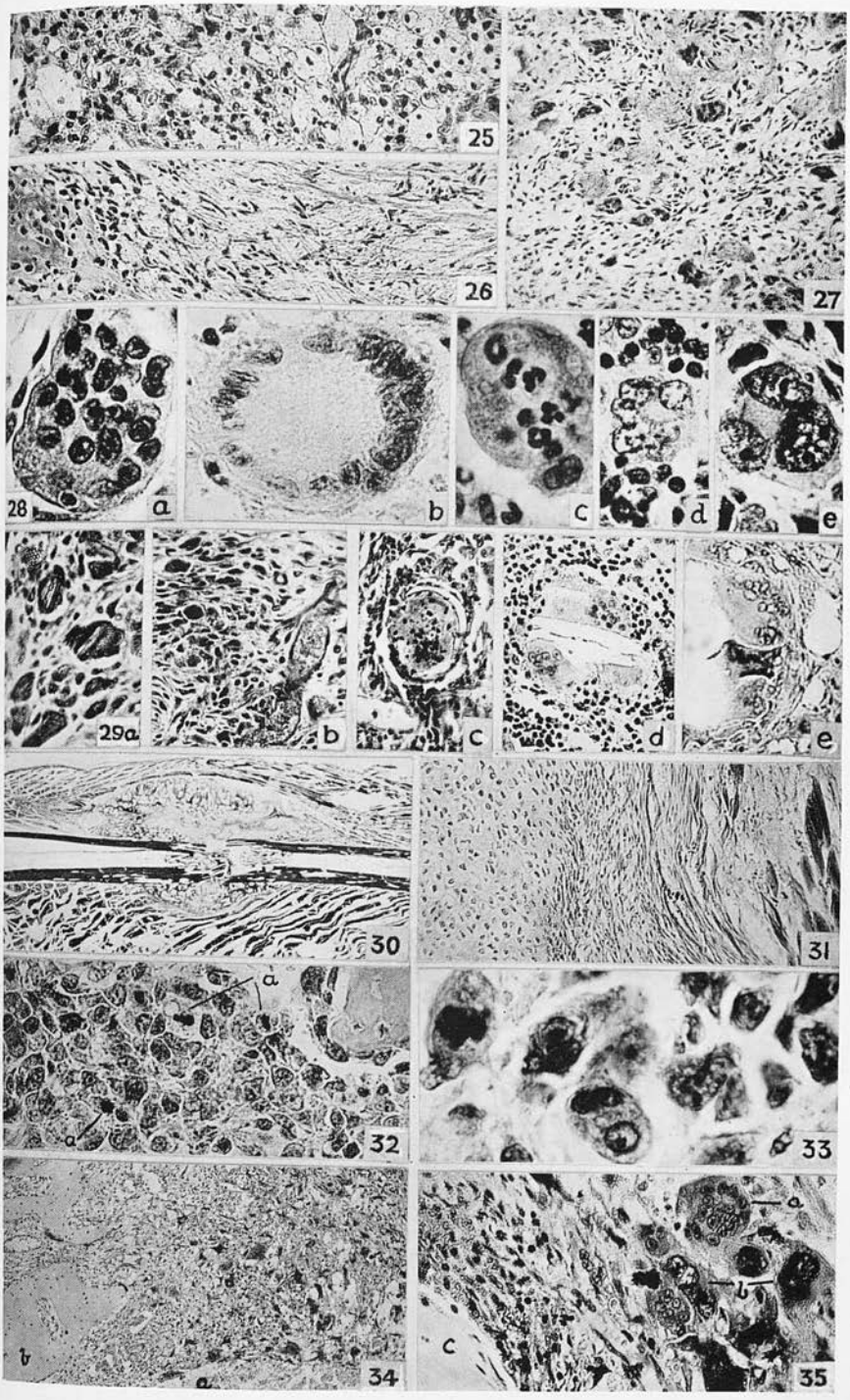
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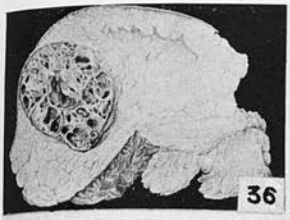
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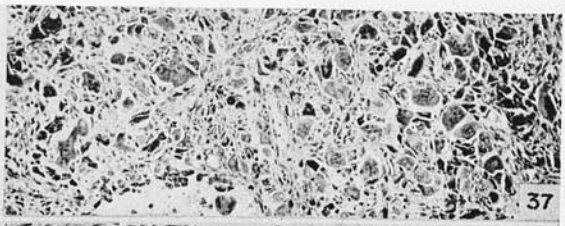
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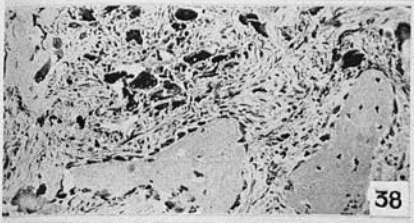
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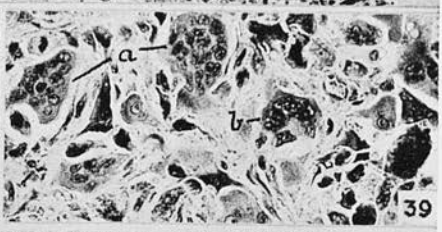

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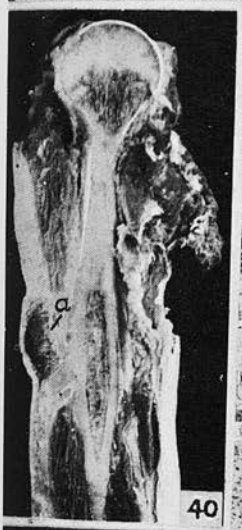

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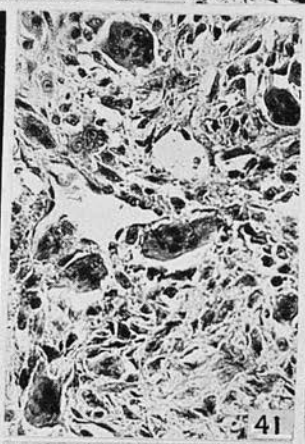
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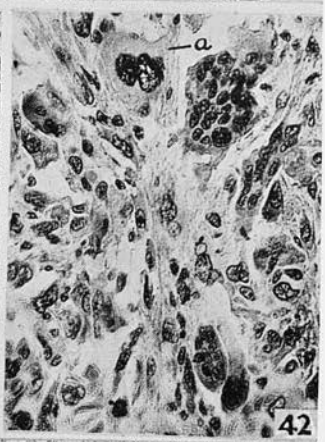

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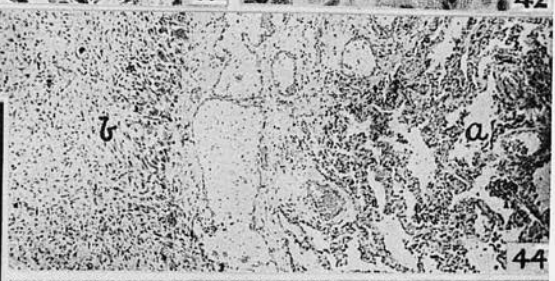

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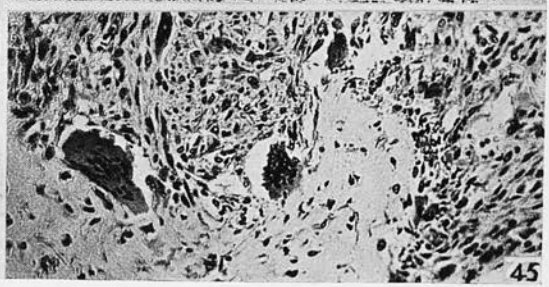
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DEBATABLE TUMOURS IN HUMAN AND ANIMAL PATHOLOGY.

VI. Meningioma.

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DEBATABLE TUMOURS IN HUMAN AND ANIMAL PATHOLOGY.

VI. Meningioma.*

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Definition.—The meningioma is a tumour chiefly of adult life, usually attached to the dura mater and exhibiting a characteristic whorled or stream-lined arrangement of tumour cells with little or no intercellular supporting substance. It is essentially benign and in its expansion presses into the brain tissue without infiltration.

Description.—The history of the nomenclature of the meningioma is illuminating, as it shows how ideas regarding its origin have evolved. It has been called tumeur cancéreuse des méninges (Cruveilhier, 1835), myeloid tumour (Paget), psammoma (Virchow), endothelioma (Golgi), cylindroma (Billroth), neuro-epithelioma (Roussy and Cornil), epithelioma (Robin), dural endothelioma, arachnoid or meningeal fibroblastoma (Mallory, Penfield), meningioma (Cushing), meningoblastoma (Oberling), and exotelioma (Río-Hortega).

Meningiomas are clinically usually dural tumours, although they probably arise from a primitive cell-type persisting in the adult and common to both dura mater and pia-arachnoid. The dural attachment is firm, broad, or slender and may even consist of only a few vascular strands. Its vascular supply is dural and very abundant. In their growth these tumours are expansile, depressing the underlying brain (Fig. 2), but not penetrating the pia mater. Thus separation of the tumour from the brain can be effected, though hæmorrhage is often considerable. A certain amount of penetration may take place into the cancellous marrow spaces of the overlying bone and the bone may undergo pressure atrophy and erosion. This penetration is not so much invasion of bone as expansion along the lines of least resistance, that is, the marrow spaces. Another consequence of the involvement of bone may be stimulation

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of the more peripheral bone with development of extracranial hyperostosis (hemicraniosis), an appearance which may be very characteristic for the radiograph (Fig. 34). A somewhat restricted localisation of the meningiomas to the regions where pacchionian or arachnoid granulations are specially distributed in and adjacent to the intradural venous sinuses and lacunæ has been frequently noted; these are, in particular, the sagittal, transverse, sphenoparietal, and cavernous sinuses. The tumours are found also in relation to the falx cerebri, the meningeal vessels, the sheath of the optic nerve, the olfactory groove and, perhaps, to the periosteum of the orbit which is continuous with the dura mater. Although they arise mainly from the meninges over the cerebral hemispheres, they may also occur anywhere throughout the length of the spinal cord and may even be extradural in position. The arachnoidal granulations or villi are essentially arachnoidal tissue; they are macroscopically most conspicuous in certain definite regions where they bring the cerebrospinal fluid into intimate relation with the venous sinus system (Fig. 21). Microscopically, they are more widely distributed still and may be mainly cellular plaques. The intracranial tumours tend to be larger than those of the spinal cord. Size, however, is very variable and may range from microscopical growths to tumours weighing as much as 400 grammes. Meningiomas are benign tumours and though they may recur they are said never to metastasise. The rare malignant forms are characterised by a more rapid growth and a greater cellularity of more primitive cell-type.

The microscopical picture of the meningioma is very typical and, as a rule, easy to diagnose (Figs. 4, 5, 6, 7). These tumours are composed of elongated cells which, if compressed, are long and narrow, and if rapidly and freely growing, are large-bodied, with finely-chromatic, large nucleus, and pale eosinophile abundant cytoplasm (Fig. 4). In architecture they are characteristic, for the component cells arrange themselves in concentrically tunicated spheres and whorls or in streamlined, fasciculated bands. On the whole, there is little stroma between these whorls and bands, except that around the nutrient blood vessels of the trabecular scaffolding (Figs. 29, 30). A striking feature of the tumour is the whorling; it may indeed be said that if the nature of these whorls could be settled, so would the origin and nomenclature of the tumour. The position is much the same for the argyrophile sheath of Schwann and its derivation from neurilemma cell or fibroblast.

Are these whorls mesodermic or are they neuroectodermic sensory end-organ structures like the Wagner-Meissner or Pacinian corpuscles ? A whorl may show in the centre one or two hyaline degenerated cells or, more rarely, what is described as a blood capillary. In the fully-developed whorl the tumour cells are arranged in an imbricated or stratified manner and, if calcification occurs, there results a " psammoma " body. The tumour indeed was originally called a psammoma. Such calcified spherical bodies are usually isolated but may occasionally be so numerous as to stud the whole field (Fig. 7). The common form of tumour is cellular, may contain an occasional mitosis, and shows few calcospherites (psammoma bodies), though there is hyalinisation of tumour cells. A fibro-vascular stroma tissue, quite distinct from the tumour cells, is easily demonstrable (Figs. 29, 30). The presence of specially dense collagenous tissue, a common feature, is usually original dura and not fibrosis (Fig. 9). Degenerative changes may take place in tumour cells of a hyaline (" corpora amylacea "), fatty, necrotic or colliquative type.

As the origin and nature of the tumour are much disputed, it may be well to point out that the pacchionian body, which figures prominently in all discussions of the meningioma, may show a cap of cells exactly similar to those of the growth (Fig. 22). Proliferated groups of cells over the arachnoid generally, giving rise to " granulations," " villi " or irregularities on its surface, are also similar in cell-type and architecture to the tumour. The formations are reminiscent of pleural villi and peritoneal " milk spots." Such groups of cells may also be found within the dura itself. One might expect that the meninges were as likely to be subject to malformation or malarchitecture as the skin and that " meningiomatosis " may be as common as angiomatosis of the skin. The clinical condition, again, known as Lindau's syndrome, which is familial, may be linked up with the more familiar vascular aberrations (due to " malinduction ") present on every skin surface. Reference is also made in this connection by various observers to the tunicate or lamellar cell formations of pacinian corpuscles (Fig. 26) around sensory nerve-terminations and to the outer structure of the " muscle spindle " around nerve-endings in muscle (Fig. 25), both of which are regarded as either perineurial or neural, according to the theory favoured.

In this description we have made no mention of the varied

forms of the tumour described as fibromatous, endotheliomatous, osteomatous, chondromatous, melanomatous or sarcomatous. The malignant forms, that is sarcomas, seem to depend for their characterisation on whether they are locally recurrent, bone-invading or eroding, or cellular with mitoses, and not on metastatic development. Melanomatous meningiomas, as illustrated, appear very similar to the melanosis seen in some animals (Fig. 24), which is probably representative of the primitive pigmentary sheath of comparative embryology, separating tegumentary ectoderm and neural tube. We suggest that the following classification, a modification of Globus's, with a preliminary division into the major categories of benign and malignant, is a logical and helpful one. A first subdivision is into non-differentiated (protomeningioma) and differentiated tumours. The full number of *histological* types which then emerge is :—

I. Protomeningioma, with primitive undifferentiated type-cells (" meningioma indifferentiale ").

II. (*a*) Periosteomeningioma (with bone development); (*b*) Pachymeningioma (" fibroblastic meningioma "); (*c*) Arachnomeningioma (the whorled " leptomeningioma "); (*d*) Piomeningioma (vascular, " angioblastic meningioma," " hæmangioblastoma ").

A more purely *cytological* and very useful classification is that of the fibroblastic, endothelioblastic, angioblastic, osteoblastic, lipoblastic types, with the inclusion of a less differentiated cell-type, in fact, the various cells included under " the polyblastic system of Maximow " (Turnbull).

It must be understood that, actually, mixed types are the general rule and that our classification, largely a convenience for description, is given at this stage without prejudice to further consideration of the embryogenesis of the meninges. The types are illustrated as far as possible in the plates and legends. We propose to regard the mixed pio- and arachno-types as the typical meningioma and to adopt for this combination the name leptomeningioma.

The material available for our examination included 191 cases, all of which are human. These are made up of the meningiomas proper (148) and the tumours regarded as hæmangioblastomas, especially of the cerebellum. It appears both from the literature and our own personal experience that intracranial tumours in animals are remarkably infrequent.

The well-known cholesteatoma of the horse, often erroneously referred to as psammoma, is not a meningioma. The stains used were : hæmatoxylin and eosin, Mallory's phosphotungstic acid hæmatoxylin, van Gieson's stain, Masson's trichrome stain and Wilder's silver impregnation method.

Discussion.—There are three possibilities to be discussed regarding the nature of the meningioma, expressed in the several views which consider it fibroblastic, endothelial, or neural. The verdict must be based mainly on the embryology and morphology of the meninges.

Embryologically, the primitive meninx is said to be composed of paraxial mesenchyme condensed upon the neural tube (Fig. 23). It separates into pachy- and leptomeninx, although details of further development are hard to find. The tumours which arise in the meninges should therefore be mesodermal. But there is not yet entire agreement even on the mesodermic origin of the meninges. It is indeed asserted that they are at least partly neural in their genesis and that the meningiomas, gliomas, acoustic neurinomas and neurofibromas found, for example, in von Recklinghausen's disease are all closely related tumours and that in this disease they have as their cell of origin the primitive or precursor cell which, as it migrated, differentiated peripherally into the neurilemma or nerve-sheath cell. That is to say, the immediate coverings of brain and spinal cord, the oligodendroglia accompaniment of central nerve bundles, and the satellite tissue of ganglion cells would be, more or less, the homologues of the peripheral neurilemma sheath (sheath of Schwann), which is embryologically derived from the neural crest. If, moreover, we may accept with many neurologists that the subdural and subarachnoid spaces of the central nervous system are continued over the nerve-roots to the termination of the cranio-spinal nerves, then the problem of the meningioma is automatically extended out to the peripheral nerves and the same questions arise regarding origin of certain peripheral nerve tumours as have arisen for the tumour of the central nervous system. The synonymy of the specific nerve-sheath tumour—Schwannoma neurilemmoma, neurinoma, peripheral glioma, perineurial fibroblastoma, etc.—is eloquent testimony of the debatable position of this neoplasm, which is so closely allied to the meningioma. The co-existence of melanoma in, or melanosis of, the meninges is a very important finding, but we defer its consideration to a later study.

Morphologically, cells which produce fibroglia, elastin and collagen ought to be fibroblasts and the cells composing a meningioma are said to be capable of doing so. There is difficulty here, however, of accepting a staining reaction as evidence of identity. Anilin blue stain and, even more, phosphotungstic hæmatoxylin bring out " fibroglia " in close relation to the periphery of the cells composing a meningioma. But then there is another school which goes so far as to maintain that a neurilemma cell (peripheral glia) is capable of forming not only " fibroglia " but even collagen. Morphologically, meningioma cells do not resemble fibroblasts either individually or in their general architecture. They do, however, have some quite appreciable resemblance to endothelial cells and this alone would justify consideration being given to the possibility of meningiomas being endotheliomas. We shall in a later study discuss the relation of the endothelioma to the angioma, or more precisely, of the endothelioblast to the angioblast, and hope thereby to reconcile divergent views expressed in such distinctive terms as dural endothelioma and intracerebellar hæmangioblastoma. It may suffice here merely to refer to the possibility that the subdural space is a sero-vascular space comparable with the pleural, peritoneal and synovial spaces, which are lined by mesothelium. Precursor cells to this lining are endothelial and mesenchymal and are " determined " to the production of flattened littoral cells and subendothelial vessels ; they may also remain as localised groups of undifferentiated cells.

A third possibility has already been considered, that the leptomeninx is partly glial in nature, a sheath of the brain and spinal cord and comparable with the neurilemma sheath to the axons of nerves.

Tissue cultures of meningiomas, which produce true tumour cells from the explant, appear to furnish a growth of cells of the known fibroblast type.

The morphological argument is intimately bound up with an embryological outlook but embryologists are themselves divided on the question of the origin of the meninges. The primitive meninx may possibly be neural or may become " infiltrated " with neural cells (glial) from the neural crest, but it is more generally regarded as an entirely mesenchymal perimedullary condensation. If, firstly, the cells of which the meningioma is composed, or from which it has arisen, are originally derived from the neural crest, the meningioma will

be glial, as will also the neurinoma. If, secondly, they are mesenchymal, the component cells may be endothelial or fibroblastic with, we may add, the possibility of lipoblastic, chondroblastic and osteoblastic participation. A third possibility may form basis of still another argument and that is the view that the germinal layers are not so specifically distinct from one another, not so " inviolable " as older authors maintained. At the later stages of blastodermic development, however, it seems to be generally accepted that " cell determination " steadily becomes more defined and irrevocable. Such a conception in no way negatives our doctrine of the regular persistence into adult life of embryonal replacement cells, each determined to its own blastodermic type of development. Most authorities adopt the mesenchymal (entodermal and ectodermal) derivation of the meninges. The implantation experiments in *Amblystoma punctatum* (Urodele, amphibian embryo) of neural tube with and without neural crest cells, which showed that the leptomeninx, in contrast to the pachymeninx, would not develop without the accompaniment of neural crest tissue, although suggestive, are not sufficiently weighty to overcome that general opinion. The explanation of these interesting experiments may possibly be found in inductive embryology and the evocator influence exerted by one tissue—in this case nervous tissue—on another blastodermic tissue such as the mesenchyme.

We may, however, bring forward this suggestion, that the fixity of our ideas regarding the type-cells of mesodermic tissues requires revision. The fibroblast, as a cell productive of fibroglia, collagen, elastin and reticulin, is surely overburdened, even if it has to be admitted that " typing " is itself only of the nature of a mnemonic. Some other " types " of mesodermic cell, each with certain differential characters, might well be suggested in substitution of the all-embracing " fibroblast " cell. Indeed, it has long been a source of bewilderment to us in our daily microscopical examination of tissues how some stroma cells or their differentiated representatives can be denominated fibroblasts. The supporting tissue of the appendicular mucosa, the endometrial stroma, the ovarian stroma are instances of this sort. Are decidual cells fibroblasts and are theca lutein or interstitial cells of the gonads fibroblasts? Should they not each have a specific denomination? It is manifest that the unfolding of this argument justifies us in contending that *the meningoblast is a type cell, not exactly*

fibroblastic or endothelial, but still mesodermic rather than neuroectodermic.

Although we recognise that we have only partially discussed the question, our conclusion is that the meningioma is a tumour of meningoblast cells, cells of individual type and of mesodermic origin as far as our knowledge goes. The term "meningioma" may then remain as the name of the tumour, not because it is non-committal, but because we regard it as derived from the mesodermic "meningoblast" cell ("meningocyte").

Conclusion.—The meningioma is a tumour arising from stem cells of the pachy- or leptomeninx, that is, from membranes of mesodermic origin. These stem cells are of a peculiar type, individual to the meninges. Meningiomas are not, therefore, fibroblastomas, endotheliomas, hæmangioblastomas or gliomas, but are derived from a type-cell of meningeal, mesodermic origin, which we name specifically a meningoblast or meningocyte.

It may be well to emphasise that the legends to the illustrations contain text matter.

We are grateful to the Carnegie Trust for the Universities of Scotland for generous help towards the cost of illustration. We are indebted to Mr Norman Dott, F.R.C.S.E., for much material and the loan of some of his valuable drawings, photographs and radiographs for reproduction. We acknowledge again the great assistance given us by Mr David Aitken of the Royal College of Physicians' Laboratory in preparing the photomicrographs.

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(*To be continued.*)

NOTES, CLINICAL AND HISTOLOGICAL, ON ILLUSTRATIONS.

FIGS. 1, 2, and 3.—*Meningioma.* Macroscopic, schematic and microscopic illustrations of an occipital, parasagittal meningioma, woman, aged 53. Present 3 years; symptoms, fits with visual aura. Showing—Fig. 1, the lobulated tumour after removal, with comparatively smooth surface, indicating that it could be shelled out except for dural attachment. Fig. 2, diagram of the tumour, which has dipped deeply into the brain without transgressing the pial capsular barrier and which has not invaded bone or produced any hyperostosis. Fig. 3, microscopic appearance of the tumour, in which the cells are arranged as solid alveoli rather than the usual whorls. 1296/37.

FIG. 4.—*Meningioma.* Showing type cells of the meningioma, with oval vesicular nucleus, diffuse chromatin and abundant cytoplasm. 4327/31. ×250.

FIGS. 5 and 6.—*Meningioma.* Male, aged 51. Meningioma in region of orbital plate; symptoms 6 months, focal epilepsy and papillœdema. Showing the most typical form of arachno- or leptomeningioma with cellular whorl formation of very uniform tumour cells, without mitoses. The supporting framework is scanty but is well brought out by Wilder's impregnation method (*cf.* Figs. 29 and 30). The whorls are solid and their centres are occupied by several large cells round which the remaining cells are arranged in tunicate formation. 5271/38. ×100 and 250.

FIGS. 7 and 8.—*Meningioma.* Woman, aged 46, with symptoms of blindness in right eye, headaches for 3 months, vomiting and migraine since puberty. Showing "corpora amylacea" and psammoma bodies. These terms seem to be used synonymously. We may restrict the first designation to the hyalinised stage and psammoma to the calcified sphere. The psammomatous spheres of dark coloration are calcified in concentric arrangement, with actual cells or cell remains at the centre. Phosphotungstic acid hæmatoxylin. 4972/38. ×100 and 250.

FIG. 9.—*Meningioma.* Left parietal pachymeningioma. Woman, aged 46, duration 6 years, with aphasia, weakness of right hand, vomiting and papillœdema. Showing dense fibrous structure, much of which represents remains of dura (*a*). Phosphotungstic acid hæmatoxylin. 5561/38. ×100.

FIG. 10.—*Meningioma.* Optic nerve tumour, female aged 42, with proptosis and venous congestion of retinal vessels. The growth formed a dense firm grey tumour (*a*) round the optic nerve (*b*). It had the microscopical characters of a meningioma and exemplifies the known fact that the sheath of this nerve is meningeal, not neurilemmal. 324/36. ×40.

FIGS. 11 and 12.—*Meningioma.* Showing the vascular type of tumour or angioblastic meningioma. Such types approximate to the so-called hæmangioblastomas of the cerebellum; they provide, with their allies of the cerebellum, argument for those who would call meningiomas hæmangioendotheliomas. Solid alveolar whorled cell units are, in parts, evident (*a*). These angioblastic tumours grow rapidly and tend to recur after removal. 164/77. I.A.P. and 3244/37. ×100.

FIG. 13.—*Meningioma.* Woman, aged 49. A cortical tumour invading the dura; 4 months symptoms of severe headache, sickness, paresis of right arm and leg and slight instability. Showing a type suggestive of neurinoma, with intertwining fasciculi of tumour tissue and a tendency to palisading of nuclei. Other parts showed characteristic calcified whorls and the meningioma type of cell. 5564/32. ×100.

FIG. 14.—*Meningioma.* "Tumour size of tangerine orange bulging into right lateral ventricle, producing localised hydrocephalus of the temporal horn." Showing one of many widely-scattered "xanthomatous" cell areas. These lipoid foam cells may be tumour cells in process of colliquefaction; it does not seem probable that they are the lipophage histiocytes known as "compound granular corpuscles" which are so apt to collect in relation to necrotic areas. 7084/38. ×100. Courtesy of Mr A. A. McConnell, F.R.C.S.I., Dublin.

FIG. 15.—*Meningioma.* Large soft left parietal tumour, woman aged 42, with right hemiparesis, 12 months, increased intracranial pressure, 5 weeks. Showing a stream-line fibrillary type, with psammoma body. 1550/37. ×250. Courtesy of Mr A. R. D. Pattison, F.R.C.S., Newcastle.

FIG. 16.—*Meningioma.* Higher power view of an area of lipoid foam cells. *Cf.* Fig. 14. 2174/37. ×250.

FIG. 17.—*Meningioma.* Right parasagittal meningioma. Woman, aged 43; 3 years Jacksonian attacks, beginning in left foot; 6 months emotionalism and personality changes. Showing a distinct thick vascular and fibrous meningeal capsule (*a*) separating tumour (*b*) from brain substance (*c*). 9885/36. ×70. Courtesy of Mr A. R. D. Pattison, F.R.C.S., Newcastle.

FIG. 18.—*Meningioma.* Showing tumour (*a*) separated from brain substance (*b*) by loose pial tissue (*c*), but not penetrating it. CG. 40. ×70.

FIG. 19.—*Meningioma.* Spinal cord tumour. Illustrating the occasional occurrence of new bone formation (*a*) in this tumour, the "periosteo-meningiomatous" type. Such a development is almost to be expected within the cranium, where the dural meninx is partly periosteal in function. In this case, however, the tumour occurred in the spinal cord region, where the dura is entirely separate from the periosteum lining the vertebral column. CG. 13. ×80.

FIG. 20.—*Meningioma.* Showing tumour invading the haversian spaces of the cancellous bone of the calvarium. The bone itself is little changed nor does the tumour appear, microscopically, different from the ordinary benign meningioma. As an invading tumour, however, even if that be invasion only in the sense of expansion along paths of least resistance, this tumour might be entitled to the designation sarcomatous type of meningioma. Tumours may, rarely, penetrate to such an extent as to appear under the tissues of the scalp. 6962/29. ×25.

FIG. 21.—*Pacchionian bodies.* Showing pacchionian bodies as nodular projections of enlarged arachnoidal granulations into the sagittal venous sinus and parasagittal lacunæ. They may consist simply of localised accumulations of cells of the single-cell endothelial mantle of the subdural space or as projections of the whole pia-arachnoidal cerebrospinal-fluid system. 106/45. I.A.P. ×12.

FIG. 22.—*Pacchionian body.* Showing the cap of cells (*a*) with which these lepto-meningeal projections may be covered; the cells have a strong resemblance to the tumour cells of the meningioma. This resemblance to tumour structure is often enhanced by the presence of cell whorls (*b*) or psammoma bodies. 106/45. I.A.P. ×100.

FIG. 23.—*Embryo meninx.* Periaxial mesenchyme in a 2·6 cm. (crown-rump) embryo. Showing the mesenchymal laminar condensation (*a*) from which the separate meninges are still to be "evoked." On the upper surface, the very thin layer of tegumentary epithelium (*b*) can be made out, and below is the tissue of the cerebral enlargement of the neural tube (*c*). Some suggestion of a split into the two primary meningeal layers may be imagined. 5614/38. ×100.

FIG. 24.—*Meningeal melanosis.* Brain, sheep. Showing superior surface of brain with the pia-arachnoid stripped off from the non-melanotic areas. This meningeal melanosis is comparatively common in sheep and horses. The melanomatous meningioma is usually included in all type classifications of the meningiomata, but generally with some hesitation, in view of the importance of the phenomenon for settlement of the sharply conflicting opinions on the ectodermic (tegumentary or neural) or mesodermic origin of the nævomelanoma. Melanin-bearing cells in greater or less abundance are to be found in the sclera, choroid and meninges of adult mammals and are a feature of the periaxial mesenchymal primitive sheath of the central nervous system in fishes and urodele amphibia. I.A.P.

FIG. 25.—*Muscle spindle.* Included as a nerve termination in muscle to show perineurial sheath.

FIG. 26.—*Pacinian corpuscle.* The laminated sheath which surrounds the axial nerve fibre of these bodies has some resemblance in architecture to the meningioma whorl. It is commonly regarded as perineurium.

FIG. 27.—*Sympathetic ganglion.* Presacral, child of 13 years; megaureter, ganglionectomy. Showing entering nerve (*a*) and capsules of ganglion cells cut superficially. The satellite cells are arranged in whorl formation (*b*), reminiscent of the meningioma. These satellite cells are continuous with the neurilemma sheath cells of nerves. 7215/38. ×250.

FIG. 28.—*Hæmangioblastoma.* Cerebellar tumour. Female, aged 24. Six years cerebellar symptoms; deeply-placed vascular tumour. Showing a meshwork of blood vessels, with intervening tumour cells of endothelial or meningeal type. This type of tumour is included by us in the angioblastic meningioma. 3327/37. ×100.

FIGS. 29 and 30.—*Meningioma.* Gross architecture and stroma arrangement. Showing how the fibrovascular supporting tissue does not, so far as these silver impregnation (Wilder) specimens indicate, enter into the essential tumour formations. The solid alveolar masses are cut transversely and longitudinally. CG. 36 and 1631/7. I.A.P. ×100.

FIG. 31.—*Meningioma.* Child, aged 4½. Left parasagittal tumour; acute increase of intracranial pressure without localising signs, 3 months. Death after exploration. Showing distribution of tumour (*a*) in the perisinus tissue; some tumour also in sinus thrombus (*b*). 5620/38. ×2.

FIG. 32.—*Meningioma.* Same tissue as in Fig. 31. Showing a very undifferentiated small cell type. 5620/38. ×250.

This illustrates what we have named "protomengioma," a type covered by various already existing terms, such as mesenchymal meningioma (Bailey), meningioma indifferentiale (Globus) and "sarcomatosis" of the meninges. The possibility of medullablastoma seeding itself along the meninges must also be considered. The autopsy record speaks only of supratentorial tumour.

FIG. 33.—*Meningioma.* Clinical picture, temporal vessels of the scalp in meningioma. This dilatation of the vessels would seem to indicate an obstruction to the return of blood from the meninges and a compensatory dilatation. Penetration of the cancellous spaces of the calvarium by tumour is usually accompanied by dilatation of vessels of the diploe; these vessels communicate with those of the pericranium externally.

FIG. 34.—*Meningioma.* Radiograph showing pericranial hyperostosis (*a*), the result of meningioma. In its growth, the meningioma appears to stimulate overlying bone as well as ultimately to penetrate its haversian spaces (*cf.* Fig. 20). The resulting external growth of bone, with a peculiar radiating arrangement of spicules is very valuable for diagnosis. A certain amount of erosion or pressure atrophy of the bone has to be reckoned with also as accompaniment of a meningioma, but the main feature is hyperostosis or hemicraniosis.

FIG. 35.—*Acoustic neurinoma.* Cerebellopontine angle tumour. Showing a fairly uniform composition of elongated cells in fasciculated arrangement and palisading of nuclei. The acoustic neurinoma may be single or it may occur along with neurofibromata of the skin and meningioma, a fact which is used in support of the contention of the identity or close relationship of all three types of growth. 6357/35. ×100.

FIG. 36.—*Neurofibromatosis.* Optic nerve. Child, aged 8. Some sight still remaining; "café-au-lait" spots, v. Recklinghausen's disease but no subcutaneous nodules. Showing (*a*) transverse section of tumour of optic nerve and (*b*) nerve behind the swelling. Condition in (*a*) is strongly suggestive of overgrowth of optic nerve fibres and at the same time of marked hypertrophy of its meningeal sheath. One contention regarding the neurofibromatosis of v. Recklinghausen is that all the component tissues of an involved optic nerve may be affected, both glial and sheath elements. 3981/37. ×2½.

FIG. 37.—*Stump neuroma.* Child, aged 13. Humerus removed for osteogenic sarcoma 18 months previously; subsequent development of stump neuroma of brachial plexus. Showing new nerve fibres developed from the central portion of a cut nerve in interlaced formation. These bulbous formations are not true tumours but, with their axis cylinders, proliferated neurilemma cells and their connective tissue sheath participation, are closely allied to the true neuromas. They are distinguished from the neurinomas and the neurofibromas by the presence of axons. Their relationship to the meningioma consists in their histogenesis. The bulbous development of the distal portion of a cut nerve resembles the neurinoma. 1227/37. ×40.

FIG. 38.—*Neuroma.* Male, aged 20. Tumour size of "pigeon's egg," right peroneal nerve, present some years, growing. The actual name to be given this tumour, neuroma, neurofibroma or neurinoma, need not concern us. It is shown here because of the whorling arrangement (*a*) of tumour cells, resembling that seen in meningiomas. The growth was apparently solitary. 6895/35. ×100.

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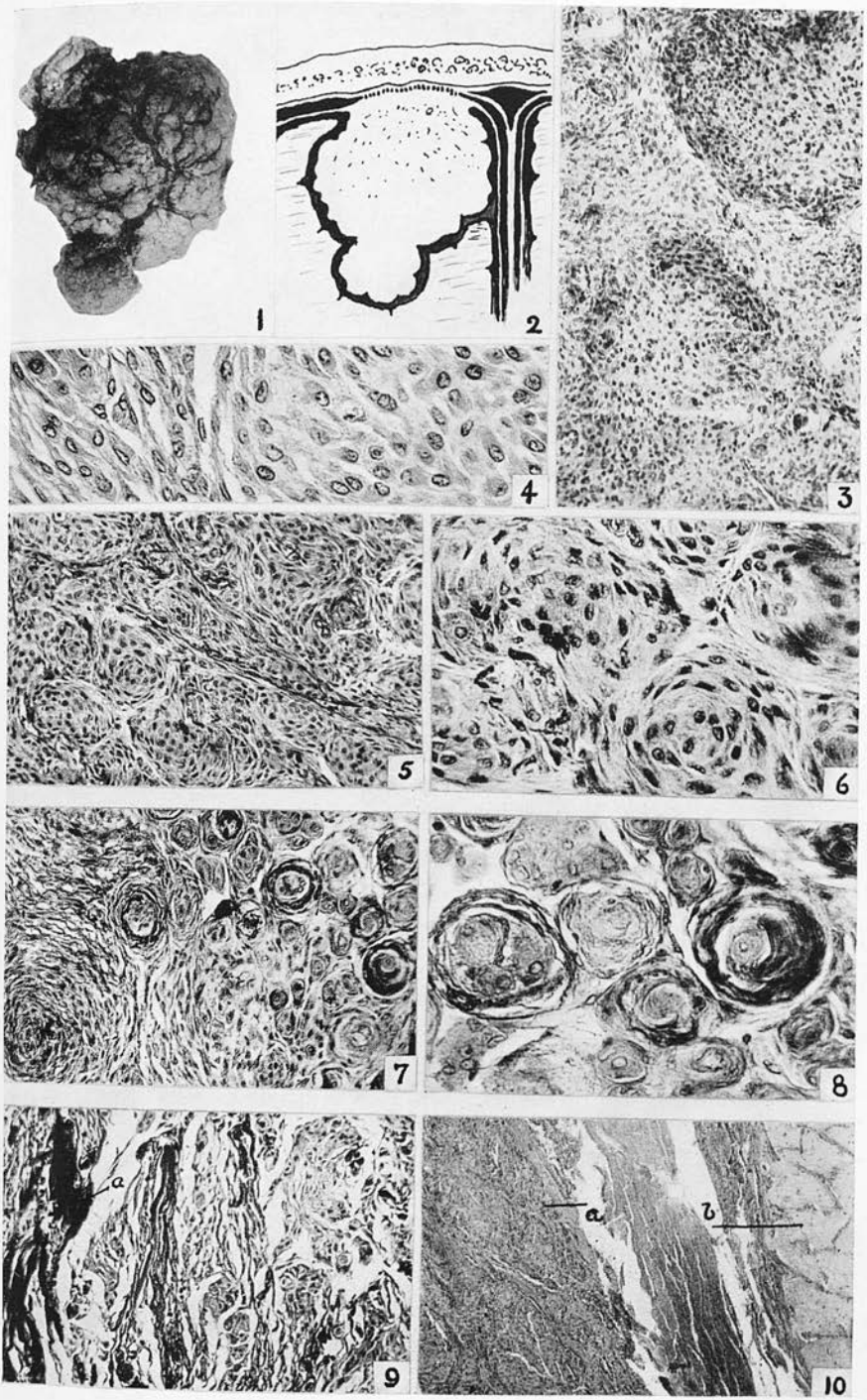
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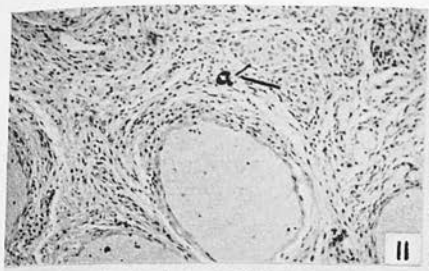

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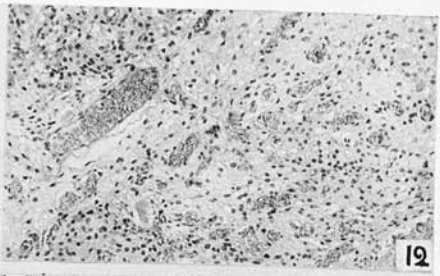

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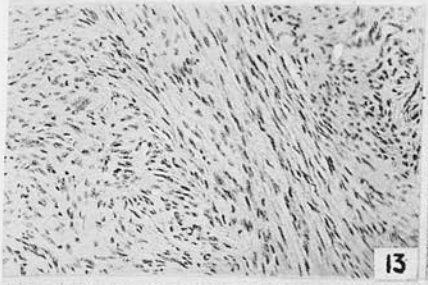

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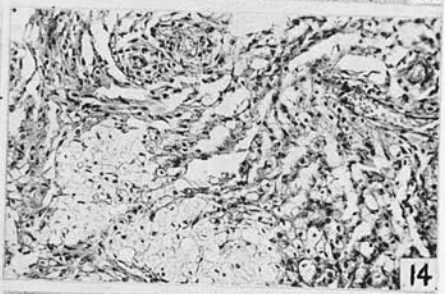

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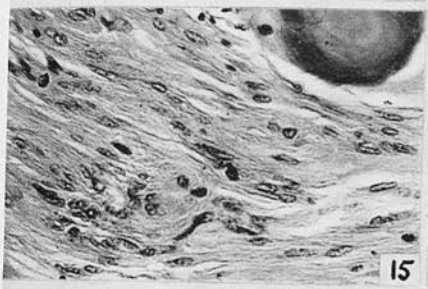

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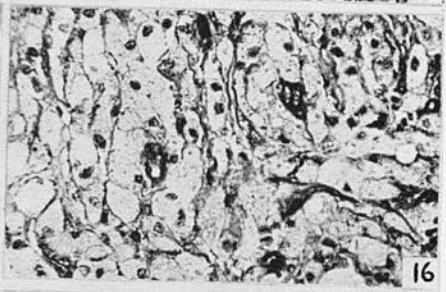

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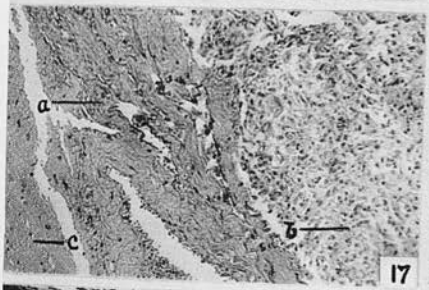

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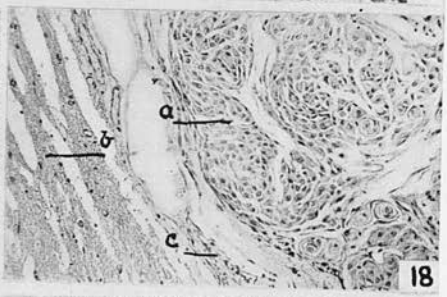

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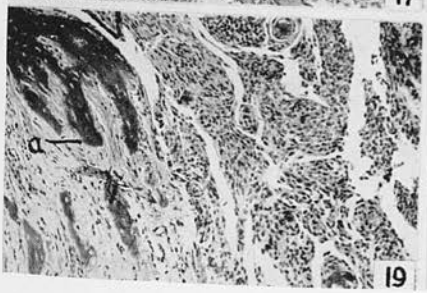

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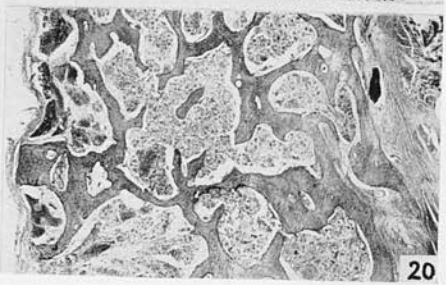

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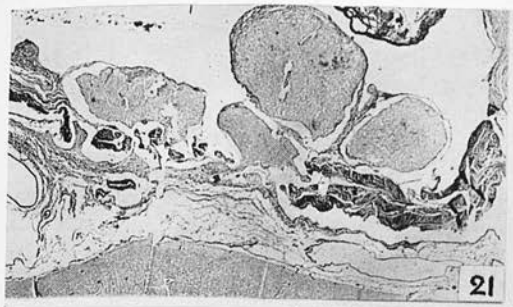
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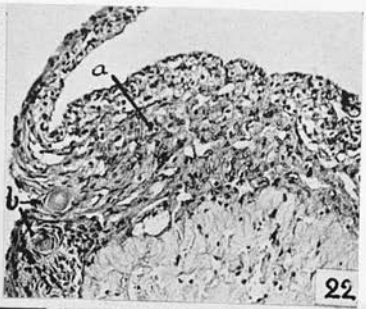

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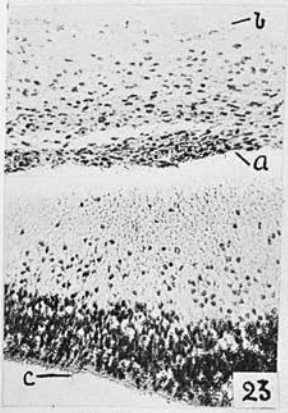

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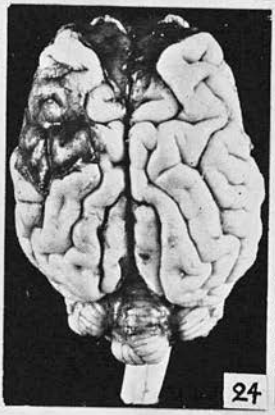
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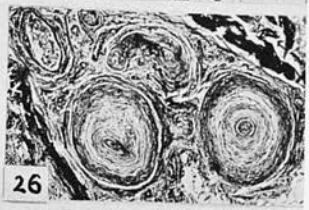
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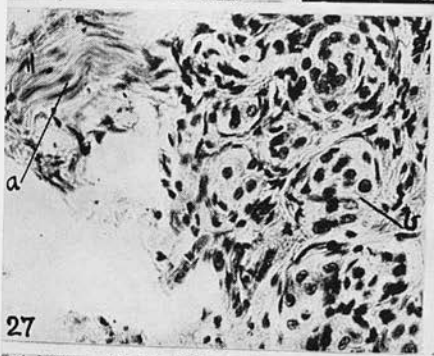

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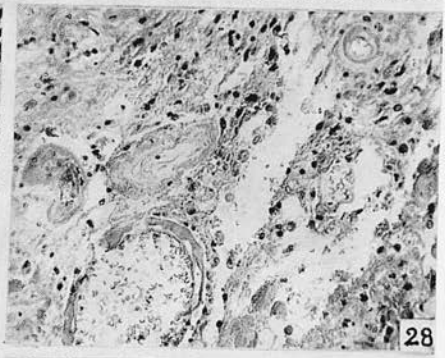
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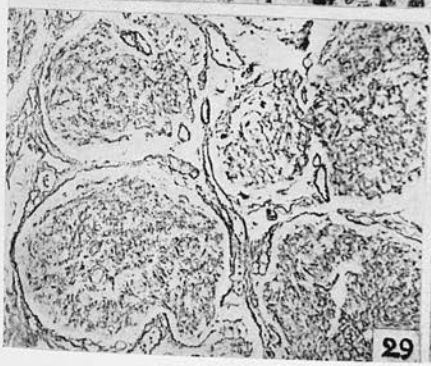
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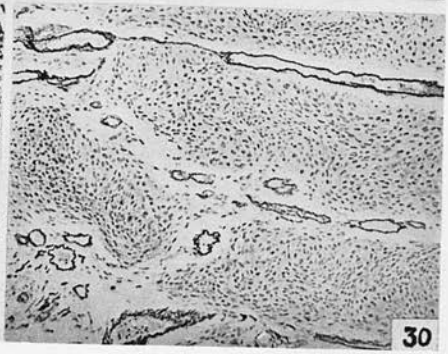
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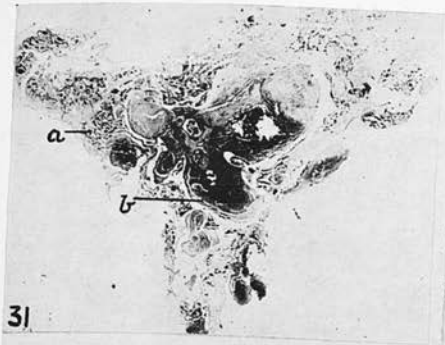

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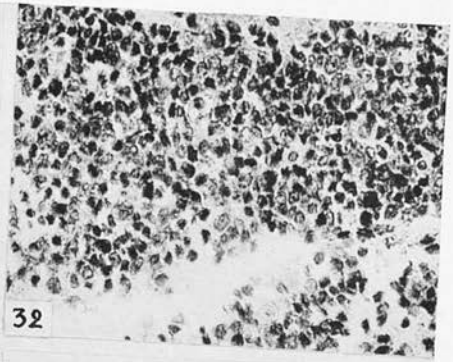
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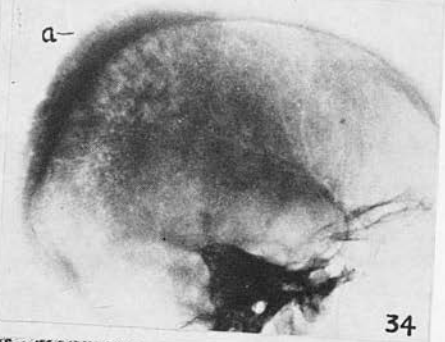

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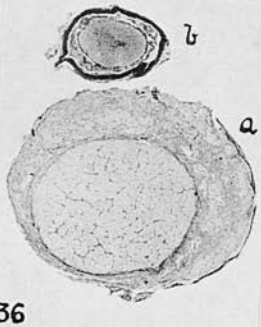

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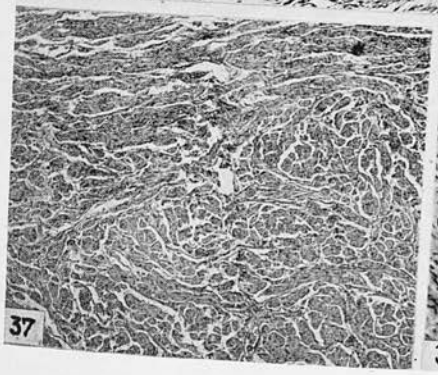
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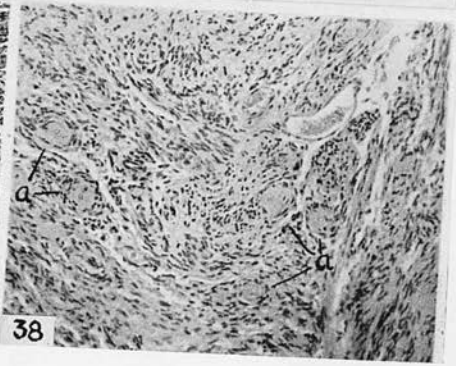

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DEBATABLE TUMOURS IN HUMAN AND ANIMAL PATHOLOGY.

VII. Granulosa-cell Tumour of the Ovary.

Debatable Tumours in Human and Animal Pathology

VII. Granulosa-Cell Tumour of the Ovary

BY

W. F. HARVEY, E. K. DAWSON, AND J. R. M. INNES

(From the Research Laboratory of the Royal College of Physicians, Edinburgh, the Cancer Control Organisation of Edinburgh and South-East Scotland, and the Institute of Animal Pathology, University of Cambridge.)

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DEBATABLE TUMOURS IN HUMAN AND ANIMAL PATHOLOGY.

VII. Granulosa-Cell Tumour of the Ovary.*

By W. F. HARVEY, E. K. DAWSON, and J. R. M. INNES.

(*From the Research Laboratory of the Royal College of Physicians, Edinburgh, the Cancer Control Organisation of Edinburgh and South-East Scotland, and the Institute of Animal Pathology, University of Cambridge.*)

Definition.—The granulosa-cell tumour is a solid or partly cystic, smooth, encapsuled epithelium-like growth of uniform cell type, with more or less folliculoid, solid alveolar, trabecular, or diffuse cell pattern. It is characteristically œstrogenic and is very rarely truly malignant.

Description.—This tumour, often described as rare, arises in the ovary at any age, involves more or less the whole ovary and, as its name implies, resembles in its architecture the tissue of the mature or immature Graafian follicle. Various names have been given to the tumour, such as folliculoma malignum ovarii, carcinoma folliculoides, granulosa-cell carcinoma and basal-cell growth. The Brenner tumour (" oophoroma folliculare ") is frequently grouped with it, rather than with the cystadenomas. We, however, provisionally exclude the Brenner type (Figs. 35-39) from our granulosa-cell tumour group.

The Macroscopical Picture.—Granulosa-cell tumours are, with rare exceptions, unilateral, of size varying from a few grammes to many kilogrammes in weight, and maintain the shape of ovary within a smooth fibrous capsule, thus forming what has been called a " giant " or exuberant ovary (Fig. 7). On transection, they are fleshy, solid growths, usually with yellow or orange streaks and a tendency to form small cysts; necrosis is not an obvious feature.

The Microscopical Picture.—1. Histologically, the tumours show a considerable variation. The essentially diagnostic pattern is the so-called *folliculoid*, with tumour-cell masses in which are scattered small microscopic spaces, some in a " rosette " formation, reminiscent of and probably identical with the Call-Exner body of the Graafian follicle. These

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spaces may be empty or may contain one or more cytolysing cells or simply homogeneous material (Figs. 9 and 12). Several spaces may coalesce to form lakes of liquor folliculi (Fig. 1, *c*) and may then resemble the tissue of the maturing Graafian follicle. A radiate, one-layered arrangement of tumour cells bordering these small spaces and some of the cysts and outlining the larger cell aggregates is very characteristic (Figs. 9 and 12), but not necessarily specific to the tumour ; it is, for example, a common phenomenon in basal-cell carcinomas, gliomas, salivary gland adenomas, some malignant mammary growths and even in a dried blood film. We have, however, found no granulosa-cell tumours in our series entirely folliculoid in architecture. They show, in other areas, a variety of patterns which give names to the other subtypes of the tumour. These are the cylindroid or trabecular, the diffuse, and types intermediate between these. The *cylindroid* (Figs. 10 and 24), a comparatively common type, shows branching columns of trabeculæ of tumour cells. The trabeculæ are separated by fibrous tissue, which may be cellular or hyaline in character. In the latter case, the old term " cylindroma " is sometimes applied. When the cell columns are very thin and anastomosing, an *intermediate* or a watered-silk (moiré) pattern is produced, with or without scattered rosette formations (Figs. 5 and 23). The formation of sheets of cells in the section with a complete lack of pattern gives the type described as *diffuse* ; this is sometimes diagnosed as sarcoma (Figs. 4 and 26). Diagnosis may be difficult where the section available shows mainly trabecular, or diffuse, or gyriform tumour growth and careful search must then be made for the characteristic folliculoid pattern. The complete absence of lymphocytic infiltration of the stroma is a helpful diagnostic feature. Examination of whole sections of the ovary or of several different areas is sometimes necessary before an opinion can be arrived at.

2. Cytologically, the component tumour cell is remarkably uniform for any particular pattern of tumour growth. The main forms are the polyhedral, the radiate columnar, and the fusiform, all with large, variably chromatic, nucleus. Mitotic figures are found but are rare.

The Clinical Picture.—It is agreed that the granulosa-cell tumour is very rarely truly malignant. This view is supported by the long survival after operation in most reported cases and the rarity of local recurrence or metastasis.

The pathological evidence of benign character includes the dense smooth encapsulation, the uniform tumour cell type and the sparse or absent mitoses, even though the tumour tissue appears to be diffusely infiltrating the ovarian stroma. Cases of bilateral tumour or metastases suggest the probability of a primary or secondary malignant growth of another type.

Recent advance in knowledge of the hormones has removed some of the difficulty in diagnosis of the granulosa-cell tumour, as a characteristic feature is its œstrogenic activity. This is evidenced by moderate uterine enlargement, endometrial polyposis and cystoglandular hyperplasia (Fig. 27), menstrual aberrations shown as post-climacteric bleeding or pre-climacteric amenorrhœa or menorrhagia, and the occurrence of precocious puberty symptoms in subjects of young age. In the fully reproductive age period, however, the menstrual upset may be insignificant.

The microscopic diagnosis of the tumour from other primary ovarian growths, such as fibroma, sarcoma, cyst-adenoma, carcinoma, and teratoma is dependent on histological and cytological characters some of which are illustrated in the plates. Metastatic growths often cause much difficulty.

The material available for our examination and comparison includes 47 cases of granulosa-cell tumour, of which four are animal (calf), and more than 500 benign and malignant ovarian tumours of other types.

Discussion.—A study of the slides of this material in order to extract tumours of the granulosa-cell group convinced us that here was an assortment of types under different names. That study has, at the same time, convinced us that this one tumour possesses a recognisable unity of architecture and some indication of specifically organoid differentiation. The designation granulosa-cell tumour is itself an admission of origin for the tumour. It is not surprising that we should have great diversity of tissue types in tumours arising even in so small an organ as the ovary, for, apart from the ovary being the seat of the totipotent sex cells, it is a derivative of that rather anomalous germinal layer of the embryo, the mesoblast; anomalous in the sense that it gives rise quite definitely from its own primordial tissue to both of the oppositely determined tissue types—the epithelial and the connective. Some of the confusion indeed which overlies the concept of the granulosa-cell tumour is inherent in either the rigidity or

confusion in the use of the terms epithelium, mesothelium, endothelium, and fibroblast. It is a general practice to speak of splanchnic mesoderm, cœlomic epithelium, and germinal epithelium when describing the development of the gonad glands. The granulosa cells, representing the hormonogenic parenchyma of the ovary, are said to arise by proliferation of the cœlomic *epithelium* of the genital ridge, the stroma of the ovary from an ingrowth of the mesenchymal element of the mesoderm. A growing body of opinion, however, regards the cœlomic epithelium or mesothelium as a covering layer only (" lepidic ") and the gonad as arising entirely from a " hylic," mesodermic primordium. It is this view which seems to us reasonable and which makes, therefore, both granulosa-cell and fibroblast into derivatives *in situ* of the mesoderm. This is equivalent to acceptation of the derivation of both epithelial and connective tissue from the gonadal mesodermic blastema, as is the case with the nephrogenic mesoderm. The way is thus paved for the view that the epithelial cell and the fibroblast are not so distantly connected in the case of the gonads as they seem to be in the case of ectodermic and entodermic tissues. It is also borne out by the finding of granulosa-cell tumour and fibroma in conjunction. A few workers have raised the question, or asserted positively, that the granulosa-cell is merely epithelioid and is derived from the stroma cells of the ovary. This would make of it an intermediate cell type between epithelium and fibroblast. This view, with which we concur, looks upon the granulosa-cell as the resultant, at a given stage of embryogeny, of a specific determination in mesodermic cell character. A glance at the primordial follicles of an ovary shows the undeveloped, single-layered granulosa cells, circumferential to the ovum cell, with features very similar to the stroma cells surrounding them and, apparently, in continuity with them. Absence at this stage of any membrana propria enhances the appearance of continuity (Figs. 42-44). The persistence of argumentation on the real nature of the granulosa cell and its relation to the stroma cell at least supplies some explanation of the liability to confusion in practice of granulosa-cell tumours with fibromas, sarcomas, and carcinomas. Decisions on some of the points here raised lies with the embryologist, perhaps with the experimental embryologist, rather than with the morphologist. The ordinary cycle of development of the

Graafian follicle to maturity gives us some clues to the appearance of the granulosa-cell tumour (Figs. 42-46). Not only is the likeness of the follicular cells to the tumour cells striking, but one can pick out in the more typical tumours the Call-Exner body and the development of the liquor folliculi. It is possible even in a typical granulosa-cell tumour to select areas which conform to the accepted subdivisions of folliculoid, cylindroid, and diffuse tumours with their follicular, trabecular, cord, moiré, and ill-defined cell patterns. It does not appear to us necessary, therefore, to emphasise too much these simple variations in architecture.

We are prepared to classify the tumours of the ovary tentatively into certain groups which become more or less intelligible by a study of embryology and by our adherence to the doctrine of (1) development of all tumours from an embryonal type of normal cell which, however, may have a decided direction of tissue determination already impressed on it before its assumption of tumour character and (2) the persistence into adult life of a residue or reserve of embryonal cells as ordinary replacement cells. One division in this suggested classification of ovarian tumours is made up of the gonadal group proper and is set up against the cystadenomas, fibromas, sarcomas, carcinomas, and teratomas. In the ovary we have distinct evidence of development of tumours which may be called essentially female (the granulosa-cell tumour), essentially male (the arrhenoblastoma) and essentially neuter (the dysgerminoma) and this also raises the important possibility of mixture of these types. This would constitute the gonadal group proper. The question still remains whether the granulosa-cell tumour, as one of the gonadal tumours, is benign or malignant. The tumour in its development remains within the ovarian limits and indeed the tumour as a whole often preserves very largely the ovarian form. In the great majority of cases the tumour appears to be benign and does not recur on removal. There is, however, no reason to suppose that it may not develop malignant characters. In that case the tumour becomes very difficult to diagnose from other primary carcinomas of the ovary and from the metastatic tumours to the ovary.

There is no doubt that the comparatively recent addition to the clinical armamentarium of functional hormone tests for the diagnosis of gonadal tumours in the male and in the

female and the new ideas that are current as a result of experimental embryology have been responsible for considerable reversion to embryological considerations as explanation for primary ovarian and testicular tumours in general. A tumour of the gonad which produces the giant ovary, secretes in glandular fashion the most important female sex hormone, and is built up in its most typical form of apparently normal gonadal cell elements immediately raises the question whether any neoplasm can be regarded as other than a purely local somatic overgrowth.

Conclusion.—The granulosa-cell tumour is a tumour of primitive ovarian follicle-cell origin. It may be of very varied histological appearance, may differentiate to organoid architecture or remain diffuse and is characterised by œstrogenic activity. It is one of a definite group of gonadal tissue tumours and may even present, in part, some of the features of one or other of the chief members of the group, the arrhenoblastoma and the dysgerminoma.

It may be well to repeat that the legends to the illustrations contain text matter.

We are grateful to the Carnegie Trust for the Universities of Scotland for generous help towards the cost of illustration. Valuable material has been sent us from numerous sources, some individual acknowledgment of which is made in the notes to illustrations. We acknowledge again the great assistance given us by Mr David Aitken of the Royal College of Physicians' Laboratory in preparing the photomicrographs.

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(*To be continued.*)

NOTES, CLINICAL AND HISTOLOGICAL, ON ILLUSTRATIONS.

FIG. 1.—*Granulosa-cell tumour.* Female, aged 11 ; menstruation since 9 years. Showing a typically " folliculoid " tumour, with areas of closely packed, uniform cells, in islets and strands (*a*), embedded in a more ill-defined (moiré) tumour tissue (*b*). The cellular masses are studded with small organoid spaces of Call-Exner type which form one of the most characteristic and diagnostic features of the " folliculoid " granulosa-cell tumour. Fusion of these spaces may result in the formation of lakes of liquor folliculi (*c*). The sparse stroma shows dilated vessels (*d*). The distinction in these tumour cell masses between what may be regarded as immature follicle formation and Call-Exner body is ill-defined. " Case 11." ×40. Courtesy of Dr Freda Pratt, Oxford.

FIG. 2.—*Granulosa-cell tumour.* Same section as in Fig. 1, showing folliculoid (*a*), trabecular (*b*), and moiré (*c*) arrangement of tumour cells of very uniform type, without mitotic figures. The stroma is inconspicuous. ×150.

FIG. 3.—*Granulosa-cell tumour.* Another area from the same section as in Figs. 1 and 2. Showing diffuse areas of tumour cells separated by broad intervening septa of hyaline, almost acellular stroma, a tumour type sometimes described as cylindromatous. There is little or no tendency to " folliculoid " formations in this area and such a picture may suggest a diagnosis of sarcoma. ×100.

FIG. 4.—*Granulosa-cell tumour.* Same section as in Figs. 1 to 3. Showing a higher power view of the diffuse tumour-cell areas of Fig. 3. The cells, although without definite pattern, have some indication of linear arrangement reminiscent of the immature gonad. ×250.

FIG. 5.—*Granulosa-cell tumour.* Female, post-menopausal abdominal swelling. Smooth tumour, 4 ins. diameter, bosselated surface, solid on transsection, with central hæmorrhage. Showing a very striking and directly recognisable form of the growth, in which the tumour cells are arranged in uniform, labyrinthine strings consisting of little more than a single layer of cells. This is an extreme form of the so-called moiré type, very diagnostic of granulosa-cell tumour. Other areas showed trabecular and diffuse cell architecture. 6214/35. ×80.

FIG. 6.—*Granulosa-cell tumour.* Female, aged 64, unmarried. " Vaginal bleeding ; twisted cyst, size of orange, very hæmorrhagic." Showing a varied picture with both folliculoid (*a*) and moiré (*b*) areas. In the adjacent stroma there is a deposit of the striking yellow pigment (*c*) so frequently observed macroscopically in these tumours. 6231/35. ×40.

FIG. 7.—*Granulosa-cell tumour.* Showing a section of an enlarged (" giant "), cystic ovary, entirely occupied by tumour and surrounded by a definite thick capsule. " Case 9." ×1. Courtesy of Dr Freda Pratt, Oxford.

FIG. 8.—*Granulosa-cell tumour.* Same section as in Fig. 7. Showing the thick capsule (*a*), sharply delimited from the tumour ; folliculoid formations of varied size (*b*) and still larger cyst formations (*c*). ×25.

FIG. 9.—*Granulosa-cell tumour.* Same section as in Figs. 7 and 8. Showing spaces of Call-Exner type, containing one or more degenerating tumour cells and bordered by characteristic radially arranged cells. In the centre a fibro-vascular core (*a*) is also surrounded by radially arranged cells. ×250.

FIG. 10.—*Granulosa-cell tumour.* Showing a " cylindroid " type of cellular growth, with diffuse tumour areas intersected by stroma trabeculæ. There is no evidence in this section plane of the diagnostic folliculoid or Call-Exner formations. " Case 10." ×40. Courtesy of Dr Freda Pratt, Oxford.

FIG. 11.—*Granulosa-cell tumour.* A typical tumour, associated with hyperplastic cystic endometrium (Fig. 27). Showing two characteristic folliculoid masses bordered by radially arranged cells. A cyst (*a*) shows a similar radiate border. 5599/38. ×80. Courtesy of Dr Leila Hawksley, Royal Cancer Hospital (Free), London.

FIG. 12.—*Granulosa-cell tumour.* Same section as in Fig. 11, to show at higher

magnification the characteristic radiate arrangement of the cells and their tall columnar form. ×150.

FIG. 13.—*Granulosa-cell tumour.* Same section as in Figs. 11 and 12. Showing a solid alveolar arrangement of tumour masses, some of which contain folliculoid formations. ×40.

FIG. 14.—*Granulosa-cell tumour.* Same section as in Figs. 11 to 13. Showing fasciculated, stream-lined, fusiform-cell characters, suggestive in the section of gonadal cords. ×40.

FIG. 15.—*Granulosa-cell tumour.* Same section as in Figs. 11 to 14. Showing higher power view of Fig. 14. ×150.

FIG. 16.—*Granulosa-cell tumour.* Calf. Tumour, 15×10 cm., found in the wall of a large ovarian cyst, with some small additional intracystic papillary ingrowths. The soft yellowish tumour portion showed solid alveolar structure, with "serous" cysts and wide-spread calcific deposit (*a*). This area shows two contiguous tumour masses, one of smaller cell type (*b*), the other of larger cell type (*c*) suggestive of a lutein change. "Case IV." 119/46, I.A.P. ×40. Courtesy of Professor R. Baumann, Vienna.

FIG. 17.—*Granulosa-cell tumour.* Calf. Same section as in Fig. 16. Showing a higher power view of the smaller cell type area (*b*), rather hypernephromatoid in appearance. ×100.

FIG. 18.—*Granulosa-cell tumour.* Calf. Same section as in Fig. 16. Showing the larger cell type area (*c*), suggestive of a lutein change in the cytoplasm (*cf.* Fig. 41, human). ×250.

FIG. 19.—*Granulosa-cell tumour.* Calf. Bosselated unilateral tumour of ovary, weight about 5 lb., size 20×15×15 cm. Strikingly yellow in colour; solid with pin-head cysts. Showing folliculoid areas bounded by nuclei radially arranged to a central space. The uterus showed glandular and muscular hyperplasia. "Case V." 120/46, I.A.P. ×80. Courtesy of Professor R. Baumann, Vienna.

FIG. 20.—*Granulosa-cell tumour.* Calf. Same section as in Fig. 16, showing higher magnification of two folliculoid formations. ×250.

FIG. 21.—*Granulosa-cell tumour.* Calf. Same tissue as in Figs. 19 and 20. Showing in some isolated areas the deposition of calcium in the central content of the folliculoid formations which are characteristic of the granulosa-cell tumours. 120/46, I.A.P. ×250.

FIG. 22.—*Granulosa-cell tumour.* Calf. Tumour right ovary. Weight 10 lb., soft consistency with clear fluid in larger cysts; yellow colouration with hæmorrhagic areas. Hypertrophic uterine mucosa with dilated gland elements. Showing solid alveolar tumour type (*cf.* Fig. 13, human). "Case VI." 121/46, I.A.P. ×40. Courtesy of Professor R. Baumann, Vienna.

FIG. 23.—*Granulosa-cell tumour.* Female, aged 56, 9-para. Uterine hæmorrhage recurring every month and almost continuous for last 9 months. Tumour left ovary, 3×2¾ ins. Hæmorrhagic cystic growth. Showing crowded folliculoid and moiré character. 8316/38. ×100. Courtesy of Professor Biggart, Belfast.

FIG. 24.—*Granulosa-cell tumour.* Female, aged 54, 7-para. Menopause at 51 years. Periods have returned for last 6 months. Abdominal swelling 2 weeks. Tumour left ovary, weight 5 lb. 7 oz.; yellowish and studded with small cysts. Marked endometrial hyperplasia. Showing characteristic cylindroid formations with a meshwork of fine cell trabeculæ separated by degenerated stroma. 8315/38. ×80. Courtesy of Professor Biggart, Belfast.

FIG. 25.—*Granulosa-cell tumour.* Same section as in Fig. 24. Showing massive tumour-cell trabeculæ. 8315/38. ×80.

FIG. 26.—*Granulosa-cell tumour.* Same section as in Figs. 24, 25. Showing an area where trabecular formations have merged into diffuse tumour growth. 8315/38. ×80.

FIG. 27.—*Uterus.* Same case as in Figs. 11 to 15 of typical folliculoid granulosa-cell tumour. Showing glandulo-cystic endometrial hyperplasia and hypertrophic myometrium. 5599/38. ×2. Courtesy of Dr Leila Hawksley, Royal Cancer Hospital (Free), London.

FIG. 28.—*Arrhenoblastoma.* Female, married, aged 41; hirsutism, marked obesity, partial amenorrhea, duration 4 years. Bilateral ovarian tumour and hyperplasia of adrenals. Showing "giant ovary" almost completely occupied

by tumour with a few cysts. 5300/35. ×1. Courtesy of Prof. J. H. Biggart, Belfast.

FIG. 29.—*Arrhenoblastoma.* Female, aged 27, married 2 years 8 months; no children, very irregular menstruation with long intervals of amenorrhea. Some hirsutism, marked obesity. Yellowish encapsulated tumour in right ovary, 3¼×2¼ ins.; small uterus. Showing strand formation in a matrix of tumour cells, suggestive of the sex cords of the early male gonad (*cf.* Figs. 57 and 58) and to a less degree of the cylindroid form of granulosa-cell tumour (Fig. 24). 8317/38. ×40. Courtesy of Professor Biggart.

FIG. 30.—*Arrhenoblastoma.* Same section as in Fig. 29. A higher power view of the anastomosing cord formation, characteristic of the arrhenoblastoma. These cords are said to be the precursors of the seminiferous tubules. 8317/38. ×150.

FIG. 31.—*Arrhenoblastoma.* Same case as in Fig. 28. Showing more differentiated seminiferous cords than in Fig. 30, with some indication of lumen. Other areas of this section showed features of the granulosa-cell tumour and the dysgerminoma. 5300/35. ×100.

FIG. 32.—*Dysgerminoma.* Unmarried female, aged 24, Unilateral mobile ovarian tumour size of small melon, bosselated surface. No menstrual upset; uterus healthy; duration 6 weeks. Showing typical dysgerminoma (seminoma), with numerous focal necrotic areas, which, however, do not appear in the illustration. Large tumour cells, many mitoses, little intercellular stroma and small aggregations of lymphocytes. 8648/38. ×150. Courtesy of Dr Fahmy, Edinburgh.

FIG. 33.—*Granulosa-cell tumour.* Female, aged 33, 1-para. Amenorrhea 3 years ago, lasting 2½ years; irregular menstruation 6 months; metrorrhagia 6 weeks, not yet stopped. Tumour right ovary, with preservation of shape. On transsection, brilliant orange streaks and small cysts. Microscopically sections showed cylindroid and diffuse tumour areas with some follicular architecture. Showing a cylindroid area, with vascular fibrous septa. 7961/38. ×80. Wilder's silver impregnation. Courtesy of Dr Jane Redman, The Women's Hospital, Birmingham.

FIG. 34.—*Ovary in pubertas præcox.* Child aged 3 years 11 months, with fits and facial weakness. Menstruation since a year old. Mammary tissue of puberty stage. Autopsy showed basal cerebral tumour. Showing part of a follicular ovarian cyst, lined by abundant granulosa cells in the midst of which are Call-Exner formations (*a*) and bordered by a distinct radiate single-cell layer. 2070/30. ×150. Courtesy of Mr Norman Dott, F.R.C.S.Ed.

FIG. 35.—*Brenner tumour.* Typical "oophoroma folliculare." Showing solid and cystic alveolar masses, mostly round or oval in cross-section, embedded in hyperplastic ovarian stroma. 6859/38. ×80. Courtesy of Dr Freda Pratt, Oxford.

FIG. 36.—*Brenner tumour.* Same tissue as in Fig. 35. Showing a solid "cell-nest" of undifferentiated epithelioid tumour cells, sharply demarcated from the surrounding stroma. 6859/38. ×150.

FIG. 37.—*Brenner tumour.* Same tissue as in Fig. 35. Showing a "cell-nest" with early cyst formation. One of these (*a*) contains a free nucleated cell in degeneration, which was taken originally, but erroneously, to be the nucleus and nucleolus of an ovum cell. The cells bordering the other cyst (*b*) tend to columnar shape. 6859/38. ×150.

FIG. 38.—*Brenner tumour.* Same tissue as in Fig. 35. Showing other stages of cyst formation in a "cell-nest," with remains of degenerate cells. 6859/38. ×150.

FIG. 39.—*Brenner tumour.* Same tissue as in Fig. 35. Showing a further stage of cyst formation with a definite columnar cell lining and basal nuclei, suggestive of a mucinous gland. The interest of this formation lies in this, that Brenner tumours, although classed by many as granulosa-cell tumours, tend to be found in association with pseudomucinous cystadenomas. ×150.

FIG. 40.—*Granulosa-cell tumour.* Female, unmarried, aged 49. Still menstruating, but periods lately irregular. Showing an admixture of diffuse granulosa-cell tumour without follicles (*a*) and areas highly suggestive of luteinised cells (*b*). Courtesy of Dr E. C. Fahmy, Edinburgh. 675/36. ×40.

FIG. 41.—*Granulosa-cell tumour.* Same section as in Fig. 40, at higher

magnification to show the two types of cells, granulosa (*a*) and lutein (*b*). Other areas showed the gradation of the former cell (*a*) into the latter type (*b*). 675/36. ×150.

FIG. 42.—*Normal ovary*. Child aged 8 months. Showing numerous primordial follicles containing ova. Some of the deeper ones show differentiation to Graafian follicles. 7689/38. ×25. Courtesy of Dr Wm. Liston, Edinburgh.

FIG. 43.—*Normal ovary*. Same section as in Fig. 42. Showing four primordial follicles containing ova set in stroma, without any accompaniment of differentiated granulosa cells. 7689/38. ×250.

FIG. 44.—*Normal ovary*. Same section as in Fig. 42. Showing one primordial follicle (*a*) with stroma-like cells inside the perimeter and another (*b*), with commencing granulosa-cell differentiation. 7689/38. ×250.

FIG. 45.—*Normal ovary*. Same section as in Fig. 42. Showing a further stage in granulosa-cell production, with early proliferation within the follicle, but no liquor folliculi. 7689/38. ×250.

FIG. 46.—*Normal ovary*. Same section as in Fig. 42. Showing a maturing Graafian follicle with abundant granulosa cells, cytoplasm of ovum (*a*) and two Call-Exner bodies (*b*). The peripheral granulosa cells show a definite radiate arrangement. 7689/38. ×250.

FIG. 47.—*Malignant ovarian tumour*. A serous cystadenocarcinoma, primary in the ovary. To show how an invading area of primary malignant ovarian growth may resemble granulosa-cell tumours in their folliculoid architecture and radiate cell perimeter. Other areas of this malignant growth had their own characteristic features of intracystic papillary or gyriform structure with numerous cell mitoses. C.G. Ovary. 76. ×40.

FIG. 48.—*Malignant ovarian tumour*. Female, aged 47. Complaining only of flatulent distension. At operation both ovaries involved, as were also intestine (the primary site) and peritoneum. Showing a diffuse metastatic adeno-carcinomatosis of the ovary—to be contrasted with the granulosa-cell tumour in its architecture. 7048/29. ×80.

FIGS. 49 to 58.—*Embryos*. A series of sections of embryos, to show the development of male and female gonads and to illustrate the tissue of the gonadal tumour group.

FIG. 49.—*Embryo*. Pig, 6 mm. Showing large bilateral mesonephroi (*a*), Wolffian bodies, with the earliest indication of development of the genital ridge (*b*). ×25. Courtesy of Professor H. A. Harris, Anatomy Department, Cambridge University.

FIG. 50.—*Embryo*. Same section as Fig. 49. Showing a high power view of the proliferating genital ridge (*a*). ×250.

FIG. 51.—*Embryo*. Pig, ♀, 14 mm. Showing definite bilateral gonad formation (*a*). ×25. Courtesy of Professor H. A. Harris, Anatomy Department, Cambridge University.

FIG. 52.—*Embryo*. Same section as in Fig. 51. High power view of area (*a*) in Fig. 51. Showing gonadal blastema with sex cells (*a*), covering cœlomic "epithelium" (*b*), and mesenchymal primordium (*c*). ×250.

FIG. 53.—*Embryo*. Pig, ♀, 21 mm. Showing further enlargement of bilateral gonads (*a*) and adjacent mesonephric tissue (*b*). ×25. Courtesy of Professor H. A. Harris, Anatomy Department, Cambridge University.

FIG. 54.—*Embryo*. Same section as in Fig. 53. High power view, showing small-cell mesenchymal primordium (*a*) and the larger-cell gonadal blastema (*b*). ×250.

FIG. 55.—*Embryo*. Human, female, 78 mm. Showing one of the gonads (*a*), mesonephric remains (*b*) and Müllerian duct (*c*). 8156/38. ×24. Courtesy of Dr W. F. T. Haultain, Edinburgh. ×25.

FIG. 56.—*Embryo*. Same section as in Fig. 55. Showing female gonadal tissue with definite sex cells (*a*). 8156/38. ×250.

FIG. 57.—*Embryo*. Human, male, 30 mm. Showing male sex *cords* in contrast to female diffuse primordium. ×25. Courtesy of Professor H. A. Harris, Anatomy Department, Cambridge University.

FIG. 58.—*Embryo*. Same section as in Fig. 57. High power view, showing development of tunica albuginea (*a*) and male sex cords (*b*). Compare these latter with the similar appearances in the arrhenoblastoma (Fig. 30). ×250.

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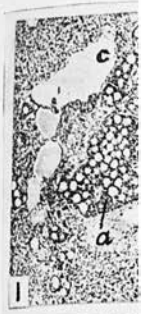

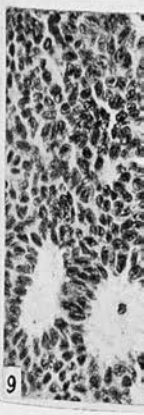

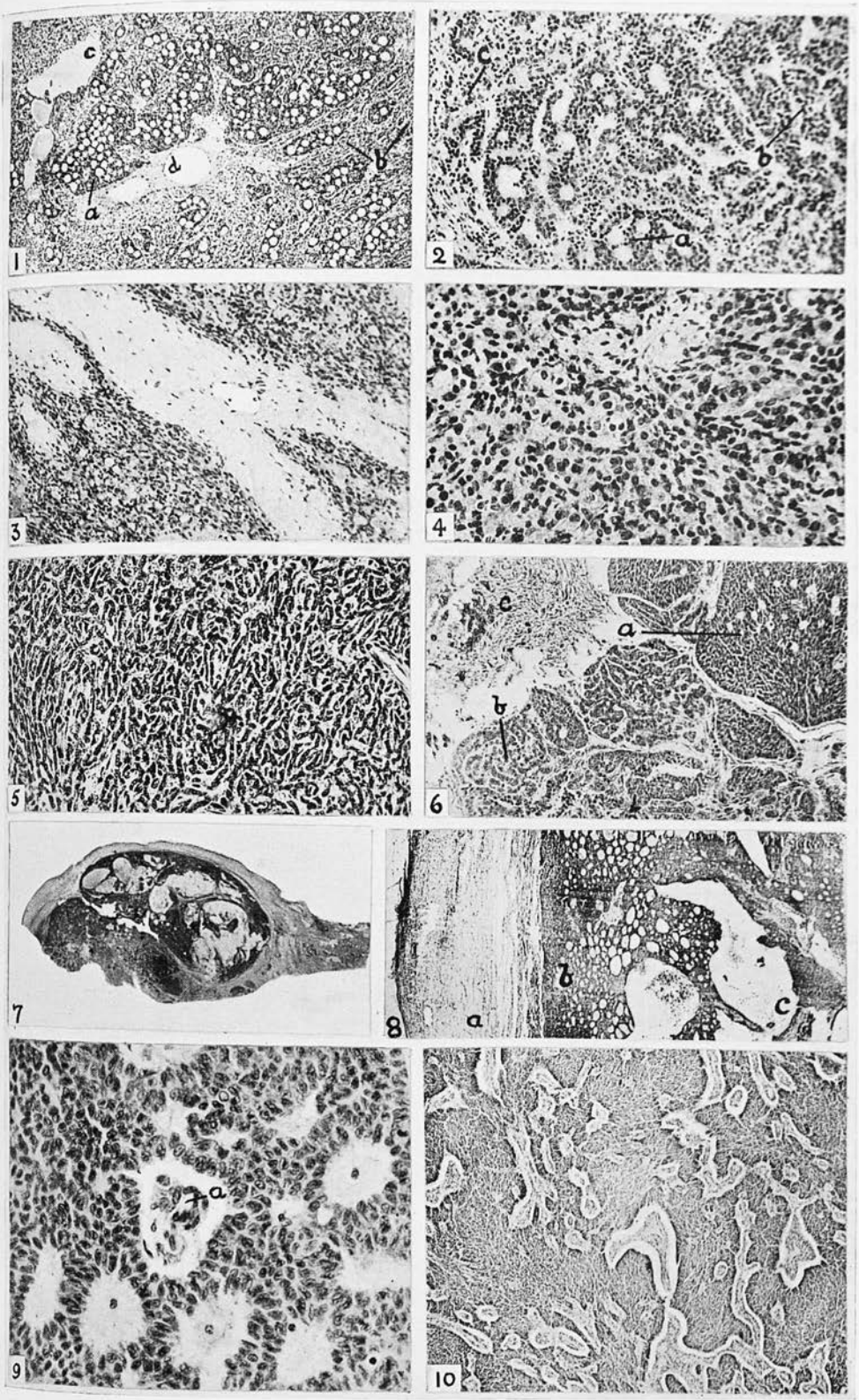
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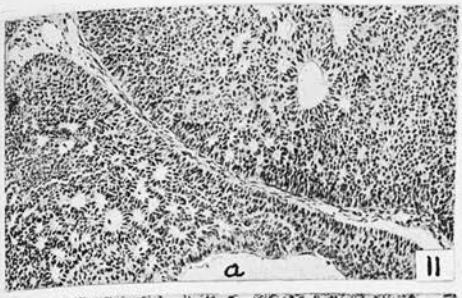

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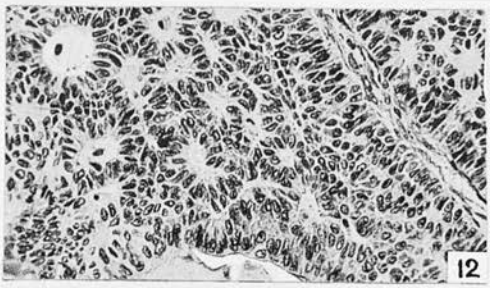

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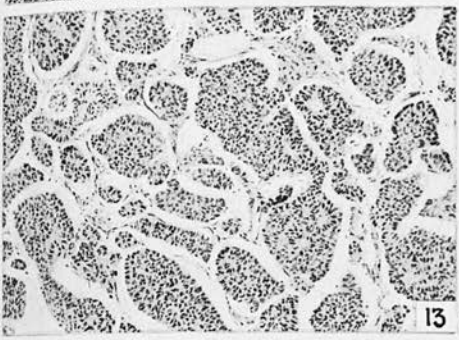

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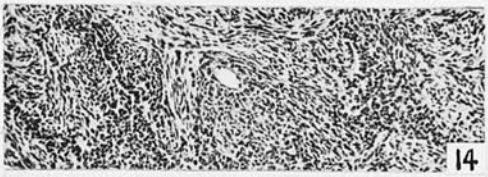

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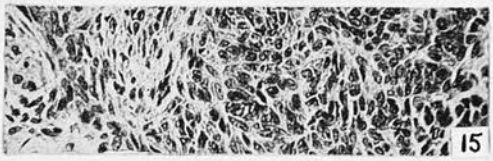

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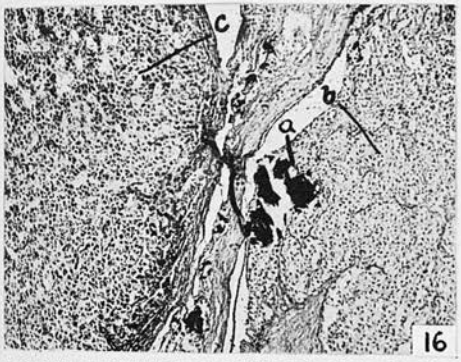

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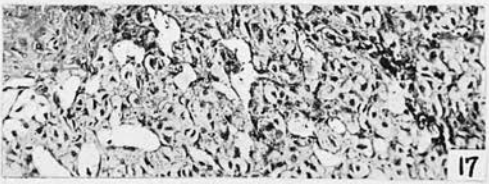

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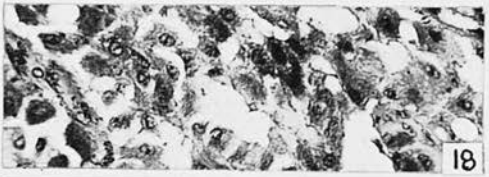

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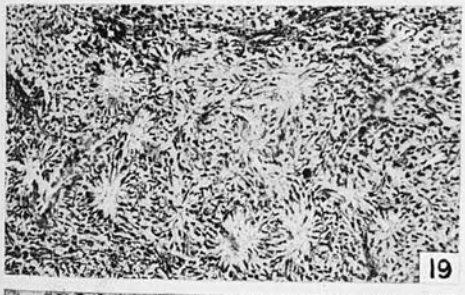

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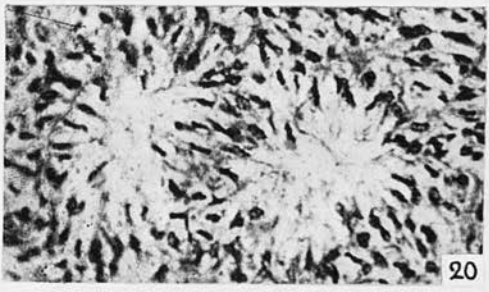

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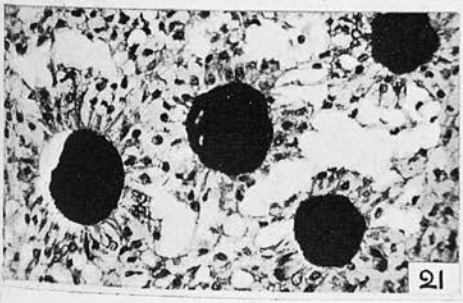

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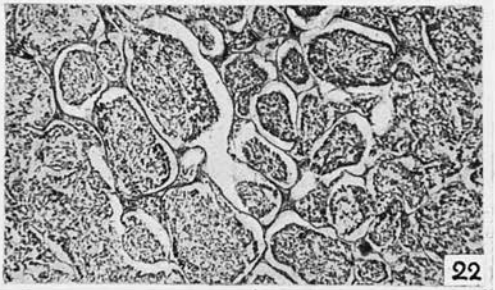

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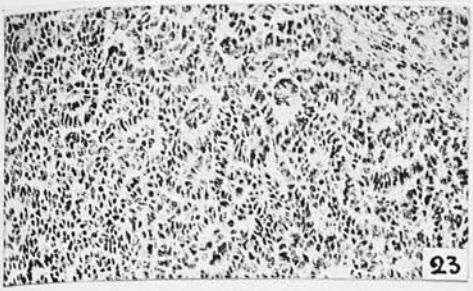
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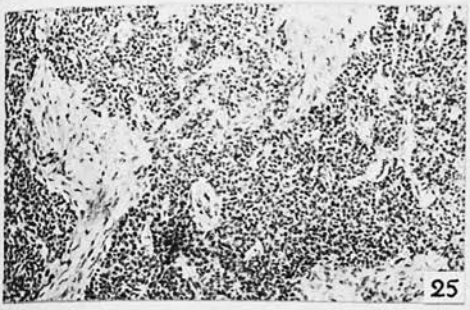
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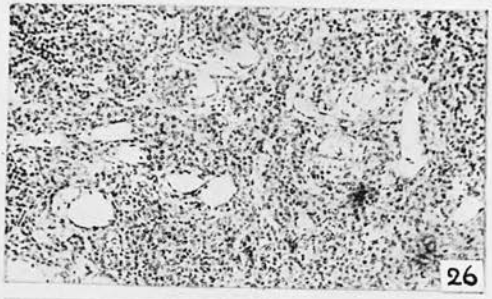
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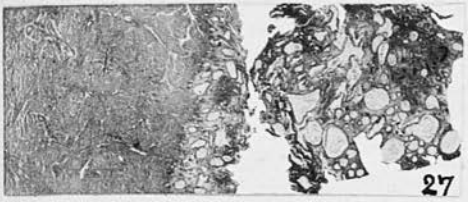
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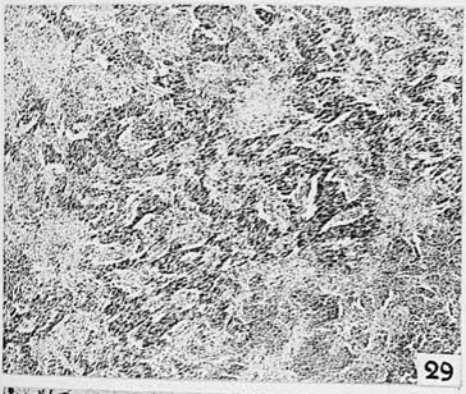
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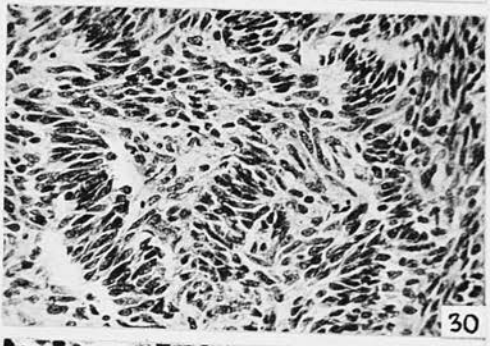
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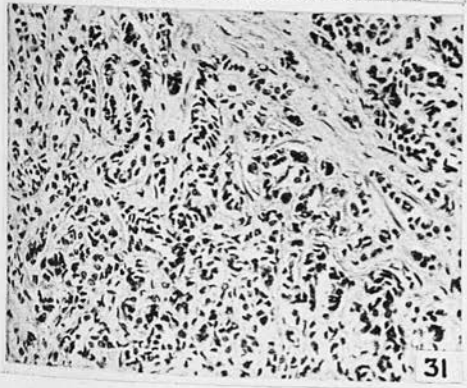
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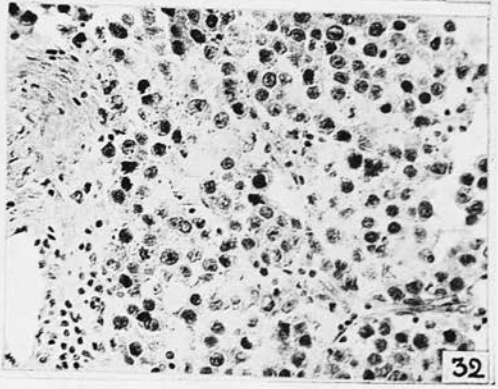
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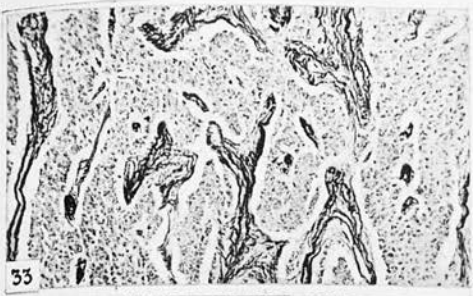
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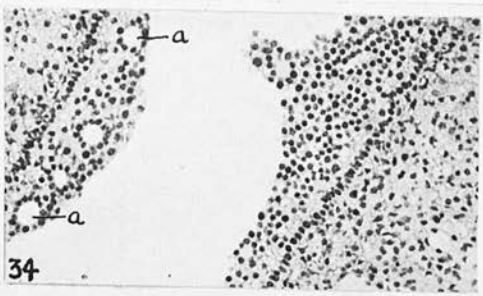

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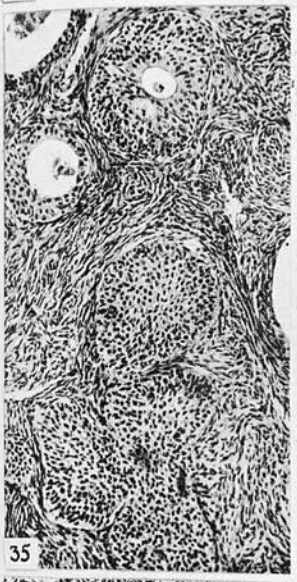
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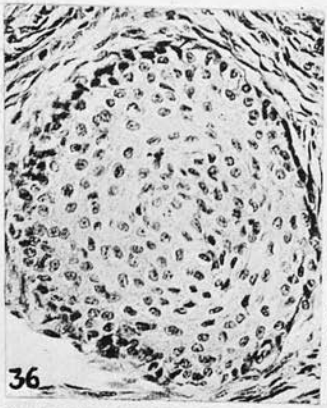
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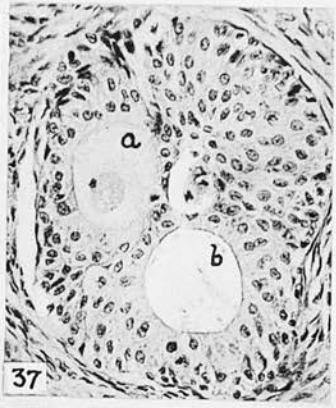

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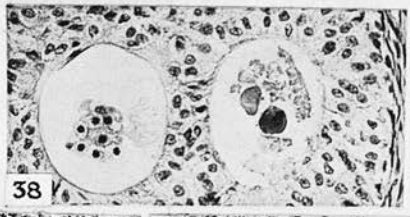
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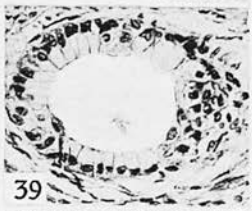
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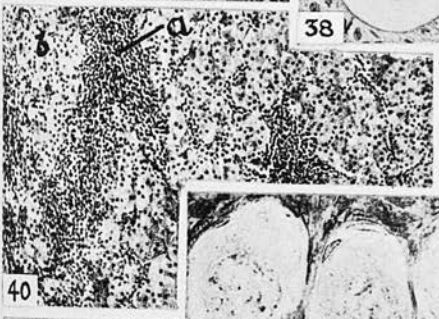

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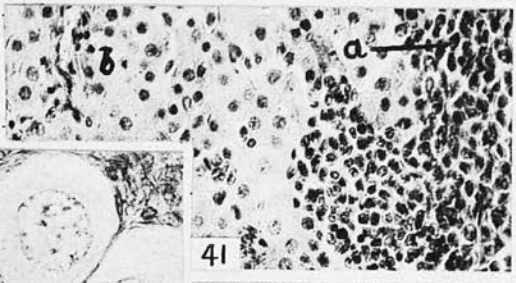

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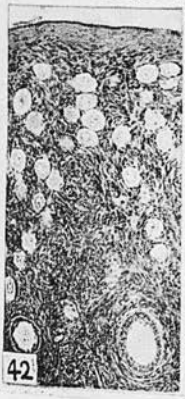
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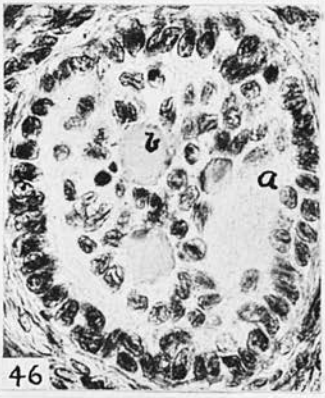

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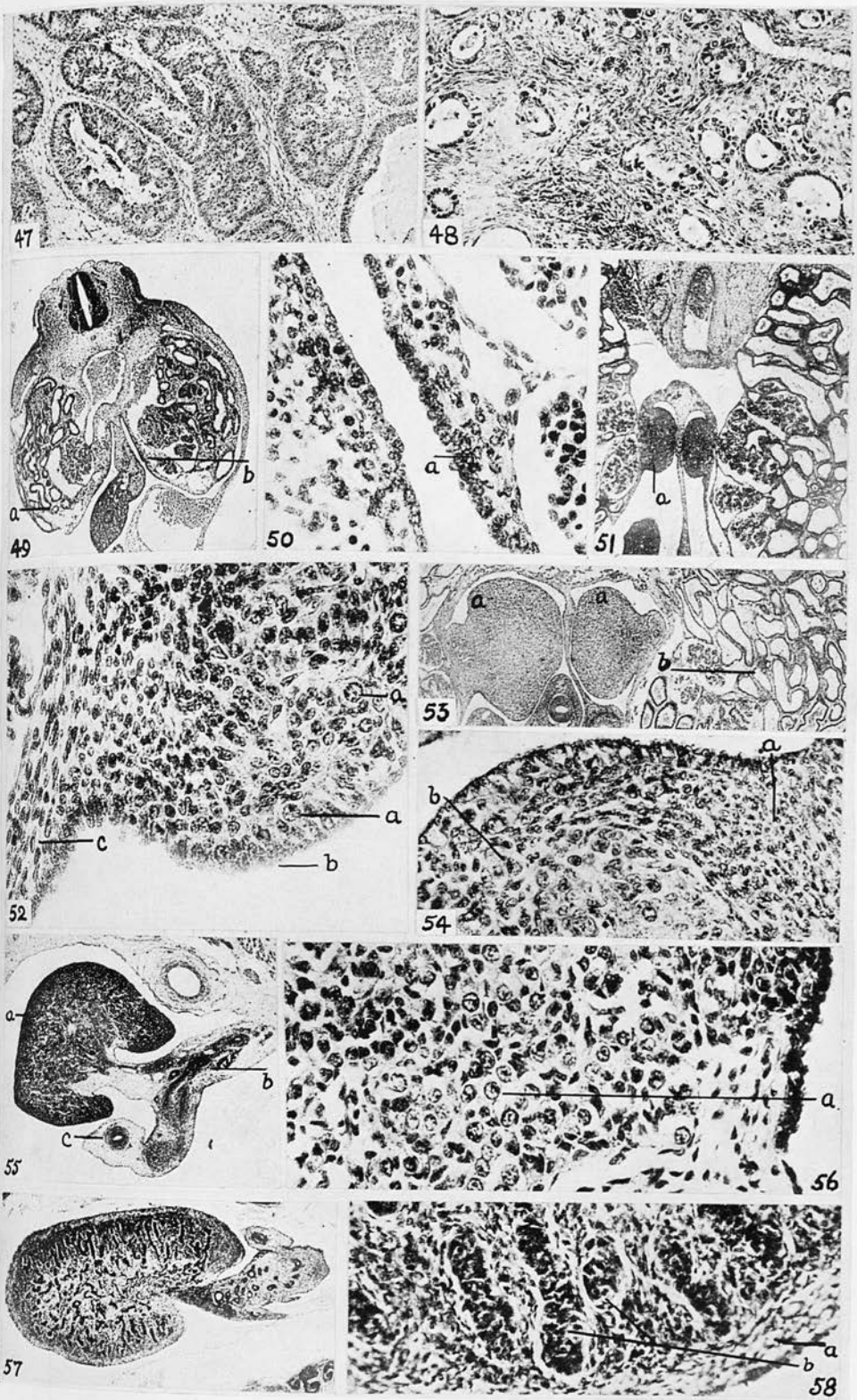
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DEBATABLE TUMOURS IN HUMAN AND ANIMAL PATHOLOGY.

VIII. Melanoma.

Debatable Tumours in Human and Animal Pathology

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BY

E. K. DAWSON, J. R. M. INNES, AND W. F. HARVEY

(*From the Research Laboratory of the Royal College of Physicians, Edinburgh, the Cancer Control Organisation of Edinburgh and South-East Scotland, and the Institute of Animal Pathology, University of Cambridge.*)

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DEBATABLE TUMOURS IN HUMAN AND ANIMAL PATHOLOGY.

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(*From the Research Laboratory of the Royal College of Physicians, Edinburgh, the Cancer Control Organisation of Edinburgh and South-East Scotland, and the Institute of Animal Pathology, University of Cambridge.*)

Definition.—The melanoma is a complex pigmented tumour. In its benign form it is essentially a malformation ranging from a purely tegumentary pigment fleck or an acanthotic epidermal disarray to one compounded of epidermic, mesodermic and neural tissue, occurring in the skin, eye and elsewhere. Biologically the benign melanoma represents a dysontogenetic field in more or less stable equilibrium. The most characteristic component cells are the " nævus cells," and these are melanoblasts situated in basal epidermis and appendages, or in the dermis, or in tissues which are their homologues. Intracellular melanin pigment is usually present in greater or less amount. The malignant melanoma in man is a carcinoma.

Description.—A description of the melanoma requires some reference to the point of view which we adopt. This has been built up gradually and tentatively by a close examination of the large amount of microscopical material, human and animal, at our disposal and by comparison of the views of others, as expressed in the literature, with our own findings and reflections.

It may be said, briefly, that we look on the mole, a pigmented or non-pigmented " nævus-cell " growth, as a complex malformation in which epidermis, dermis, blood vessels and nerves participate in greater or less degree. The growth of the mole may be proportionate to that of the body as a whole, remaining in a state of more or less equilibrium and to that extent represent a malformation. If the malformation shows

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disproportionate growth characters, it may then be called tumour or, in some cases, progressive melanosis. We regard the malignant melanoma in the human subject, until proof to the contrary is forthcoming, as carcinoma, whatever be the constitution of the benign condition. It has only in certain cases a partial resemblance to sarcoma and little or none to neurogenic sarcoma. In speaking of melanocarcinoma we take into account the ocular tumour, which must be called neuroectodermic carcinoma, as distinct from the skin tumour which we consider a tegumentary ectodermic carcinoma. Our description covers the benign melanoma of the skin and mucosæ derived from the skin, the benign choroidal or uveal melanoma and the melanocarcinoma of the skin and eye, and necessitates definition of our terminology.

Three precise terms suffice for the broader description—the melanoblast, the melanocyte and the melanophage. The *melanoblast* is a primitive cell of the type to which we have often referred in our previous studies as being the foundation of all tumour growths, since in our view all tumours have their origin in a primitive type of stem cell constantly present throughout life and ordinarily destined to replace already fully differentiated functioning cells when they die. These primitive stem cells, usually of a definite order of " determination," necessarily undergo differentiation. In the present case the primitive cell would be initially non-pigmented and might or might not contain the oxydase required to convert the circulating chromogen substrate into melanin ; this melanoblast would be predominantly the important cell type of the malignant melanoma and capable of proliferation. The *melanocyte* may be defined as a differentiated cell with melanin content and as derived from the melanoblast by the development and exercise of its melanin-forming function. The process of transformation from melanoblast to melanocyte is demonstrated by the dopa reaction (Figs. 66, 67). Melanin itself is not dopa-positive although it is strongly argyrophile. By means of this test the oxydising ferment contained in active melanoblasts may be demonstrated *in vitro* by treating thin slices of frozen tissue with the substrate dioxyphenylalanine (" dopa "), generally regarded as the final stage in the production of melanogen, or a chemical substance very closely allied to it. The reaction product is " dopa-melanin." It is understandable that there may be potential melanoblasts which do not possess a developed

oxydase and also that there would be all degrees of melanin content between the melanoblast and the melanocyte. The melanocyte would then be the pigmented epithelial cell of the epidermalists and the chromatophore of the mesodermalists. Formed melanin reacts with silver to give " silver-melanin," which is a decidedly blacker product than dopa-melanin. The silver reaction is very useful to demonstrate melanin-containing cells when they are sparse as in Mongolian flecks or in the choroid of the eye. Melanoblasts and melanocytes may be epidermic or dermic, choroidal, meningeal or retinal, and are not regarded by us as migratory in adult tissues, whatever they may be in tissue culture. The cells which grow out from a tissue fragment of a Harding-Passy mouse melanoma (Fig. 70) are described as macrophages, fibroblasts and dendritic melanoblasts. The essentially dermal melanoblast or melanocyte may perhaps best be seen in the skin of lower vertebrates, especially fish and amphibians (Fig. 75). In man apparently one of the few places where they persist in mesodermal form is the choroid (Fig. 60). The *melanophage* is a macrophage or phagocytic cell of mesenchymal origin and often named a reticulo-endothelial cell. It is perhaps better to regard it as of the mesenchymal polyblast cell series. It takes up by active ingestion melanin particles, just as it would the particles of a tattoo marking or blood pigment and is indeed sometimes called a tattooed cell. Proliferation of the polyblast cell in cutis or in lymph sinus may occur concurrently with ingestion of melanin and thus account for the dense aggregations of melanophages which are sometimes seen in sections. The difficulty remains how to distinguish the melanocyte from melanophage other than by defining the former as a cell-forming pigment, the latter as a cell-ingesting pigment. Morphologically the mature melanophage is in general a larger cell than the melanocyte, contains more, coarser and more globoid, melanin pigment and has a smaller, non-vesicular, often rather pyknotic, central or excentric nucleus (Fig. 39). The pigment taken up by the phagocyte would be that set free on the death of the melanocyte or, if such occurs, its active depigmentation either by extrusion of particles, " clasmacytosis " or dissolution. The melanophage may be a fixed tissue cell and not always an actively wandering cell.

The Benign Cutaneous Melanoma.—We may take as synonyms for this malformation the hard and soft mole, the

birth-mark and the pigmented nævus. They fall into two main forms, the nævus-cell type proper and the acanthotic. If we adopt a tumour nomenclature—and the stage at which such malformation passes over into benign tumour is regarded by us as somewhat arbitrary—we may speak of the benign melanoma, the melanocytoma, or the quiescent nævus-cell tumour. In its macroscopic appearance it varies from an epidermal brown macula of greater or less extent to more or less raised structures of varying size and pigmentation, with or without an overgrowth of hair. Moles which are not raised, not macular, but deeply placed in the dermis constitute the "blue" nævi and may even be so extensive as to produce "blue melanosis" (Fig. 58). The so-called Mongolian spots are also of this nature. The uniform slate-blue colour of some monkeys and the skin coloration of many lower animals is often due to the depths at which the dermal melanocytes are placed. Indeed a widespread distribution of melanotic pigment in the tissues of the body is a very marked feature in some fish and amphibian species (Fig. 75) and in some invertebrates (Fig. 76). Nor is this to be wondered at when the actual ovum cell of many of these species is already pigmented in the ovary. Examples of such ova are to be found in the common frog (*Rana temporaria fusca*) and the axolotl (*Siredon pisciformis*).

Sometimes a mole has a peculiar arrangement which suggests the distribution of a cutaneous nerve or some relation to the embryo clefts of the body. It was not, however, possible for us to substantiate any such distinctive distribution of moles of the face (Fig. 26), which were recorded for the purpose and represented over 100 individuals met with in daily travel.

Microscopically the congenital soft mole of the adult shows a very striking aggregation of "nævus cells" in the dermis, often reaching into the papillæ, and sometimes down to the subcutaneous tissue. If they do reach into the papillæ the epidermis overlying the widened and globular summit is often thinned and stretched (Fig. 57), while the interpapillary epidermal processes may be reduced to a 2-cell thickness. A certain amount of hyperkeratosis is usually present (Fig. 1). This may show in the acanthotic nævus or hard mole not only superficially but as "horn cysts" (Fig. 6). The fully developed nævus cell is comparatively large with vesicular lightly chromatic nucleus and is more or less polyhedral in shape (Fig. 3). In embryonic life or early infancy it is apposed

to neighbouring nævus cells, although often in very loose fashion ; in later life the nævus cells are often separated from one another by a homogeneous substance or tissue, especially if they have been present long enough after birth to permit of this development or intrusion. The cells may be pigmented or non-pigmented ; active or atrophic ; polygonal, dendritic or fusiform, and they have often an epidermoid nucleus and cytoplasm (Fig. 3). Their aggregate arrangement tends to be alveolar (Fig. 2), so that the nævus-cell area as a whole or in its parts is demarcated from the supporting tissue which surrounds and traverses it. The pigmented cells tend to occur in greater number in the more superficial areas. The supporting tissue is not, as a rule, very abundant within the denser cellular areas, but in the deeper parts of the growth a diminution of the nævus-cell groupings, with their assumption of strand formation, is accompanied by corresponding increase of fibrous tissue. Nerves and blood vessels are associated with the nævus-cell aggregates and may be so predominant as themselves to constitute part of the malformation. We must not, because of this, confuse the neurofibroma (Fig. 64) or the angioendothelioma with the nævus-cell tumour proper. Many and sometimes puzzling variations occur from the typical nævus-cell growth. The epidermis and appendages may show hyperplasia, large hypertrophic hairs (Fig. 5) and excess of hair follicles (Fig. 8). In addition to the nævus-cell aggregates in the dermis there may be projection downwards of hyperplastic epithelial processes (Fig. 2) and their marginal cells may show loosened nævus-cell character. We do not know how far the lower animals show moles of the characteristic human nævus-cell type.

The second benign human form, the hard mole, which may also be an animal form, is the important " acanthotic nævus " (Figs. 4, 6, 7 and 9). Its importance lies in this, that there are usually no nævus cells whatever present in the dermis. The line of demarcation of the growth from the dermis is sharp, straight and often in line with the normal deep epidermal edge (Figs. 4, 7). The composing cells are unmistakably epidermal, although mainly of a rather immature squamous type. They have pavement arrangement but usually lack intercellular bridges. The intracellular pigment is usually rather sparse but may be abundant (Fig. 9). There may be very marked hyperkeratosis and horn cyst formation (Fig. 6) in the acanthotic

nævus, and the malignant tumour which may develop from it is a definite squamous-cell carcinoma.

The architecture of the nævus-cell tissue in the dermis is sometimes reminiscent of neural elements ; in the deeper parts it suggests neurofibromatosis, while more superficially it may form caricatures of neural tactile terminations, the Wagner-Meissner corpuscles (Figs. 12, 13, 27, 28, 29). As a rule only a few of the nævus-cell aggregates show more than a partial approach in appearance to these concrete and striking structures. It has to be remembered, however, that Wagner-Meissner corpuscles are not common structures, are mainly restricted in distribution to the palm and soles and the pulp of fingers and toes. One argument, therefore, against the essentially Wagner-Meissner composition of the benign melanoma is that the tumour does not commonly develop in situations where these sensory tactile terminations are most numerous.

Malformations other than pigmented moles are very common in the skin, and we have angiomatous, myomatous, lipomatous and neurofibromatous growths which are likewise given the designation nævi or birth-marks. They may be combined with the melanotic nævus, thus forming the complex or mixed nævus. It is better, however, to restrict the term benign melanoma to the single-type nævus-cell growth. The melanotic cell itself, as has been mentioned, may have a dual origin and be epidermal or dermal. We have purposely refrained from any description of the cells of Langerhans as cells of tactile sensation or melanotrophic function and of the Merkel-Ranvier tactile epidermal cells (Fig. 74), as having any proven connection with nævus cells or nævus-cell malformations.

The Benign Ocular Melanoma.—The ocular melanoma has to be considered in close conjunction with the cutaneous melanoma, for in the eye we have two sets of conditions in the human being which are apparently different from those which exist in the skin and which must be linked up histogenetically with melanoma in general. The eye itself is an outgrowth from the neural tube (Fig. 72) ; it is therefore an outcrop of the brain itself. Any epithelium, except that of the lens, which enters into the constitution of the eye is neuroepithelium and its retinal melanoblasts (Figs. 72, 73) are neuroectodermic. The other feature of the architecture of the human eye which demands a pronouncement is the presence of diffusely, more

or less evenly distributed pigment-bearing cells in the choroid (Fig. 60). The pigmented choroid, in our view, represents in the human and animal eye the mesodermic homologue of the periaxial and perimedullary, often perivascular or pericœlomic, pigmented sheath (Fig. 73) of the lower vertebrates. It is comparable, in the eye, with the leptomeninx of the cerebrum ; in both and in many other situations a hyperpigmentation, a melanosis or " melanoblastosis " may occur, for example in the sheep and horse (Fig. 71). The dendritic pigment-bearing cells of the choroid we regard as melanocytes—chromatophores in the terminology of Ribbert—not as segregated or wandering cells. In the conjunctiva the conditions of development of moles are merely those of a modified skin and may vary from a conjunctival " freckle " to a more pronounced mole. It may be said generally that in situations in which melanosis can be demonstrated there may be abnormal collections of melanoblasts and melanocytes. We have few examples of benign melanoma of the conjunctiva (Fig. 53), although malignant growths are not uncommon (Fig. 54). In the iris abnormal pigmentation is observed in man, and in some animals, such as the horse, benign melanoma formation at the pupillary border is very common (Fig. 69).

Benign growths of the retina have very much the same characteristics as superficial forms ; the component melanoblasts of the retina are, both embryogenetically and morphologically, epithelial. The epithelium is now, however, neuro-epithelium. In the human embryo pigmented cells are not recognisable in the choroid until the fifth to seventh month or later and make their first independent appearance in its outer layer beneath the sclerotic. Retinal neuro-epithelial melanin pigmentation is already apparent in the outer wall of the optic cup of the six-weeks (10 mm.) human embryo.

Malignant Melanoma.—These may be considered as mainly cutaneous and ocular. In our view and in our material, in spite of a puzzling polymorphism, these have been, in the human being, all malignant epithelial tumours ; that is to say, the heading of this section might be melanocarcinoma. We exclude the probability of melanosarcoma.

The polymorphic characters of these malignant tumours are well illustrated by a comparison of the cytology and architecture in Figs. 19-22, 30-39. This polymorphism, often exemplified in one and the same tumour (Figs. 19-22),

makes it difficult to describe a *typical* malignant melanoma. In general, it is a tumour composed of cells with vesicular nuclei and often with a prominent nucleolus. The cells frequently form a loose pavement epithelium even when, as in the sarcomatoid type, they are individually fusiform and collectively fasciculate. The cytoplasmic staining reaction is also unlike that of the truly sarcomatous fibroblast, and this facilitates the diagnosis of the malignant growth as epithelial. Genetic stages—so important in the diagnosis of the type of a malignant growth—can often be traced in the skin (Figs. 40-43, 46, 47) and provide a positive argument for the origin in epidermis or its appendages of the malignant cutaneous melanoma. In the eye the malignant melanoma is often described as choroidal, and it is not always as easy in the eye to find epithelial genesis as it is in the skin. The malignant ocular growth is more commonly, in the first instance, intra-ocular with detachment of the retina (Fig. 59), although in time it involves the choroid.

The architectural arrangement of melanocarcinoma at the periphery is mainly alveolar (Fig. 22) so far as the relation of tumour cells to invaded supporting tissue goes. Necrosis may occur and a very characteristic perivascular, often called peritheliomatous, appearance is produced, which is due to survival of those cells which are closest to the blood supply (Fig. 32). The perivascular arrangement of the melanoma may, however, occur without necrosis (Fig. 31). If the decision of the nature of the melanoma rested with the common human malignant forms, the continued controversy on the subject would probably settle down overwhelmingly in favour of a carcinoma, even if portions of a malignant tumour may appear fibrosarcomatous (Fig. 21) or even leiomyomatous (Fig. 38). The verdict in favour of an epithelial origin of the malignant growth is borne out by the common mode of spread by lymphatic vessels (Fig. 63) to regional lymph nodes, although spread by the blood vessels is also common in the late stages. The metastases themselves, in human cases, are as epithelial in appearance as the primaries (Figs. 49, 50). The important histological diagnosis of malignancy is often a difficult one and particular emphasis must be laid on any clinical note that increase in size of a mole has recently taken place, with or without local causal irritation. A quiescent mole removed for purely cosmetic reasons may show features microscopically

which raise suspicions of malignancy. Histological appearances which raise or confirm such suspicions are : signs of increasing pigmentation, epidermal downgrowth or spray formation into the cutis ; solid alveolar and perivascular (perithelial) arrangement of cells ; cellular instability extending beyond the obvious tumour area and formation of numerous epidermal loose cells or " nests " ; cellular polymorphism and increase in cell size ; hyperchromatism and mitotic figures. A peripheral lymphocytic stroma infiltration is a helpful diagnostic feature in cases of early melanocarcinoma, as it is in other forms of tegumentary carcinoma. We have no personal experience of the rare condition known as progressive blue melanosis, which may, it is said, end in death with metastasis. The manifold morphological appearance of the melanoma is such an outstanding feature and the architectural patterns produced—solid alveolar (Fig. 30), floral (Figs. 34, 36), perithelial (Figs. 31, 32), papillary (Figs. 20, 35), fascicular (Figs. 21, 38)—that one might almost say, " When in doubt regarding the nature of a skin or ocular tumour, suggest melanocarcinoma even if no melanin is present." The cytology of melanocarcinoma has as extensive a range as the histology. Melanoblasts, the precursor or stem cells of the tumour with little or no pigment and melanocytes, the pigmented, fully differentiated cells incapable of further proliferation, are both represented in the malignant tumour. Melanocytes may be present in large numbers even in the very malignant forms, and this fact is shown in the extreme blackness of many primary growths and metastatic deposits. In all these tumours, however, the melanophage is also a constant feature. It is admittedly difficult to distinguish from the melanocyte. The matter becomes especially important when the diagnosis has to be made of the extent of involvement of regional lymph nodes by metastatic true tumour cells or merely phagocytic cells ; some of the pigmented cells in the node need not be tumour cells but " reticulo-endothelium " with ingested pigment ; the presence of melanotic cells in a node argues, however, in favour of metastasis.

Great differences exist in the degree of malignancy of the melanomas. Cutaneous recurrences may continue to appear in the primary drainage area and yet the final widespread dissemination may not take place for 10 to 15 years. In very rare cases regression and detachment of tumours have been observed (Figs. 16, 17). In other cases, spread may occur

with incredible rapidity. The patient may develop a widespread melanomatous distribution (Fig. 23) and new nodules appear in the skin overnight. This possibility indicates the necessity of extreme care in interfering with moles and of wide removal when dealing surgically with those suspected of being malignant.

Our illustrations with their legends will supplement these descriptive sections. The material we have examined for this research includes: 799 human cases, of which 283 were benign, 516 malignant; and 66 animal cases. The localisation of the human tumours is as follows: skin, 571; eye, 76; other sites, 152. In addition we have examined much tissue illustrative of melanin pigmentation in general.

Discussion.—We consider it our duty, although a departure from our rule, to acknowledge obligations to that detailed, fully illustrated, carefully reasoned monograph from this laboratory on "The Melanomata," by the late Dr James W. Dawson as the source of much of our knowledge and an inspiration to this discussion. The idea of a wholly neural origin for the melanoma necessitates also a tribute to the creative brilliance of P. Masson and his disciples.

The problem of the melanoma is still debatable. Modern embryology, which deals with "organisation," "morphogenetic fields," "induction" and various stages of cell "determination" and potency may bring the best solution and place the explanation not only under the heading of malformation in a purely morphological sense but also of malinduction and mal-determination in the sense of misdirection of growth energy. It may be well to clear the ground for our discussion by stating that we divide the purely unitary theories of origin of the melanoma into the epidermalist, neuralist and mesodermalist. It seems further desirable to separate the localities of occurrence of primary human melanomas into the main categories of skin, eye and, possibly, meninges.

The epidermalist thesis is essentially one of sequestration and detachment of epidermal tissue to form the quiescent nævus-cell tumour of the skin; the neuralist is the extension of the concept of neurofibromatosis to explain the nævus-cell tumour as either neuroma, neurinoma, or "neuroneurinoma" of tactile nerves, while the mesodermalist is contention for a specific melanoblast of purely mesodermal origin which would

justify the creation of the special term " chromatophoroma " for the nævus-cell tumour. The epidermalist view restricts the explanation of melanoma ontogenesis in the skin to tegumentary ectoderm and its appendages, for, with the transference of discussion to the ocular melanoma, it follows necessarily that the derivation must be from the neuroectodermic retinal pigment layer of the eye. According to the neuralist view, we are asked to accept a great variety of difficult propositions designed to emphasise the wholly neural character of the nævus-cell tumour, involving the inclusion in the tumour composition of specifically named cells or organs, such as the Langerhans' cells, the sensory intraepidermal tactile cells of Merkel-Ranvier, the dermal Wagner-Meissner corpuscles, neurilemma sheath cells and medullated or non-medullated nerves. In the mesodermalist view there is the simplicity of one specific tumour cell type, the " chromatophore." This " chromatophore," although etymologically a pigment-carrier cell, is regarded as a melanoblast and a specific cell type. The cutaneous, choroidal and meningeal melanomas are thus all easily included in one tumour type, the " chromatophoroma," at least as long as they remain benign. The difficulties of this theory, as also of the neuralist view, appear to us to arise when we have to explain the human malignant melanomas and their metastases as being other than carcinomas.

If we ourselves recognise, however, that in each of these three views there is an element of truth which should be reckoned with, it may render it easier for us to put forward a reasonable view of the nature of the melanoma which still upholds the genetic relationship of nævus cells in the human being to embryonal ectodermic cells, and explains the cutaneous nævus-cell tumour as one or other grade of a malinduction process in skin and appendages. There is also the distinct possibility to be considered that melanogenic function is possessed by several types of cells, such as the basal epidermal epithelium, retinal epithelium, dendritic melanoblasts in the cutis and ganglionic nerve cells, although these cell types do not necessarily belong to the same blastodermic layers.

All three theories seem to agree substantially on this, that the mole or benign nævus-cell tumour is a malformation or dyscrasia of antenatal origin. The disputation centres round the cytology and histology of this peculiar malformation. In our view the nævus-cell tumour is a complex, malformed,

morphogenetic field in more or less stable equilibrium, in which the component cells or structures are developed or continue to develop *in situ*. We conceive that the structures concerned may be: (1) epithelial and representative of malinduction of epidermis and its appendages—hairs, sebaceous and sweat glands; (2) in one type—the acanthotic nævus—frankly tegumentary epidermis, which is present in excess and has failed to develop appendages; (3) dermal elements, by which we mean connective tissue and the vascular and neural elements found in the dermis. This last category is one of our concessions to the neuralist and mesodermalist views and may explain occasional features of the benign skin melanoma. Even in the human subject mesodermal melanocytes may account for the blue nævi, Mongolian spots, benign choroidal and meningeal melanosis and for melanosis in various other situations. Melanogenesis in two blastodermal layers has been a source of difficulty to epidermalists, mesodermalists and neuralists alike. In the human being and some of the higher mammals we regard the ordinary melanoblast and melanocyte of the skin as epidermal; the epidermis has become the host tissue of these cells and they have become, thereby, wholly epithelial cells. We conceive that there has been a progressive advancement in man of the melanoblast to purely epithelial status, or a simple evolutionary disappearance of the specific dermal melanoblast of the lower vertebrates.

Though we regard the human melanotic malformation or tumour as essentially epithelial, this position is quite compatible with participation in melanoma architecture of neural or mesodermal elements. We do not, however, elevate either of the latter elements into the position of the chief or only agent in its production in the case of man. We conceive that the mechanism of production of the nævus-cell tumour in the skin is the presence of a primordial morphogenetic field from which should emerge epidermis, hair follicles, sebaceous and sudoriparous glands, and dermis with its blood vessels and nerves. Owing to malinduction, these fail in greater or less degree to develop normally, and we are left with the products or vestiges of that failure or with the actual nævus-cell tumour. The malinduction process, though usually antenatal, may continue slowly acting into adult and old age. The important melanoblasts represented in fish, amphibians, reptiles and many subhuman species by large, specific, dendritic, epithelioid,

pigmented cells situated in the mesodermal tissue of the skin and only to a lesser extent by epidermal cells, have in man become entirely epidermal in appearance, function and situation. There is no reason to suppose that the melanin-bearing cell of the basal epidermis is not truly epithelial or that it is not independently elaborating its own melanin. With failure of the morphogenetic field to produce its normal structures, there is left only imperfect structural development with much scattered but still regular cellular debris and often monster elements, such as multinucleated giant cells, hypertrophic hairs and sebaceous glands. Loosening of epidermal cells is an indication of epidermal instability. That these cells should round off and lose their processes and intercellular bridges is only what we should expect of any detached epithelial cell. The actual visible continuity, in many cases, of epidermal cells and interpapillary epithelial processes with the underlying tumour cells is strong evidence that they are identical cells (Figs. 40-42). We do not subscribe to any attempt to identify the mole with a neurofibroma. If that view were adopted it would be but a step to call all cutaneous malformations neural and thus to extend very widely the term "neurofibromatosis" of von Recklinghausen. That to us seems altogether too simplifying and over-emphasises neural participation.

The evidence for ectodermal tumour genesis is even stronger when we consider the definitely malignant type of growth. Although malignancy does develop in the mole of the skin and the eye, we are of the opinion, from examination of our own abundant material, that in the human being the malignant forms were carcinomas only, epidermal or neurocular carcinomas and not sarcomas. The carcinoma character of the malignant melanoma is brought out in its purity in many metastases. The metastases may attain far greater dimensions than the primary tumour, and this fact alone demands that special search be always made for an insignificant primary before adjudicating on tumours in unexpected sites as primary. Although we do not deny the dictum that where there is melanosis there may be melanoma and that primary choroidal and meningeal melanomas may occur, we find, as we have said, in our own human malignant material only the evidence of carcinoma. We cannot speak from any experience of the malignant meningeal melanoma, nor have we ever heard of a

melanotic tumour of the substantia nigra of the brain. The condition of melanosis of the meninges, even when pronounced, is scarcely a neoplasm. Recently published work has certainly brought out very carefully considered evidence of primary meningeal melanoma, but we think it is still safe to say *not* positive proof.

Some of the details of our views will be found in our legends and illustrations, and we have endeavoured, by the use of specific staining methods and selected material, to give due weight to other theories. It seems justifiable to state that no specific staining method can distinguish between the nerve sheath cell and the fibroblast. We have tried to be impartial in presenting illustrations: in the skin of Wagner-Meissner corpuscles, Merkel-Ranvier cells, neural-like melanomas, etc., while in the case of the eye, meninges and connective tissue, we seek to illustrate the occurrence of melanosis and the possibility of occurrence of mesodermal melanoma. We admit tumour origin from neuroectoderm and possible origin from a mesodermal "chromatophore" and the combination of neural elements in the malformations, but not the description neural tumour for the skin melanoma. If a nævus tumour has a preponderating neural character it had better be called neurofibroma or neurinoma and not melanoma, even if pigmented cells accompany it.

The whole subject of melanin formation and function with its normal and pathological manifestations, as in the skin patterns of animals; the melanosis of mammals, fishes and amphibians; xeroderma pigmentosum; lentigo maligna, vitiligo and albinism; scars; etc., has great importance for genetic and inheritance problems and almost as great an importance for the theory of neoplasia. Many of the melanoses, well demonstrated in certain hybrid fishes, may represent an inherited endocrine dyscrasia, possibly adrenal; such a condition may also imply a hyperplastic or neoplastic tendency liable to eventuate in a progressive melanosis and in certain conditions to become a melanomatosis. The melanin or melanogen oxydase is probably not entirely specific and is almost certainly closely allied to tyrosinase and phenoloxydases. Melanin is on occasion found in unusual localities such as the intima or adventitia of blood vessels, the lung, the cells of the liver, the kidney (Fig. 52), the intestine and some of the cells of the nervous system (Fig. 68). Here its presence may constitute

a form of " melanosis " or may represent phagocytosis by fixed histiocytes. It is, however, necessary in this connection to refer to the chemical difficulties of distinguishing between melanin, lipochromic and hæmatogenous pigments. The term melanin is still somewhat loosely applied. It is a brown to black pigment which microchemically gives no iron reaction, no fat reaction, may be demonstrated and produced by the interaction of the amino-acid dihydroxy-phenylalanine and its specific oxydase, and can be bleached by hydrogen peroxide.

In terminating this discussion, we may sum up the difficulties which must be faced. These are that, embryologically, some workers trace origin of melanomas to tegumentary or neuro-ectoderm, to residue of invagination of the neural tube, to either of the two blastodermic layers, ecto- or mesoderm, and, histologically, to cells situated in epidermis, connective tissue, retina, choroid and meninges respectively, or to structures representing nerve sheaths of tactile nerve terminations. It is improbable that any of these origins can be correct in a purely unitary sense.

Conclusion.—The benign melanoma or nævus-cell tumour of the skin is a complex malformation of which the principal component cells are melanoblasts or melanocytes ; these are pigment-forming cells and, in man, in whom melanogenic potency has become restricted (" determined ") to epithelium, the common nævus-cell tumour is a manifestation of mal-induction of the epidermis and its appendages. One form of the nævus-cell tumour, the acanthotic nævus, is wholly epidermal. In the human eye and meninges there are found choroidal and meningeal melanotic cell aggregations of the mesodermic pigment-cell type. Malignant melanoma in the human being is a carcinoma, epidermal melanocarcinoma in the skin, neuroectodermal carcinoma in the eye. Gaps still exist in our knowledge of mesodermal melanoma and a strict unitary conception of the melanoma may have to be abandoned, nor may the existence of neural melanoma be summarily dismissed.

It may be well to emphasise that the legends to the illustrations contain text matter.

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(*To be continued.*)

NOTES, CLINICAL AND HISTOLOGICAL, ON ILLUSTRATIONS.

FIG. 1.—*Nævus-cell tumour.* Benign melanoma. Female aged 30. " Small wart on chest wall which has become blacker past 2 months." Showing nævus-cell aggregation in dermis and hyperkeratosis. 516/39. ×2.

FIG. 2.—*Nævus-cell tumour.* Same section as in Fig. 1. Showing (*a*) nævus-cell groupings in " nest " and strand formation occupying papillæ and upper dermis; (*b*) cap of keratinised epithelium; (*c*) elongated rete pegs; (*d*) straight edge of nævus growth against collagenous dermal fibrous tissue and absence of lymphocytic infiltration. 516/39. ×100.

FIG. 3.—*Nævus-cell tumour.* Same section as in Figs. 1, 2. Showing nævus-cell grouping in solid " nests " of pigmented cells, with some tendency to whorl formation. 516/39. ×250.

FIG. 4.—*Acanthotic nævus.* Benign melanoma. Hard mole. Female aged 55. " Wart increasing in size." Showing straight edge of the growth against the dermis and in line with the basal layer of normal epidermis on either side; several horn cysts; no nævus cells in dermis. 170/36. ×2.

FIG. 5.—*Nævus-cell tumour.* Hairy pigmented mole. Benign melanoma. Boy aged 14. " Hairy mole on forearm present since birth, expanding proportionately as child grew." Showing hyperplastic, hypertrophic hair emerging from a nævus-cell growth. Courtesy of Dr R. L. Sutton, Jr., Kansas City, U.S.A. 5820/38.

FIG. 6.—*Acanthotic nævus.* Benign melanoma. Man aged 70, with pedunculated dark-brown tumour, 1 inch diameter, on abdominal wall. Microscopically there was much pigment, hyperkeratosis and acanthosis, numerous horn cysts and sharp delimitation against dermis. Showing some of the horn cysts. 7497/35. ×25.

FIG. 7.—*Acanthotic nævus.* Benign melanoma. Man aged 53. " Pigmented tumour over scapula, no deep attachment, present many years." Microscopically moderately pigmented with very keratotic surface layer. Showing early " pearl " or " nest " formations; surrounding tumour cells of primitive epidermoid type; straight edge against dermis. The cells of the nests show vesicular nuclei with prominent nucleoli. 799/34. ×150.

FIG. 8.—*Nævus-cell tumour.* Benign melanoma. " Cyst of face, duration some months." An example of nævus malformation of pronounced hair-field type. Showing numerous hair shafts, many abortive, some hypertrophic; aggregates of nævus cells throughout the dermis, mostly atrophic; some pigmented cells. 9467/39. ×40.

FIG. 9.—*Acanthotic nævus.* Benign melanoma. Male aged 70. "Tumour from skin of thigh; present many years, recent enlargement." Showing pavement and epidermoid character of the tumour; in this area much intracellular pigment present, thus characterising growth as melanoma. Melanin pigment is often sparse in the typical acanthotic nævus. 7242/38. ×300.

FIG. 10.—*Pigmented fleck.* Man aged 31. "Pigmented spot on lower lip, extending past 3 weeks; first noticed a year ago; no thickening palpable in area of spot." Showing deeply pigmented basal layer of tegumentary epithelium, with no special nævus-cell character. 1510/34. ×800.

FIG. 11.—*Nævus-cell tumour.* Man aged 58. "Slow-growing tumour on skin of neck." Showing nævus cells around and in the course of a hair shaft. Illustrative of the dysontogenetic hair field. Courtesy of Dr R. L. Sutton, Jr. 8915/38. ×40.

FIG. 12.—*Nævus-cell tumour.* No history. Showing the most pronounced neuro-nævoid structure of the nævus-cell tumours in our collection. C.G. 172. ×100.

FIG. 13.—*Nævus-cell tumour.* Same section as in Fig. 12. Showing structures with striking resemblance to Wagner-Meissner tactile corpuscles. C.G. 172. ×250.

FIG. 14.—*Nævus-cell tumour.* Same section as in Figs. 12, 13. Showing a superficial area of pigmented nævus-cell "nests," with the component cells in close apposition (pavement character). C.G. 172. ×250.

FIG. 15.—*Malignant melanoma.* Man aged 41. First lesion noticed, 18 years previously, was a small reddish-brown warty mole on skin of upper abdominal wall. Ten years later this was as large as a walnut; was irradiated and disappeared but the skin was burnt. Four years later a number of small secondary tumours appeared at margin of burnt area; these and the ulcer were excised. Three months later abdominal tumour recurred with evidence of widespread cutaneous secondaries (almost a hundred). These, apart from the abdominal lesions, showed a very unusual tendency to regress by pedunculation or sloughing, leaving no trace or only an area of skin pigmentation. Melanuria. Death about 19 years after appearance of initial lesion. Courtesy of Dr G. H. Percival and Mr J. J. M. Shaw, F.R.C.S., Edinburgh. 7236/29.

FIG. 16.—*Malignant melanoma.* Same case as in Fig. 15. One of the smaller tumours removed. Microscopically very malignant, but fibrosing at the base. 7236/29. ×2.

FIG. 17.—*Malignant melanoma.* Same case as in Figs. 15, 16. Showing one of the smaller secondary tumours which sloughed off. A striking example of spontaneous regression of a malignant tumour. 7236/29. ×2.

FIG. 18.—*Malignant melanoma.* Same case as in Figs. 15-17. A large section of the main fungating mass on abdominal wall (Fig. 15). Showing papillary and solid areas of the tumour still extremely superficial. The polymorphic histology of the malignant melanoma is well shown in the next four figures (19-22) taken from different areas of this large section. Other melanoma "patterns" are shown in Figs. 30-39. 7236/29. ×3/5.

FIG. 19.—*Malignant melanoma.* Same case as in Figs. 15-18. Showing an area of pavement carcinoma and epidermoid cells with vesicular nucleus and prominent nucleolus. 7236/29. ×250.

FIG. 20.—*Malignant melanoma.* Same case as in Figs. 15-19. Showing alveolar cell arrangement traversed by fine fibrovascular cores. This arrangement of large loose cells round an apparent central space with papillary architecture is, when present, very characteristic, if not diagnostic of melanocarcinoma, even if no pigment is found. 7236/29. ×150.

FIG. 21.—*Malignant melanoma.* Same case as in Figs. 15-20. Showing an area of fusiform-cell type in stream-line arrangement which might be taken for fibrosarcoma or even neural tumour. 7236/29. ×250.

FIG. 22.—*Malignant melanoma.* Same case as in Figs. 15-21. Showing a solid area, sharply defined against dermal fibrous tissue in which pigmented cells (melanophages) are abundant. The pigmentation of the tumour cells is not evident at this magnification. 736/29. ×150.

FIG. 23.—*Malignant melanomatosis.* Man aged 53. The first tumour lesion was originally considered a sebaceous cyst and excised. On admission to the Cancer Hospital patient had a subcutaneous nodule on chest; later, numerous cutaneous and subcutaneous nodules appeared over trunk and limbs; diagnosed pathologically as non-pigmented melanocarcinoma, with positive dopa reaction. No melanuria. General condition remained good, though superficial nodules continued to appear in rapid succession. Showing some of the nodules on trunk and arm. Courtesy of Dr Leila M. Hawksley, Royal Cancer Hospital, Free, London. 9816/39.

FIG. 24.—*Malignant melanoma.* Metastatic nodule in lung. Showing solid alveolar areas surrounded by fine strands of fibrous tissue or reticulum. Stained Wilder silver impregnation. 75·43. I.A.P. ×150.

FIG. 25.—*Malignant melanoma.* Pigmented tumour of the skin. Showing single tumour cells separated by fine fibrous tissue strands or reticulum. Stained Wilder silver impregnation. 71·38. I.A.P. ×250.

FIG. 26.—*Moles on face.* Chart of distribution of moles, 100 individuals. Showing no recognisable association with the embryo clefts of the face.

FIG. 27.—*Wagner-Meissner corpuscle.* Sole of foot. Showing the terminal ramifications of the nerve in a tactile corpuscle situated in a skin papilla. Silver impregnation. 452/30. ×100.

FIG. 28.—*Wagner-Meissner corpuscle.* Finger-tip. Showing the entire cylindical tactile corpuscle, consisting of cells forming transverse lamellæ at right angles to long axis. Masson's trichrome stain. 317/39. I.A.P. ×250.

FIG. 29.—*Malignant melanoma.* Female aged 70. "Wart from chest wall, present all her life; enlarging and ulcerating 4 months." Microscopically a highly malignant pigmented melanocarcinoma with varied but characteristic patterns and extensive lymphatic invasion. Showing cylindrical cell "nests" with appearance very similar to the Wagner-Meissner carcicatures in the nævus-cell tumour, but with unmistakable epidermal genesis in much of its surface. 174/39. ×250.

The following figures, 30-39, are shown to illustrate some of the special types and "patterns" of malignant melanoma. Other patterns are seen in Figs. 19-22.

FIG. 30.—*Malignant melanoma.* Man aged 36. "Pedunculated black mole right flank, present a few years, and large, hard, painless lymph node in groin of 4 months' duration." Showing a melanocarcinoma of two different architectural patterns in close proximity and abundant melanin pigment. A third pattern is shown as metastasis in Fig. 31. 5982/38. ×100.

FIG. 31.—*Malignant melanoma.* Same case as in Fig. 30. Metastasis in lymph node. Showing a melanoma pattern, the "perithelial," with numerous melanophages. The architecture, in this case, is not due to perivascular survival and intervascular necrosis. 5982/38. ×40.

FIG. 32.—*Malignant melanoma.* Eye. Advanced growth invading choroid and sclera. Showing perivascular ("perithelial") tumour cell survival and intervening necrosis, a not infrequent appearance in ocular melanoma. C.G. 2. ×60.

FIG. 33.—*Malignant melanoma.* Eye. Another area of tumour illustrated in Fig. 32. Showing sheets of tumour cells of very epidermoid character with vesicular nucleus and prominent nucleolus, in loosened pavement arrangement. C.G. 2. ×500.

FIG. 34.—*Malignant melanoma.* Metastatic subcutaneous nodule left arm. Female aged 37. "Right eye removed 4 years ago for 'tumour'; hemiplegic attack 2 months ago . . . arteriograms suggest tumour in stem of right sylvian fissure; within past few weeks widespread subcutaneous nodules have appeared; clinically suggests melanoma." Showing an unusual perivascular arrangement of tumour cells, cut longitudinally and transversely. Courtesy of Mr A. R. D. Pattison, F.R.C.S., Newcastle. 5562/38. ×150.

FIG. 35.—*Malignant melanoma.* "Tumour of foot, inguinal nodes greatly enlarged." Showing a striking melanoma picture with parallel fine fibrovascular cores separating elongated tumour areas. 9861/33. ×100.

FIG. 36.—*Malignant melanoma.* Showing a pattern of small cellular aggregates in close juxtaposition with few fibrovascular cores. 1498/39, 12. ×150.

FIG. 37.—*Malignant melanoma.* Male aged 60. "Pedunculated infected tumour of scalp; duration 6 months." Showing polymorphism and a general loose monster-cell pattern. 2369/37. ×250.

FIG. 38.—*Malignant melanoma.* Broadly pedunculated mole; its epidermal genesis is illustrated in Fig. 41. Showing fusiform-cell bundles, cut longitudinally and transversely, in dermis, very reminiscent of muscle structure; a rare pattern. Illustrated macroscopically in Monograph, J. W. Dawson, *Edin. Med. Journ.*, Plates B, 33 and C, 6. 1498/39. ×150.

FIG. 39.—*Malignant melanoma.* Lymph node. Man aged 69. "Swelling of groin gland 9 years after removal of malignant melanoma on heel; no sign of recurrence for 7 years and now no recurrence at primary site nor between heel and groin. Patient otherwise healthy." Showing large free melanophages distended with pigment (*a*) and tumour tissue (*b*). 4251/35. ×250.

FIG. 40.—*Malignant melanoma.* Skin. Showing how an epidermal process is transformed into malignant melanoma. The prickle-cell character of the malignant cells is evident (*a*) and brought out in the high-power illustration of this same area in Fig. 44. 1498/39, 12. ×100.

FIG. 41.—*Malignant melanoma.* Skin. Same section as in Fig. 38. Showing the epidermal genesis of the tumour; the deeper invading parts show the resemblance to muscle bundles noted in Fig. 38. 1498/39. ×100.

FIG. 42.—*Malignant melanoma.* Skin. Showing an epidermal process which in its lower part presents one of the characteristic malignant melanoma pictures. 198·47. I.A.P. ×100.

FIG. 43.—*Malignant melanoma.* Skin. Showing rete process with entirely pigmented unstable epithelium, at advancing edge of a malignant tumour. 1498/39, 10. ×300.

FIG. 44.—*Malignant melanoma.* Skin. Same section as in Fig. 40. Showing the prickle-cell character of the malignant cells in area (*a*) of Fig. 40. 1498/39, 12. ×800.

FIG. 45.—*Malignant melanoma.* Skin. Showing part of the malignant tumour and the surrounding skin. The skin all round the growth showed proliferation, with intra-epidermal loose cell-nest formation (Fig. 46) and what may be called cellular instability. It illustrates the necessity for great care in interfering with moles and the advisability of wide excision. 1498/39, 13. ×2.

FIG. 46.—*Malignant melanoma.* Skin. Showing the unstable skin surrounding the actual tumour of Fig. 45, with cell-nest formations, loosening epithelium and lymphocytic cell infiltration in the adjoining dermis. 1498/39, 13. ×120.

FIG. 47.—*Malignant melanoma.* Skin. Showing unstable epidermis in skin beyond obvious tumour. Its "pagetoid" appearance is striking, especially in association with the dense lymphocytic cell infiltration (*cf.* Fig. 48). 1498/39, 5. ×100.

FIG. 48.—*Paget's disease of the nipple* associated with mammary cancer. Female aged 67. Showing characteristic Paget cells in the basal epidermis and subepithelial lymphocytic cell infiltration (*cf.* Fig. 47). 218/39. ×100.

FIG. 49.—*Malignant melanoma.* Metastasis in lung, showing clear-cut edge against lung tissue: highly pigmented cells of very epithelioid character. 1498/39, 7. ×150.

FIG. 50.—*Malignant melanoma.* Lung metastasis. Showing marked variation in melanin content of the tumour cells and highly epithelioid character. 1498/39, 5. ×800.

FIG. 51.—*Generalised malignant melanoma.* Kidney. Female. Primary mole on forehead accidently scratched; a generalised melanomatosis developed with death 14 months after injury. Showing tumour cells in the capillaries of glomeruli. Tumour cells were found in nearly all the glomerular and intertubular capillaries. Melanuria is a late sign of generalised melanomatosis. 1684/37, 27. ×250. Monograph, J. W. Dawson, Plate XI.

FIG. 52.—*Generalised malignant melanoma.* Kidney. Same tissue as in Fig. 51. Showing melanin pigment in the tubular epithelium. 1684/37, 27. ×250.

FIG. 53.—*Nævus-cell tumour.* Benign melanoma. Conjunctiva. Male aged 12. "Small pigmented growth in caruncular region of right eye; there are multiple small pigmented growths of skin of body." Showing diffuse distribution of nævus cells in the subepithelial tissue. (*a*) Conjunctival epithelium; (*b*) Meibomian glands; (*c*) nævus cells. The nævus-cell tumour of the conjunctiva does not differ from that of the skin. 7016/32. ×150.

FIG. 54.—*Malignant melanoma.* Conjunctiva. Male aged 74. "Melanoma of right conjunctiva; painless pigmented swelling of lower fornix; 6 months' duration." Showing derivation of malignant growth (*a*) from conjunctiva epithelium. 7088/35. ×80.

FIG. 55.—*Nævus-cell tumour.* Benign melanoma. Skin of chest. African infant aged 6 weeks; there were a number of moles elsewhere. Showing pigmented nævus cells in dermis, which in another part of the section were continuous with the epidermis. Epidermis itself shows melanotic pigmentation of the basal epithelium. The skin of the African infant is light coloured at birth, darkens at once and is fully pigmented in a few weeks. Courtesy of Dr B. Elmes, Lagos. 4/39. ×150.

FIG. 56.—*Nævus-cell tumour.* Benign melanoma. Nose. Female aged 60. Showing multinucleated giant cells among the other nævus cells. This feature may sometimes prove useful in diagnosis. Courtesy of Dr Leila M. Hawksley. 9816/39, 12. ×250.

FIG. 57.—*Nævus-cell tumour.* Benign melanoma. Skin. Showing nævus-cell masses (*a*) distending the papillæ, with reduction of epidermis proper and its processes to thin strands. 198-42. I.A.P. ×100.

FIG. 58.—*Progressive melanosis.* Skin. Female aged 30. Slate-coloured pigmentation of skin of shoulder and upper thorax began to show at 14 years; no previous birth-mark in the area, but brown moles elsewhere. Blue area has gradually spread but no palpable change in the affected skin. Showing epidermis and abundant widespread distribution of melanocytes in the dermis. Pigment is iron-free. The condition closely resembles the melanosis of white horses and, in human beings, the "blue nævus." Courtesy of Professor Duguid, Cardiff. Published, Dr F. R. Bettley, *Brit. Journ. Derm. Syph.*, 1938, i., 181. ×100.

FIG. 59.—*Malignant melanoma.* Eye. Showing intra-ocular tumour with pigmented retinal epithelium from which it arises still intact over it. 5512/32. ×1.

FIG. 60.—*Malignant melanoma.* Eye. Same section as in Figs. 32, 33. Showing tumour arising from pigmented retinal epithelium and bounded by the lamina vitrea (*a*); choroid with pigment in its outer layer (*b*); sclera (*c*). C. G.2. ×150.

FIG. 61.—*Malignant melanoma.* Skin. Female aged 62. Dark brown nodule; patient aware of it for a few months. Showing conjunction of malignant melanoma of loose fusiform-cell type (*a*) with benign nævus-cell tissue (*b*). The malignant tissue showed extensive epidermal genesis. It is exceptional to find this conjunction in malignant melanoma sent for examination. 7170/38. ×250.

FIG. 62.—*Pigmented basal-cell carcinoma.* Skin. Man aged 62. "Hard, non-ulcerated, scaling, scar-like mass 2×3 cm. attached to sterno-mastoid muscle in centre of an X-ray atrophy; duration 10 years; X-ray treatment 10 and 8 years previously. Nodes not involved." Showing early invasive downgrowth of highly pigmented basal-cell carcinoma (*a*); fully developed typical basal-cell carcinoma (*b*); intervening zone of atrophic tissue (*c*). Courtesy of Dr R. L. Sutton, Jr. 8923/38. ×100.

FIG. 63.—*Malignant melanoma.* Skin. Same section as in Fig. 42. Showing edge of tumour (*a*); dilated lymphatic with tumour embolus (*b*). 198·47. I.A.P. ×40.

FIG. 64.—*Neurofibroma.* Skin. Male aged 42. Swellings on forearm and palm since childhood. Showing a cellular area of a neurofibromatous lesion. The neurofibrillar appearance is evident and the general cell type is that of the sheath of Schwann. It must be remembered that it was the supposed resemblance of the neurofibroma and neurinoma to the nævus-cell tumour which led to the theory of their identity. 2083/7. ×150.

FIG. 65.—*Melanosis.* Adrenal. Cow. History described multiple metastatic melanotic tumours; primary not definitely located. Showing widespread distribution of melanin-containing cells; cortical tissue (*a*); medulla (*b*). Primary adrenal melanoma has been described; it is difficult to decide in this case whether the melanin represented melanoma, melanosis or melanophagocytosis. 756/30. ×150.

FIG. 66.—*Normal skin.* Dopa reaction. Showing distribution of dopa-melanin restricted to basal epithelial cells, somewhat discontinuous. 5982/32. ×100.

FIG. 67.—*Normal skin.* Dopa reaction. Showing a basal epithelial cell with dendritic intercellular prolongations. 5982/32. ×800.

FIG. 68.—*Substantia nigra.* Human. Showing abundant melanin pigment granules in neurones. Melanin pigment in these midbrain cells is found apparently only in man and is absent even in the anthropoid apes (Le Gros Clark). Courtesy of Dr James, Repton State Institution. 917/39. ×250.

FIG. 69.—*Benign melanoma.* "Granula iridis." Horse. Showing a melanoma nodule (*a*) at the pupillary border of the iris, a common site for these small formations. 627/39. I.A.P. ×2.

FIG. 70.—*Harding-Passey tumour.* Malignant melanoma. Showing some of the features of this tumour, with epidermoid cell character, intracellular pigment and melanophages. This tumour is of particular interest because it was the first melanotic tumour to be successfully transplanted. Courtesy of Professor Passey, Leeds. 583/39. I.A.P. ×350.

FIG. 71. *Melanosis.* Horse. Showing widespread distribution of melanocytes in leptomeninx (*a*) of spinal cord (*b*). Melanosis of white horses is well known to veterinary surgeons and is apparently closely associated with melanomatosis. 1623/37, 2. ×40.

FIG. 72.—*Tadpole.* Neural tube (*a*) with optic stalk (*b*) and optic vesicle (*c*). Showing melanin pigment in cells of neural tube, in outer layer of optic cup (*d*) and in tegument (*e*). 11/39. I.A.P. ×100.

FIG. 73.—*Tadpole.* Transverse section showing eye and neural tube at a later stage of development than in Fig. 72, with separation of retinal pigmented layer (*a*) and subscleral pigmented choroid (*b*); periaxial pigment sheath (*c*); pericœlomic pigment sheath (*d*) and perimedullary pigmentation (*e*). Melanin pigment is widely distributed throughout the tissues. 13/39. I.A.P. ×40.

FIG. 74.—*Merkel-Ranvier cells.* Skin of pig's snout. Showing a group of tactile cells (*a*) with flat nuclei in the deep epidermal layers and the associated myelinated nerve (*b*). It is doubtful whether these cells, on which great stress is laid by the neuralists in the composition of melanoma, occur in man (Le Gros Clark). 45·47. I.A.P. ×500.

FIG. 75.—*Newt.* To show presence of melanin in epidermis and dermis. The perivascular distribution of dermal melanin (*d*) is well shown. 7247/39. ×150.

FIG. 76.—*Slug. Arion ater.* To show the extensive distribution of dermal melanin pigment in an invertebrate animal. 698/39. ×150.

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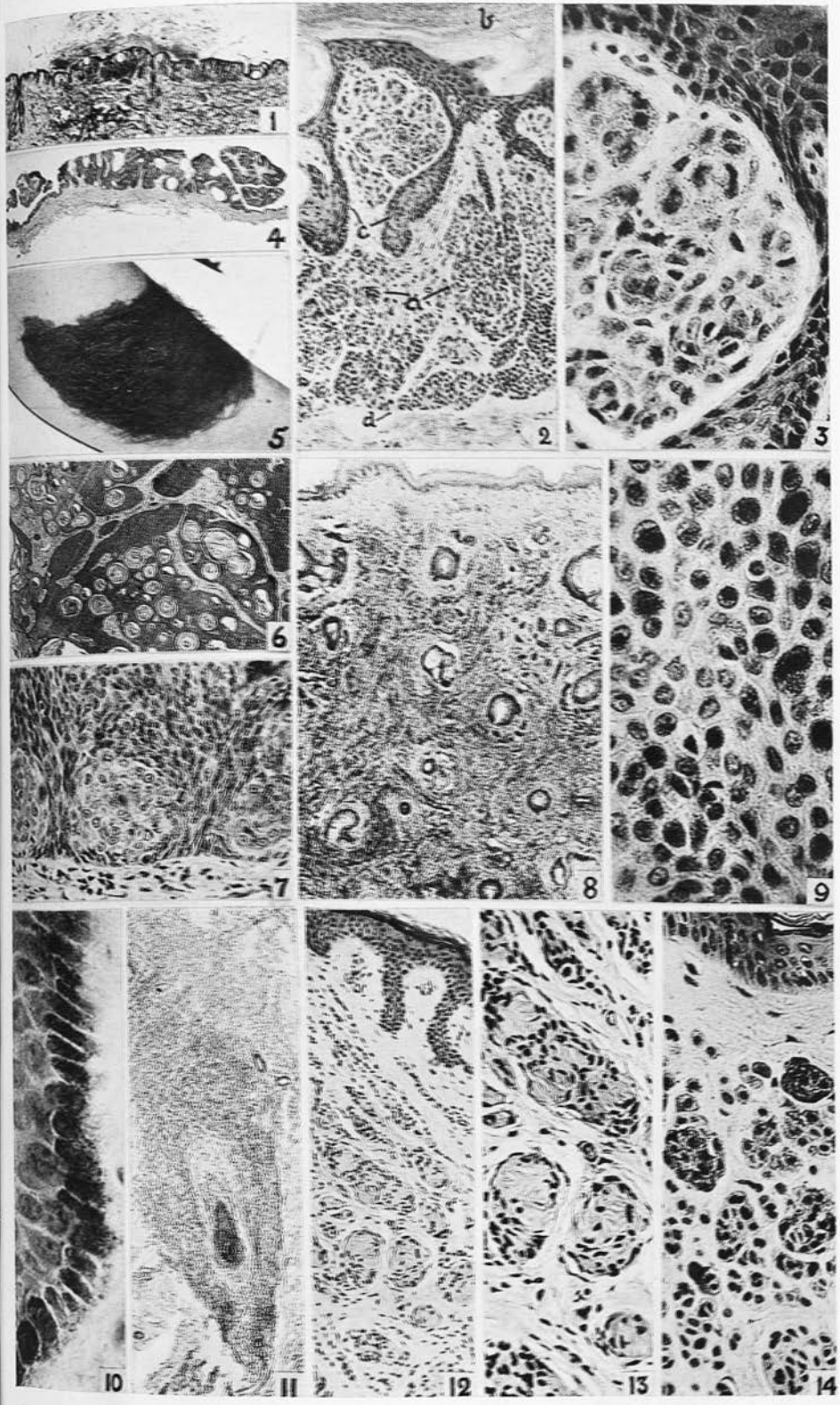


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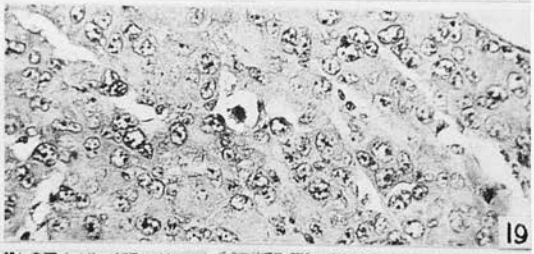
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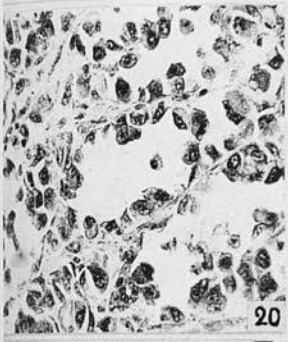
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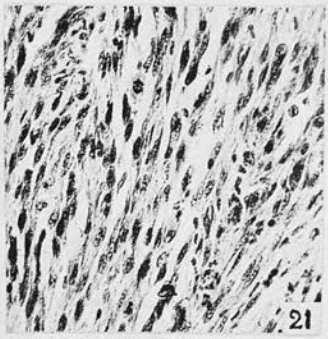
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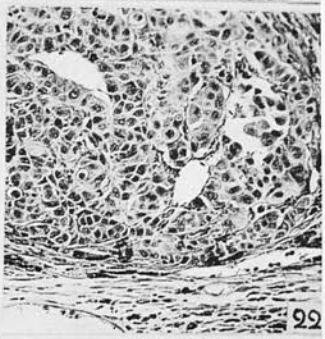
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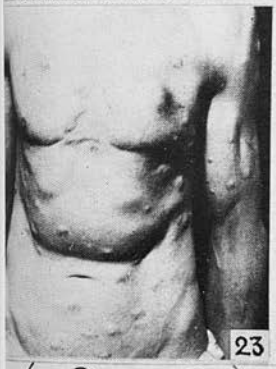
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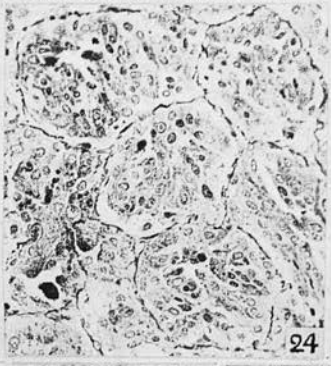
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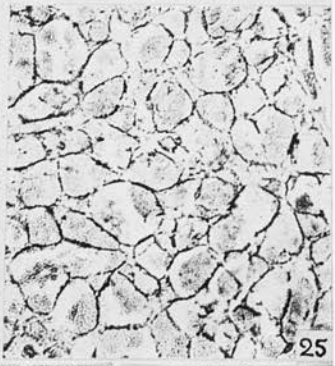
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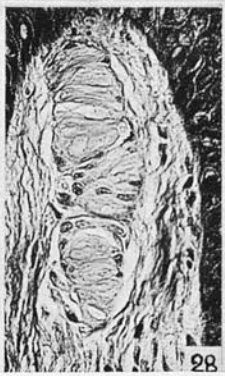
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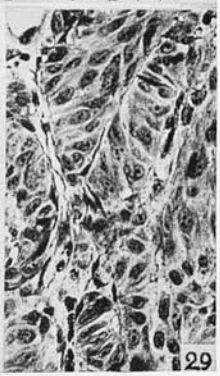
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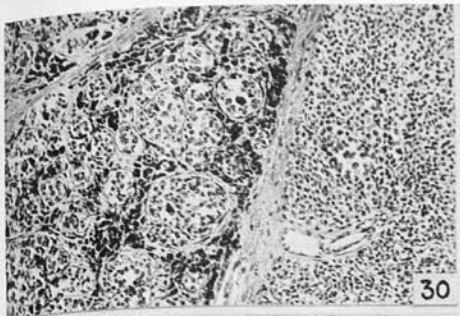
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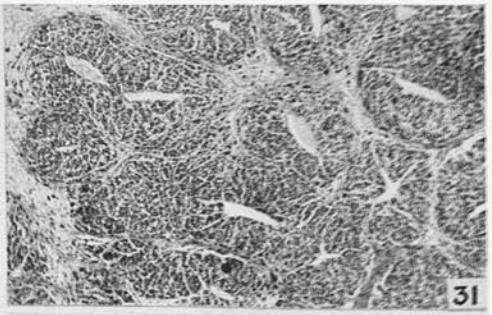
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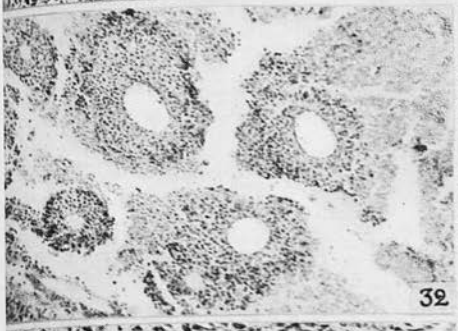
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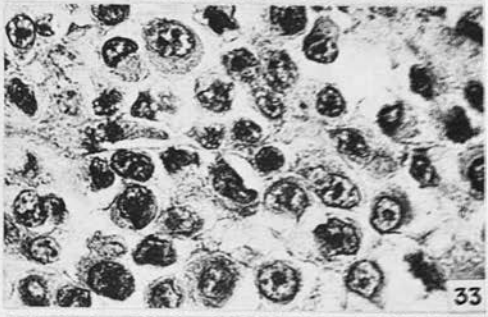
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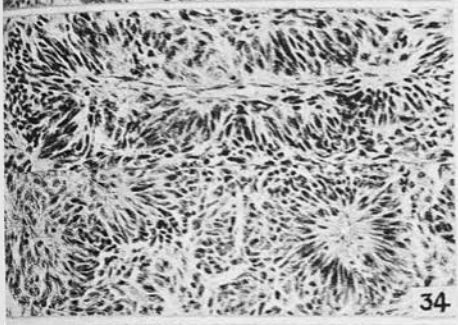
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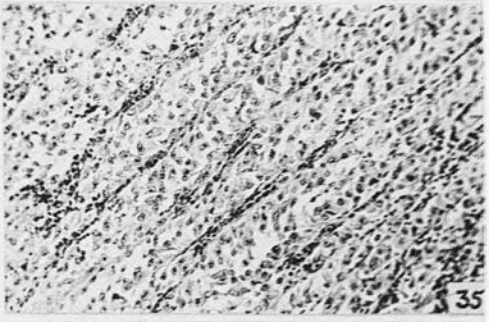
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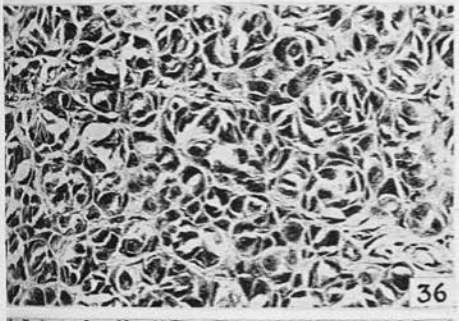
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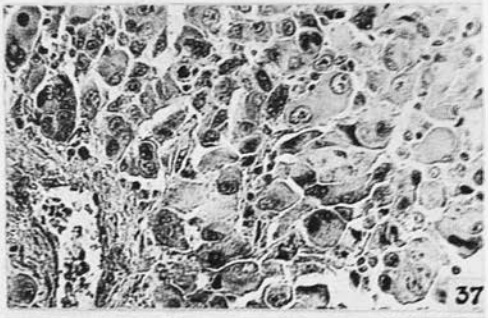
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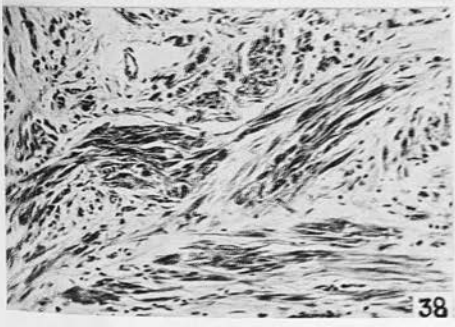
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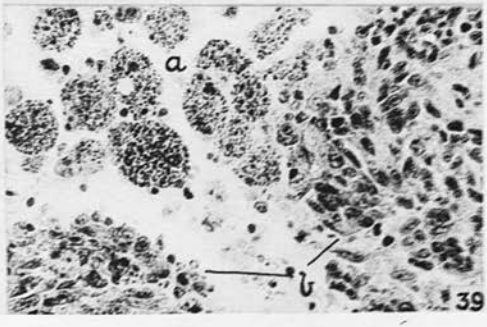

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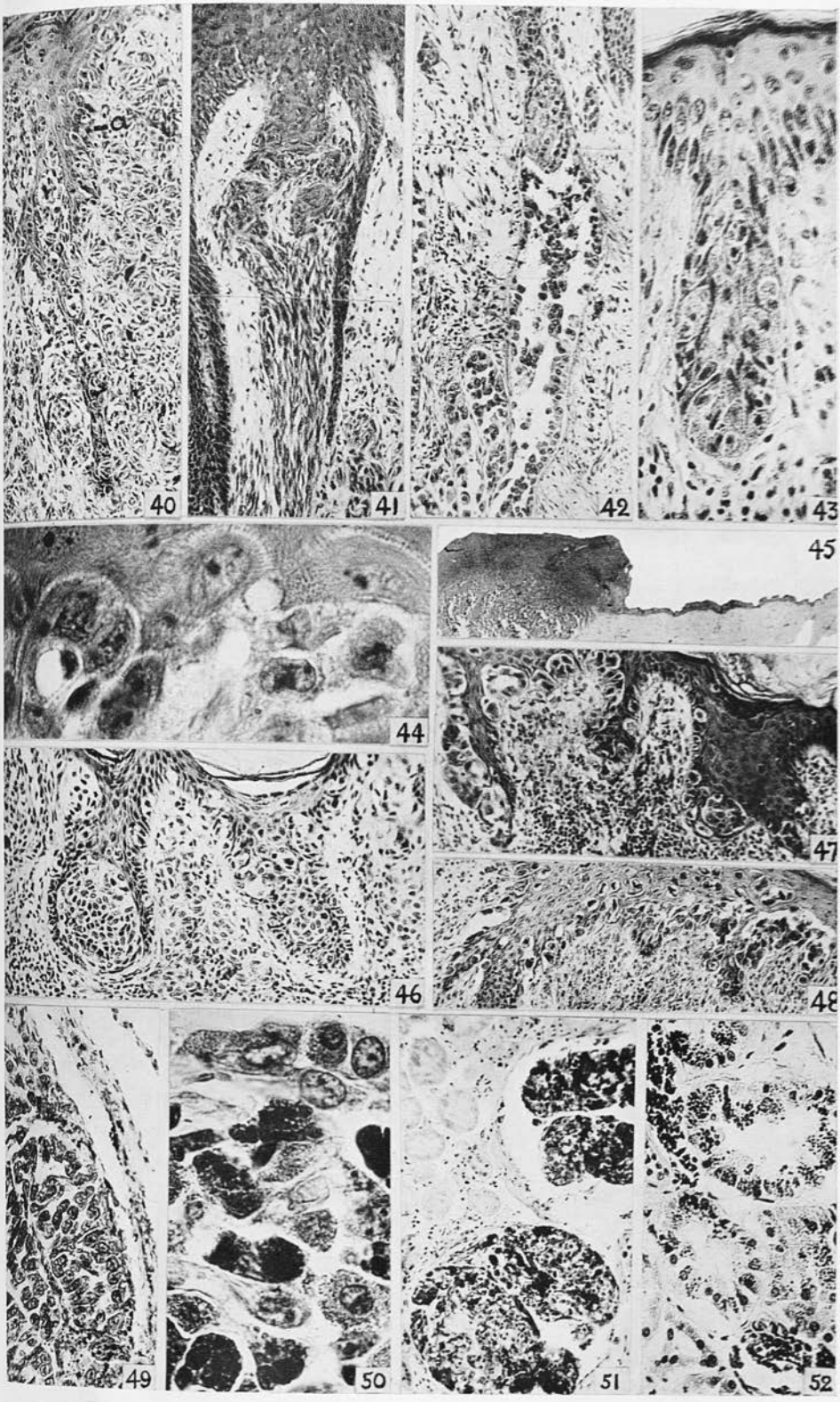
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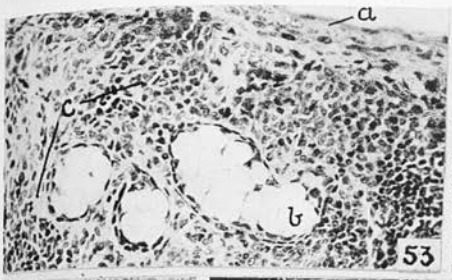

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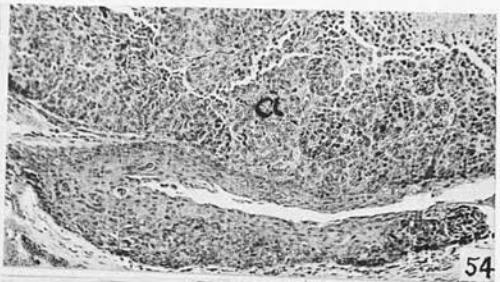

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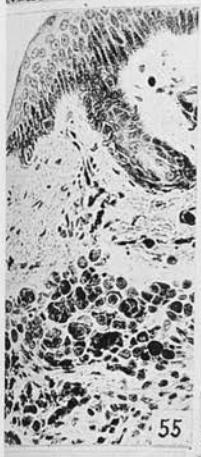
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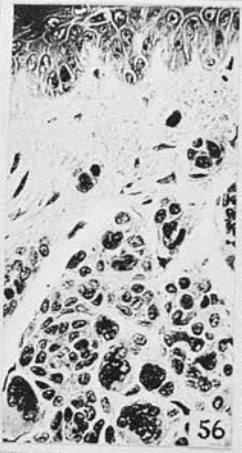
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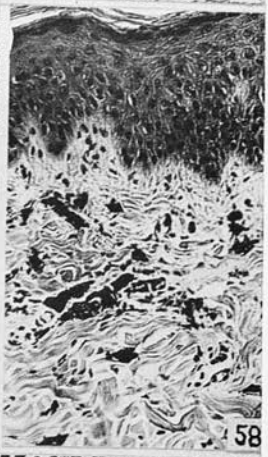
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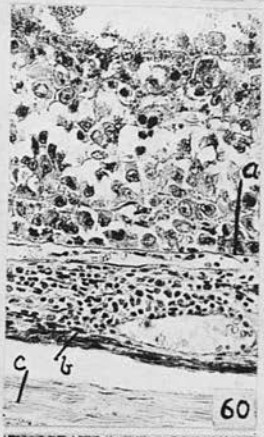

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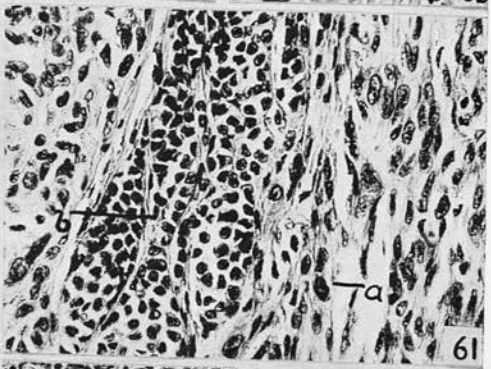

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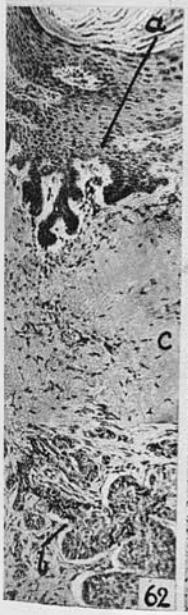

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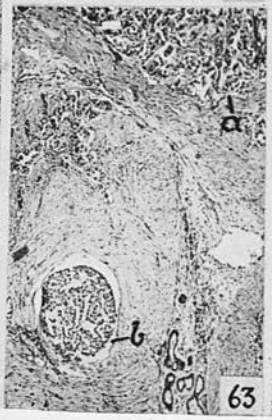

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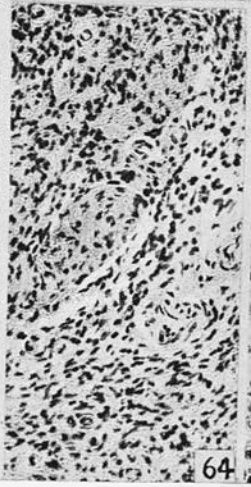
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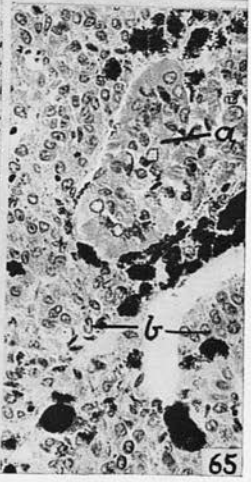

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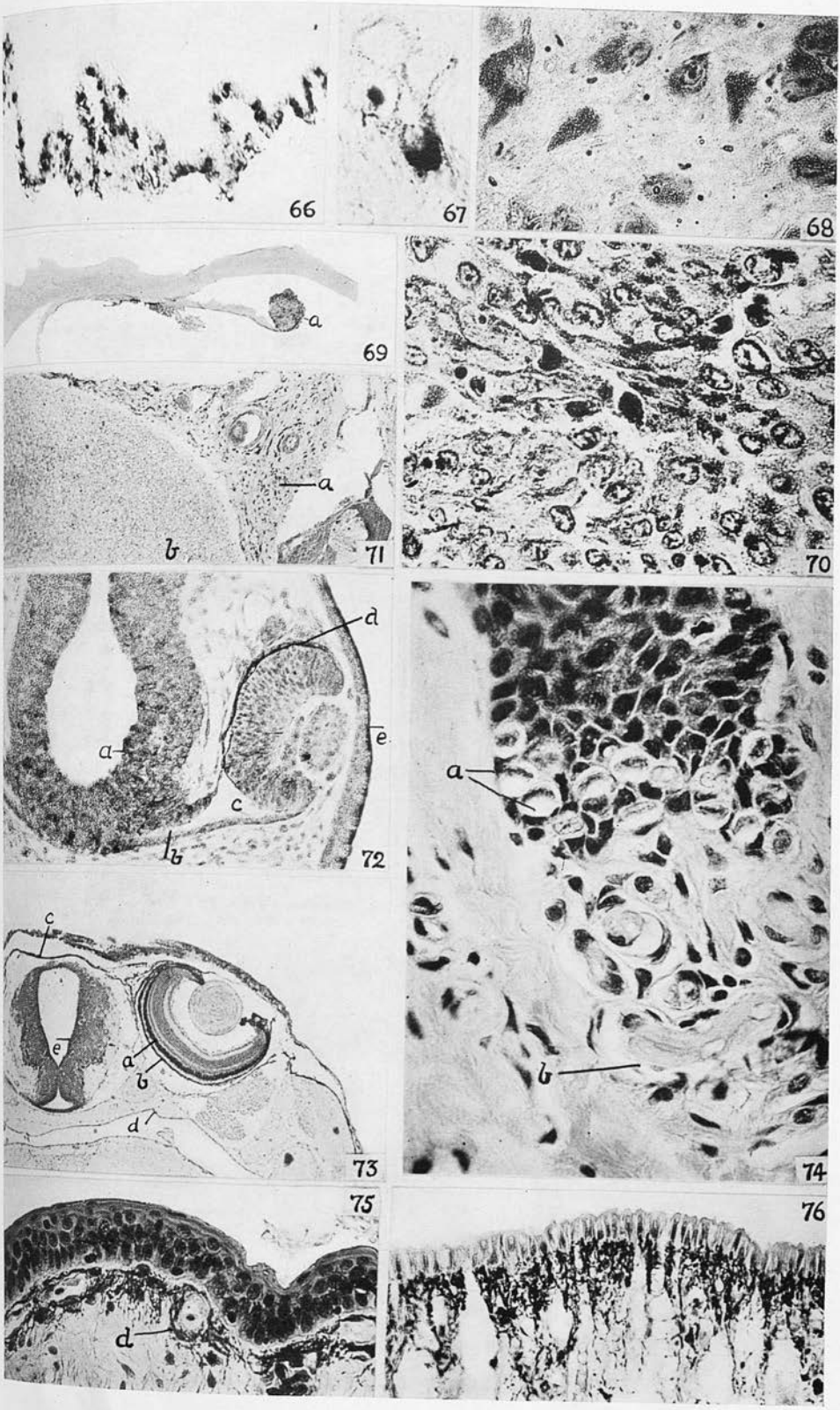
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PERMANENT STOMACH FISTULÆ IN RUMINANTS. By A. T. PHILLIPSON and J. R. M. INNES. From the Institute of Animal Pathology, University of Cambridge.

(*Issued October* 1939)

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PERMANENT STOMACH FISTULÆ IN RUMINANTS.

By A. T. Phillipson and J. R. M. Innes. From the Institute of Animal Pathology, University of Cambridge.

(*Received for publication 5th May* 1939.)

Gastric fistulæ have been used extensively as an experimental technique for studies of alimentary physiology, particularly in dogs. Problems associated with digestion in ruminants have necessitated the use of similar surgical measures in order to give access to the various compartments of the ruminant stomach.

During the past five years we have evolved methods for making permanent fistulæ in the rumen and abomasum of sheep through which samples of ingesta could be obtained, and the mechanical functions examined without disturbing the health of the animals. This paper is an account of our technique.[1]

Colin [1886] was the first to use successfully the technique of rumen fistulæ in oxen, but no attempt was made to close the opening by mechanical means. Wester [1926], Schalk and Amadon [1928], and Diernhofer [1928] have also employed the same technique in cattle. Schalk and Amadon closed the fistulæ with a block of wood; a hole was bored through the centre and the block was kept in position by a flap of leather in the inside and a flat piece of wood on the outside, the three pieces being tightly held together by wire passing through the hole. Diernhofer employed a similar method using a cork block instead of wood, and rubber flaps instead of leather and wood. The three parts were held in place by string soaked in olive oil and iodine to prevent digestion by bacteria in the rumen. These methods have the disadvantage that the whole stopping must be removed each time the animal is used for experiment.

In small ruminants, rumen fistulæ were employed by Krzywanek [1929], Weyers [1937], Krzywanek and Quast [1936], and Rathnow [1938] in sheep, and by Trautmann and Schmitt [1933] in goats.

Very little work has been done on cattle with abomasal fistulæ, although Belgowski [1912] described a method he used for making an abomasal fistula in a calf already provided with a Pavlov pouch. In sheep, abomasal fistulæ have been used by Bruggeman and Buss [1937], Krzywanek and Buss [1935],

[1] By this technique certain studies are being carried out by Dr. H. E. Woodman and Dr. R. E. Evans, School of Agriculture, Cambridge, concerning the digestion of cellulose and other carbohydrates in the sheep; other studies on the mechanism and nervous control of the ruminant stomach are being carried out by one of us (A. T. P.), while Dr. W. Berridge, Dept. of Medicine, Cambridge, has used abomasal fistulæ for direct observation of the mucosæ to determine the rugæ formation following electrical stimulation and the administration of drugs.

and Rathnow [1938], while Trautmann and Schmitt [1933] used them in goats. Krzywanek and Buss and Rathnow record that one of their animals had a normal gestation and parturition during the course of the experimental period.

Canulæ devised for Stopping the Fistulæ.

Open fistulæ, apart from the unsightly appearance, are at all times unsatisfactory for the carrying out of physiological work. An animal may live for weeks with an open rumen fistula, but it loses weight and condition and cannot be considered as normal. In sheep, death will occur within a few days unless the abomasal fistula is closed. The main difficulty encountered at first with rumen fistulæ was the devising of a mechanical stopper which would be permanent in its position, would keep the rumen from leaking and would allow access to the rumen without being removed. The method of Schalk and Amadon (a block of wood and leather flaps) was used at first both in sheep and in the cow, but was found clumsy and impracticable. Later, a valve-like mechanism was produced by the use of a football bladder held in the middle by an ivory ring which fitted the rim of the fistula. When the bladder was inflated a balloon was produced on both sides of the margin of the fistula; this proved quite an efficient method while the bladder lasted. The main reason for the difficulty in closing the fistula is the strong regular contractions of the organ with a resulting contraction and relaxation of the fistula. A stopper was devised, therefore, similar to the tracheotomy tube used in horses. It was made of brass and then electro-tinned to prevent corrosion and was so designed that the several separate parts could be fitted into a fistula much smaller than the diameter of the inside wings and the outside collar or flange. The essential feature of the apparatus was that the tube was prevented from coming out by the broad wings lying close to the inside of the rumen and by the collar or flange on the outside skin. When in position these two flaps cause the canula to sit snugly against the rim of the fistula. This was found to act as a good stopper when once it was well fitted to the fistula, and this form of canula has been used on several sheep. One of them was kept alive in excellent condition for three years. The canula can be made in various sizes depending on the subsequent nature of the experiments. This form of canula is illustrated in figs. 1, 2, 3 and 4 in pieces, assembled and fitted into the fistula of a sheep.

Dr. J. J. Quin, Veterinary Laboratories, Onderstepoort, S. Africa, informed us (personal communication) that he had used ebonite with success in making canulæ. (Ebonite is tough, non-corroded by ingesta and can be easily worked on a lathe.) Small ebonite canulæ consisting of a shaft and internal collar, that can be brought out by a stab wound, have been used for abomasal fistulæ (figs. 5, (*a*) and (*b*)). Similar canulæ of a larger diameter have also been used for rumen fistulæ.

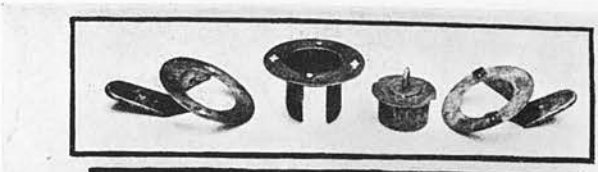


Fig. 1.

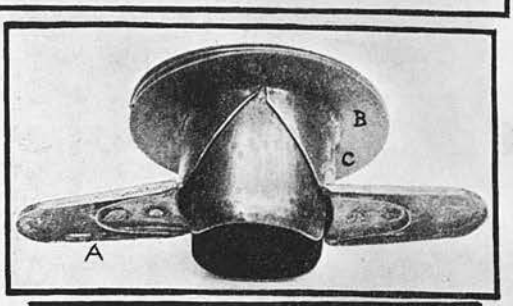


Fig. 2.

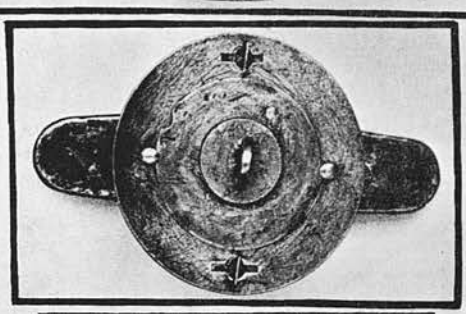


Fig. 3.

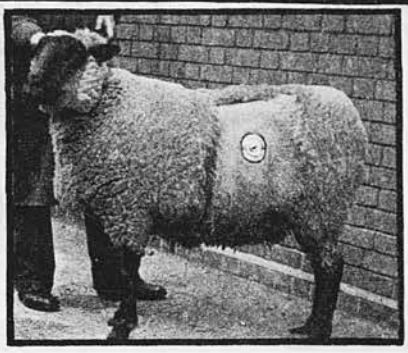


Fig. 4.

Fig. 1.—Metal canula arranged to show the individual parts. The right-hand wing and collar is inserted first into the fistula; the left-hand wing and collar is next inserted and finally the central tube. The three collars are clamped together by the buttons attached to the lowest collar. The cap can then be inserted to close the tube.

Fig. 2.—Metal canula assembled, seen from the side. Measurements: Length of side wings (A), 5 cm.; width of side wings, 3·2, taper to 2·8 cm.; width of collar (B), 2 cm.; depth from collar to side wing (C), 2·8 cm.; diameter of internal opening, 4·5 cm.

Fig. 3.—Metal canula assembled to show method of clamping collars with the buttons and of fixing in the cap.

Fig. 4.—Sheep with metal canula in place. This photograph was taken after the canula had been in place for over 2 years.

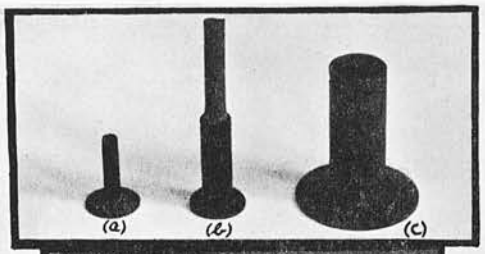


Fig.5.

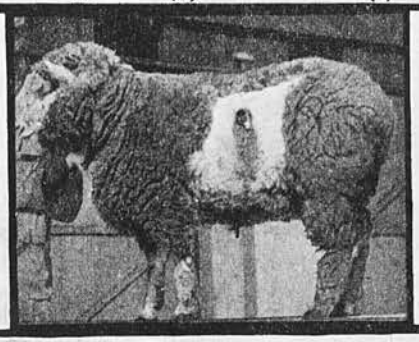
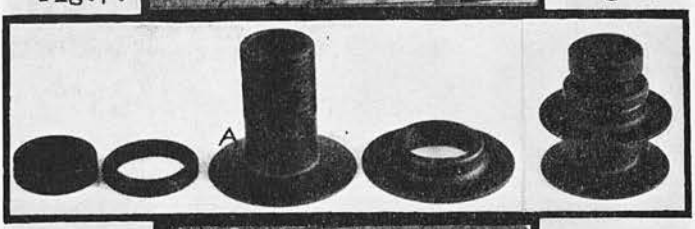


Fig.6.

Fig.7.

Fig.8.



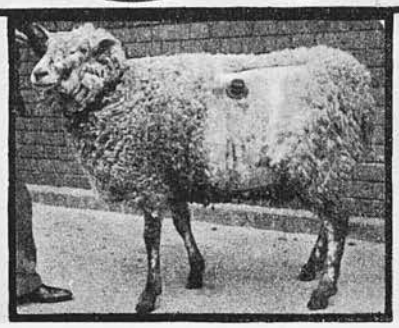


Fig.9.

FIG. 5.—(*a*) Small ebonite abomasal canula for lambs. Length of shaft, 3 cm.; width of collar, 1 cm.; internal diameter of shaft, 0·5 cm. (*b*) Larger ebonite abomasal canula for adult sheep. Length of shaft, 4·5 cm.; width of collar, 0·8 cm.; diameter of shaft, 0·8 cm. internal. (*c*) Simple ebonite canula for the rumen fistulæ. Length of shaft, 6 cm.; width of collar, 1·5 cm.; internal diameter of shaft, 2 cm.

FIG. 6.—Sheep fitted with abomasal and simple rumen canulæ. This photograph was taken 4 months after the operation.

FIG. 7.—Large ebonite canula for rumen fistula arranged to show individual parts. Width of collar (A) at the base of the shaft, 2 cm.; length of the shaft, 9 cm. (the thread covers the top, 6½ cm.); internal diameter of the shaft, 3 cm.; width of outside screw on flange, 2 cm.

FIG. 8.—Large ebonite canula assembled.

FIG. 9.—Sheep fitted with a large ebonite canula. This photograph was taken 6 weeks after the operation.

Holes were bored on both sides of the shaft so that the canula could be fixed in position by passing a piece of wire through them (fig. 5 (*c*)). Recently, the addition of a screw-on flange, a collar to lock the flange, and a screw-on cap instead of the wire have improved the neatness and efficiency of the canula (figs. 7 and 8). Sheep fitted with these canulæ are illustrated in figs. 6 and 9. A wooden stopper planned on the principle of the metal canula used in sheep was devised for the cow. but had the disadvantage that it had to be removed for each experiment,

The animals used in our own work and for the purpose of other studies by Woodman, Evans and by Berridge, have been lambs, adult sheep and one cow. In 13 animals rumen fistulæ were made and in 12 abomasal fistulæ. Four of the sheep were provided with both rumen and abomasal fistulæ; three have lived for 5, 3 and 3 months respectively, and are still alive and healthy. The fourth sheep underwent a later vagectomy which proved fatal. The longest survival period of a sheep with a rumen fistula has been three years, and with an abomasal fistula five months. The cow with the rumen fistula was kept for ten months, when it was destroyed because of traumatic gastritis due to the presence of a piece of wire.

Methods of Making Fistulæ.

Anæsthesia.

The sheep appears to be a bad subject for inhalation anæsthesia; consequently nembutal was used as an anæsthetic for all operations. For young lambs it has proved efficient, but for adult sheep the duration of anæsthesia is short and it is usually necessary to administer one or more doses during the course of the operation. The injections are given slowly into the jugular vein and anæsthesia is estimated by the character of the respirations, the loss of reflexes and by muscular relaxation. The average dose used for lambs was 27 mg. and for adult sheep from 31·5 mg. per kilo body weight, respectively.

Regional Anatomy of the Operative Sites.

The rumen fills the whole of the left side of the abdominal cavity in adult sheep. The site of operation for a rumen fistula is an area bounded cranially by the last rib, dorsally by the transverse processes of the lumbar vertebræ and caudally by the external angle of the ilium. The layers of tissue forming the abdominal wall in this region are the subcutaneous fat, *M. obliquus externus*, *M. obliquus internus* and *M. transversus abdominis*, and the parietal peritoneum. The fibres of *M. obliquus externus* are directed backwards almost in a horizontal plane, and its upper border does not extend dorsally to the lumbar muscles; in this area the so-called lumbar triangle *M. obliquus internus*, therefore, is immediately deep to the subcutaneous fat. The fibres of

the latter muscle are directed downwards and slightly forwards. In practice *M. transversus abdominis* is not noticed as it is very thin in the sheep and may be regarded as part of *M. obliquus internus*. The blood supply of these three muscles is derived from branches from the lumbar arteries and from the terminal anterior branch of the deep circumflex iliac. The latter is a large artery, and for this reason the site of incision must not be too near the crest of the ilium.

The abomasum in the adult is situated on the floor of the abdominal cavity to the right of the mid-line. In lambs, however, it is much larger in proportion to the other compartments of the stomach when full. The fundus is related immediately in front to the diaphragm and the pylorus is found in the right lumbar region. The site of operation is in the ventral part of the abdomen immediately behind the xiphoid cartilage and to the right of the mid-line. The tissues that have to be incised are skin, *M. panniculus carnosus*, *M. rectus abdominis* and the parietal peritoneum. The blood supply of *M. panniculus carnosus* and *M. rectus abdominis* is derived from the internal thoracic (internal mammary) artery. This vessel leaves the thorax between the xiphoid cartilage and last sternal costal cartilage and gives a branch, the cranial epigastric, which runs along the internal aspect of the rectus muscle, near the mid-line. The incision should not be more than 1 inch to the right of the mid-line, otherwise this vessel will be encountered.

Operative Technique.

The operative sites are clipped, shaved, cleansed and sterilised. Strict attention is paid to asepsis. It is unnecessary to starve sheep for rumen operations.

Rumen Fistula.—Method 1. This method is used if a very large fistula is required. The animal is laid on its right side. A skin incision is begun immediately below the lumbar muscles in the middle of the area described above and carried downwards and slightly backwards 4–7 inches according to the size of fistulæ desired. Subcutaneous fat, if in excess, can be removed, the external oblique muscle is exposed and the fibres are cut transversely. The most serious hæmorrhage is encountered here and it must be controlled. The exposed internal oblique muscle and the underlying transverse abdominal muscle can be split by blunt dissection in the direction of their fibres to expose the peritoneum. The latter is cut and the rumen is found immediately below. A pouch of rumen is drawn out and clamped with a pair of bowel forceps, and the edge beneath the clamp is stitched by interrupted sutures to the edges of the skin all the way round. The bowel forceps are removed and replaced by a metal clamp which is tightly screwed. The whole wound area is covered with gauze and bandaged. The second stage of the operation is done one week later when firm adhesions

between the rumen wall and skin will have formed. The dried necrotic pouch of the rumen produced by clamp pressure is then cut off along the line of the clamp; the rumen, having been opened, the metal canula is then inserted.

Method 2. The approach to the rumen is the same as in Method 1, except that the initial skin incision is started higher up and is made about 1½ inches behind the last rib. When the rumen is exposed a large pouch is pulled out and the whole area around the wound is well packed with gauze. Bowel clamps are again applied. A purse-string suture (Silk No. 2) is inserted into the pouch of the exposed rumen wall, penetrating all the coats except the mucosa. The area covered by the suture should correspond roughly to the diameter of the collar of the canula. The canula is well packed with cotton-wool or gauze. A stab wound is made in the centre of this area and enlarged by scissors until the inside collar of the canula can be slipped into the rumen. The purse-string suture is then pulled tight and tied so as to invert the lips of the rumen wound and also to ensure that only the serous coat of the rumen is in contact with the canula. It is seldom necessary to superimpose a second purse-string suture over the first. In some cases when the largest size of canula has been used, the bowel forceps have had to be released before the purse-string suture could be tied. In this case, great care must be taken to prevent contamination of the wound by escape of ingesta. The openings in the abdominal muscles are sutured in layers from below upwards, as it is important that the canula should be as high up as possible. The sutures next to the canula on both sides also include the rumen wall and thus the latter is kept in close contact with the wound until adhesions have formed. The edges of the skin wound are finally sutured together. The wire is inserted across the canula and padded and fixed in position by elasto-plast, or if a screw flange is fitted, this is gently screwed on.

Abomasal Fistulæ.—The sheep is laid on its back for this operation. A skin incision is begun in the abdominal wall immediately behind the xiphoid cartilage to the right of the mid-line and extended caudally along the abdomen for 3–4 inches. In lambs the muscle layers are thin and the abomasum is quickly exposed. Because of the highly vascular nature of this organ, compared with the rumen, great care is necessary in all stages of its handling. In adults the rumen is found lying immediately under the wound as the sheep is on its back. The abomasum lies deeper and further to the right side. It can easily be identified by the omentum which is attached to the greater curvature. A purse-string suture is inserted on the lateral surface of the abomasum as near the greater curvature as possible; the abomasum is opened by a stab wound and the canula inserted and fixed as in the rumen. This can be done in lambs without the segregation of an area by bowel

clamps, but in adults clamps are necessary to prevent escape of ingesta over the wound. The canula is then brought through a stab wound in the skin and the rectus muscle about half an inch from the original skin incision.

The muscle, subcutaneous fascia and skin of the initial wound are again closed in layers by interrupted sutures. In order to prevent escape of the stomach contents over the peritoneum, the canula is always plugged tightly with cotton wool or gauze. The above applies only to fistulæ where the canulæ are of small diameter. In two cases larger abomasal canulæ have been inserted into the abomasum of lambs. These were a modification of the larger rumen canula used in sheep. One of these lambs lived for two months.

After-care of Animals.

It is advisable to inject a prophylactic dose of tetanus and polyvalent anærobe antitoxin immediately after the operation as a precautionary measure. Apart from simple protective dressings, no after treatment of the operative site has been necessary, as healing in all cases has been by first intention. (Continuity between either rumen or abomasum mucosa and the adjacent skin is effected in a remarkably short period.) Skin sutures are removed after 14 days. Lambs and adult sheep recover rapidly from these operations and are usually feeding normally the next day.

In the earlier series of animals some leakage of ingesta occurred at the edge of the canula, but latterly remarkably little leakage in either rumen or abomasal fistulæ has been noticed. In two cases a ventral hernia has occurred at the site of the original incision for the abomasal fistula. This has not proved serious, and the sheep have remained in good health.

Experience has shown that it is inadvisable to keep an experimental sheep alone because they sometimes refuse to eat if kept in isolation.

Summary.

The technique of making permanent rumen and abomasal fistulæ in sheep is described. The canulæ used are described and illustrated.

Acknowledgments.

The canulæ were made by Mr. F. Mitchell, School of Pathology, Cambridge; photographs have been prepared by Mr. W. J. Smith, Institute of Animal Pathology, Cambridge, to whom our thanks are due.

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General Articles

THE PATHOLOGY AND PATHOGENESIS OF TUBERCULOSIS IN DOMESTICATED ANIMALS COMPARED WITH MAN

By J. R. M. INNES,
Institute of Animal Pathology, Cambridge University

Introduction

It is perhaps not always realised that there are still unsolved problems associated with the pathology and pathogenesis of tuberculosis. The variations in the reaction of different animals and man to the tubercle bacillus must be fully explained if pathology is to have that broad comparative basis so essential for its future development.

In English veterinary literature there are no papers comparable to the many excellent English and American publications dealing with the medical aspect of the disease. It is hoped that a paper, such as this, with suitable illustrations, will offer a background for those who wish to view the disease through the eyes of the comparative pathologist. For these reasons the article has been written more in the manner of a critical annotation.

The first part deals with a general consideration of the reaction of tissue to the tubercle bacillus, the pathogenesis of the disease in man and a section on the disease in dogs and cats; a second part will deal with bovine tuberculosis. The literature is not intended to be complete and is given in a "directional" form suggesting the most important publications, some of which are of historical interest.

Tuberculosis is a chronic disease process in which, even in one animal species, the anatomical variations may appear to be many, but if it is considered as a whole in different animals and man, they might seem almost endless. These variations are related to the inter-action of the destruction and repair of tissues, the factors which influence the end result being (a) the size of the infecting dose of bacilli, (b) the virulence of the latter, (c) the resistance of the host, (d) the state of allergy, (e) the path of infection, and (f) in animals particularly, the type of bacillus is of great importance. Although all are contributory, not one of these factors alone is decisive in establishing the pathogenesis of the disease. These are statements often made in the literature, but we still lack accurate information of their influence in any given tuberculous infection. As a result certain lesions have been interpreted differently, and there is still much controversy of the rôle of allergy and immunity in the development of the disease.

prepared and shaped. The name of Share Jones will always be associated with the School of Veterinary Science of the University of Liverpool for it has been largely under his guidance and rule that its present position has been attained. His interests, however, have lain and, we doubt not, will continue to lie far beyond the School itself, for he has the wide subject of veterinary science and its various ramifications so very much at heart. We cannot believe that Share Jones will simply go into retirement, though he richly deserves a well-earned rest after 35 years of activity in the University. Those who know him well cannot imagine that the veterinary world has lost one of its important champions by his retirement from the University. No! We look forward to hearing much of him and his activities in the future and we know that any new venture in which he may be found will assuredly have the interests of his profession as its one and only object.

We extend to him our very hearty congratulations on the position he has attained and the very great respect in which he is held as shown by a perusal of the proceedings published in this volume, and wish him a further long, useful spell of life when he may devote himself to the accomplishments of ambitions which we know he may be cherishing, unhampered by official duties. He has accomplished much—he will accomplish more!

To his wife, Dr. Mary Share Jones, our warmest good wishes are also extended.

THE NEW EDITORSHIP OF "THE VETERINARY JOURNAL"

It was with much pleasure that we read the Editorial published in *The Veterinary Record* of February 10, 1940, which deals with The Veterinary Journal and its newly-appointed editors. We have the interests of the veterinary profession very much at heart and it will be our endeavour to maintain the standard of the publications in this journal so that the profession shall at all times have brought to its notice recent observations which may be applied to the maintenance of health and prevention and treatment of disease among our domestic animals. The Veterinary Journal during most of its life has catered more for the practitioner than for members of the profession engaged in other aspects of veterinary science and it is the full intention of the present editors to continue on these lines. We are deeply grateful to *The Veterinary Record* for giving prominence to this recent change of editorship and we thoroughly agree that there is ample scope for the healthy existence of both journals.

The pathogenesis of tuberculosis in man and animals can be understood only when we can answer accurately the questions (a) how does the bacillus enter the body?; (b) what is the immediate and subsequent reaction of the host to one or more infections?; and (c) how does the infection spread through the body? These problems have only been partly solved.

It is necessary to review modern concepts of the disease in man as recent studies of animal tuberculosis have been constructed on a comparative basis. As far as the subject concerns the veterinarian recent work in animals has been mainly that of Nieberle and his pupils in Germany.

Effect of the Tubercle Bacilli on Tissues

It is an old observation that (a) there are differences in reaction of similar hosts to different strains of the same organism, and (b) there are similar differences in reaction when animals of the same species are infected by a type of bacillus of standard virulence. According to Rich, virulence is irrevocably linked with resistance, hence to a consideration of the former must always be appended the question " for what animal? "

With these reservations the entrance of tubercle bacilli into a tissue may be regarded as evoking a standardised reaction which soon reaches the macroscopic stage of a small nodule (tubercle). (In some animals, e.g., dog and cat, this is not strictly accurate.) The lesion in the initial stages is essentially the same whether the organism is of high or low virulence or even of a non-virulent character such as BCG — all will cause a fundamentally identical tubercle. The cellular response has been regarded by Rich as the most primitive organised type of reaction in which a vascular inflammatory mechanism is not involved and which occurs before the onset of any tissue destruction.

The bacilli, having entered the tissue, are immediately surrounded by small numbers of polymorphonuclear leucocytes and lymphocytes (Fig. 1). Soon, however, the cellular focus enlarges by an accumulation of phagocytic cells—the epithelioid cells, at which stage the leucocytes may be scanty or absent (Figs. 3 and 4).

There has been much controversy about the nature and genesis of these epithelioid cells. They are usually large, closely packed, round or polygonal cells with indistinct margins with abundant eosinophilic granular, or sometimes clear, cytoplasm, and with a small vesicular nucleus which may be at one pole of the cell (Figs. 21-24). The genesis of these cells has been worked out by Sabin, Doan, Forkner, Cunningham and others. Their immediate precursors are the mononuclear phagocytes or macrophages which are identical with the blood monocytes and which are continually wandering from the blood into tissues. By the use of vital staining it has been shown that the epithelioid cell in common with the blood monocyte possesses a cytoplasmic rosette. These macrophages or mononuclear phagocytes have been given many different names at various times, e.g., endothelial leucocytes (Mallory) said

to be derived from vascular endothelium, adventitial cells (Marchand), clasmatocytes (Ranvier), polyblasts or amœboid wandering cells (Maximow), histiocytes (Aschoff). These cells which give rise to the epithelioid cells of the tubercle are to be found in many different diseases and localities, and are referred to under other names, e.g., as scavenger cells in the nervous system following tissue destruction and known as compound granular corpuscles or *gemästete Glia*—probably microglial in origin (Fig. 24); as "heart failure" cells or alveolar phagocytes in the lungs (Fig. 22); and as macrophages in and around abscesses.

Tubercles will form in this manner either in intra- or extravascular locations, indeed they appear to form in any tissue in which mononuclear phagocytes are present, even in tissue culture. At a later stage lymphocytes appear more numerous and may form a thin layer peripheral to the epithelioid cells, (Figs. 4-7). Subsequently in man and in most species of animal, there are formed, either by fusion of several epithelioid cells or by amitotic nuclear division, the characteristic multinucleated giant cells, first described by Langhans. These may be of different shape and size and their numerous nuclei are often, but not always, arranged in a horseshoe fashion around the periphery of the cells (Figs. 8-20). Giant cells have been regarded as a post-necrotic development, but they are often found in areas totally unrelated to necrosis. They appear to be a response to the need for the phagocytosis of foreign material; the lipoid fraction of the tubercle bacilli is said to contain a maturation factor necessary for their genesis.

Silver impregnation methods can demonstrate a delicate framework of argyrophil fibrils ramifying throughout the tubercle even at an early stage of development (Fig. 25). The relationship of these fibrils to collagen is disputable, but there is good reason for believing that they represent simply a pre-collagenous stage.

The focus can now be regarded as a fully formed tubercle (Figs. 5, 6 and 7) which is characteristic, at least, for certain stages of the disease, in man and some animals; similar lesions can be provoked by non-virulent bacilli, e.g., BCG (Figs. 1-5), although the fate of the lesion may be very different. Epithelioid and giant cell lesions can also be produced experimentally by subcutaneous injection of olive oil, droplets of mercury, suspensions of agar, etc. (see Cunningham and Tomkins); the "tubercle" is not, therefore, specific for the pathogenic bacillus.

The subsequent course of the lesion varies greatly in natural and experimental conditions, and may be summarised as follows:

(1) If the numbers of bacilli are small, they may fail to propagate and/or they may die rapidly; the lesion may then resolve completely illustrating a potentiality of tissue reversion from altered character to normal which is insufficiently stressed. This must occur before connective tissue develops otherwise scar formation will result. Resolution of this kind may be commonly

seen following the intravenous injection of an organism such as BCG into rabbits, and must also occur during the natural disease.

(2) By fibroblastic proliferation and the production of collagen, the tubercle may be converted into a hyalinised mass and/or be encapsulated by fibrous tissue, while calcification may occur in the centre, which in human primary infection may form a locus for later ossification. This lesion might be regarded as obsolete or healed (Figs. 26, 28, 29, 37 and 38).

(3) The centre of the tubercle may undergo necrosis and subsequent caseation (Figs. 5, 7 and 37). The bacilli by themselves have little power to cause necrosis in the unsensitised body, nor, as was once thought, is necrosis due to the non-vascularity of the tubercle. The supposedly non-vascular nature of the tubercle is often emphasised, but as cells appear to wander in from neighbouring vessels, tissue fluid may well do likewise, otherwise all tubercles would suffer the same fate of tissue death. The development of necrosis appears to be influenced partly by the number of bacilli present and partly by the development of sensitisation of the body to the tuberculo-proteins. It is now known that of the chemical fractions isolated from tubercle bacilli, it is the lipoids which produce tubercle formation and the proteins which cause acute inflammation and tissue death. Central necrosis occurs before fibrosis and encapsulation and liquefaction may subsequently ensue.

(4) The necrobiotic process may extend centrifugally to destroy fibrous tissue as soon as it forms, in which case fresh infections into adjacent normal tissue will form contiguous or satellite tubercles (Figs. 34 and 35).

(5) Bacilli may enter the blood or lymph stream and be transported to organs near to, or distant from, the primary source and thus give rise to secondary lesions in which the above processes may be repeated.

(6) An acute fulminating inflammatory reaction may occur in response to massive infection (or reinfection) in which the intensity of tissue destruction overwhelms the "defensive" cellular mechanism.

The body, therefore, responds to infection by the production of a tubercle (Fig. 7) or by acute exudative inflammation (Fig. 32); hence, the terms "proliferative" and "exudative" inflammation, now commonly in use, and under certain circumstances either process may predominate. Factors which control this variation have been mentioned, but allergy (in the sense of Pirquet-Ranke) requires further consideration. The effects of allergy in the body is seen in the well-known Koch phenomenon. If a healthy guinea-pig is inoculated subcutaneously with tubercle bacilli of low virulence, a nodule forms which breaks down, does not heal, and the regional glands become enlarged and caseous; this is followed by generalisation of the disease and death in about 2-3 months. The effect of inoculation of a guinea-pig already infected is markedly different. A local swelling appears in a few days which ulcerates but soon heals, and the regional glands are not enlarged. This does not mean that the glands are not infected, but that the bacilli are mostly retained at the site of inoculation, and/or die in great numbers, or those which

do reach the glands find them unfavourable for free propagation. This fixation of bacilli to the site of entry is the result of an acute exudative inflammation which to some degree is characteristic of a second infection; the nodular tubercle, on the other hand, is more characteristic of a first infection. These phenomena are observed in natural disease of man and animals depending on whether it is a primary infection or a secondary (re)infection (Figs. 30, 31, 32 and 33). These facts must not, however, be accepted too strictly as it has been shown, particularly by Rich, that exceptions occur and that allergy must be separated from immunity. Rich contends that, although allergy and immunity may be coincidental, they are not identical as suggested by Krause and others. Following infection the body acquires an altered reactivity towards the bacillus, but both the normal animal, following a single infection, and the allergic animal may respond either by tubercle formation or by exudative inflammation. Which type of reaction occurs is primarily dependent on the size of the infecting dose and where the bacilli lodge. The difference is seen in the greater tendency of the allergic animal to react by exudative inflammation, i.e., with more extensive damage and tissue death. As a result of previous infection an animal is allergic to the products of the bacillus and the response is necrosis and inflammation, but there is also a development of acquired resistance which localises the bacilli to the site of entry where they may not thrive, or where the spread of viable organisms is delayed.

Tuberculosis in Man

Parrot (1867) stated that the portal of entry of the tubercle bacillus is indicated by the lesions in the regional lymph nodes, a dictum which has withstood the test of all subsequent work. As an index of primary infection the lesion must be the only lesion in the body or the oldest, and must be in a situation which is a possible portal of entry for the bacillus from outside sources. The lymph node draining the path of such a primary infection always shows a lesion, and together with the primary infection forms the so-called " primary complex " of Ranke (Figs. 34, 35, 36, 39 and 53). Particularly in mucous membranes and/or if the number of bacilli are small, entry may be " silent " and only the regional lymph node appears (at any rate macroscopically) affected; this is often referred to as the " incomplete primary complex."

Behring stated that the disease in the adult was the end stage of a childhood infection, and Ranke subsequently believed that tuberculosis was comparable to syphilis with (a) a primary infection period, (b) a secondary anaphylactic stage and metastatic spread, and (c) a tertiary stage of increased resistance expressed by limitation of the disease to the lungs.

Pulmonary tuberculosis (phthisis) of the adult is not the first manifestation of the disease but is the result of (endogenous or exogenous) reinfection. In many individuals, primary infection occurs in infancy, but in most the lesions heal, calcify and may even ossify. In a few the lesion progresses,

and the child dies, while in others infection spreads by direct extension, by the natural passages and by hæmatogenous and lymphogenous paths. Primary infection occurs in nearly every living person of civilised communities.* The path of infection in about 80 per cent. is *via* the respiratory tract, in about 15 per cent. *via* the intestine, and the remaining 5 per cent. probably comprise sites such as the nasopharynx, skin and middle ear. These figures vary in different countries; for example, in Scotland infection by the alimentary tract is probably much higher. Blacklock's figures are: primary thoracic infection, 61.1 per cent., and primary abdominal infection 35.7 per cent.; the recent data of MacGregor and Alexander are very similar.†

Primary pulmonary infection may occur as early as the first month after birth, usually in the first six years, and less frequently after ten years. Opie gives the incidence as 43 per cent. of children up to five years, 80 to 90 per cent. of those over 20, and nearly 100 per cent. of persons over 30 years.

The entry of the bacilli causes a caseating broncho-pneumonic focus which is small and sharply demarcated from adjacent normal lung (Fig. 35). The lesion may occur in any part of the lung, is usually single, and often, but not always, sub-pleural, and is the so-called Ghon lesion, although Kuss was the first to describe it (Figs. 34, 35 and 36). It is always accompanied by a lesion in a regional lymph node which can be related anatomically to the lung focus (Figs. 34, 35 and 36). The spread of the bacilli from lung to lymph node is probably by means of the macrophages which abound in all advanced tuberculous lesions. In most cases these lesions heal by fibrosis, hyalinisation and calcification, and the lung lesion, in time, even by ossification (Fig. 38). These healed forms constitute a valuable index of the first infection of an individual, and may be found in cases of chronic (adult) phthisis. Relatively rarely in children this primary infection does not heal, the caseous pneumonic process spreads through the lungs and death may result from an acute tuberculous caseous pneumonia (Fig. 41).

The mode of infection to the lung has been contested since Behring stated that the bacilli passed through the alimentary mucosa and mesenteric lymph nodes without producing any lesions, to settle eventually in the lungs—views

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* In autopsies on infants and children carried out by Blacklock in Glasgow, healed calcified lesions were not found. This is in contrast to the findings of continental, American and other British workers (Aschoff, Rich, Opie, Pagel, etc.). Blacklock states that nearly all the children in this district of Scotland when infected with tuberculous lesions of the lungs and/or tracheo-bronchial nodes die in childhood, and that he was unable to trace any obsolete calcified pulmonary foci in adults. He concludes that there must be a low racial resistance to infection in Scottish children. The higher incidence of abdominal infection must be due to the higher number of infections by the bovine type.

† "It must be clearly recognised that a consideration only of clinically manifest pulmonary tuberculosis in children gives a misleading conception of the real importance and frequency of primary tuberculosis lung lesions. The fact that they are often clinically undetectable and that illness and death may be due to secondary or metastatic manifestations of the disease have tended to obscure the truth that the lungs play a dominant role as a portal of entry and as a primary infection site of all ages of children."

which were later supported by Calmette. The evidence, however, is open to severe criticism and Blacklock, for example, states that no definite conclusions can be drawn regarding this mode of infection where tuberculosis affects only, or is most advanced in, the lungs. All available modern data indicate that primary pulmonary lesions are due to direct inhalation infection *via* the air passages.

As a result of primary infection, a state of allergy in the sense of Pirquet-Ranke, i.e., hypersensitiveness, develops. The lesion in the lymph node may progress, with infection of adjacent nodes, to produce enlarged glands—the so-called scrofula of olden times. By exacerbation of the primary focus, the individual enters the post-primary phase of generalisation. This is due to the entrance of bacilli into the blood stream with spread of the infection to a few or many different organs of the body, the lungs usually being also involved in this secondary spread. In almost every individual post-primary lesions occur in the lungs, but the foci are abortive and may heal in the same way as the primary lesion. The Ghon focus is thus often surrounded by a ring of " satellite " tubercles (Figs. 34 and 35). These lesions may progress and produce an anatomical form different from that of chronic adult pulmonary tuberculosis, " punched out " thin-walled cavities (Fig. 46) and cortico-pleural lesions being part of this process. The lesions in the visceral organs which may arise by blood spread may also be progressive—constituting the chronic post-primary disseminated tuberculosis (Fig. 47). Various bones may be affected, usually in the vicinity of the metaphysis, with production of tuberculous osteomyelitis, periostitis and arthritis; the uterus in the female, the testicle, prostate, seminal vesicles in the male, one or both kidneys, ureter and bladder, the brain and the suprarenal glands are other organs which are commonly affected. In all, the subsequent course is the same, viz., progressive caseation with contiguity spread of the infection, while the lymph nodes draining the organs concerned may also become involved. Invasion of the blood stream by a large number of bacilli may cause a fatal acute miliary tuberculosis (Fig. 43), affecting perhaps every organ in the body. (Particular mention must be made of the involvement of the brain in this post-primary stage. Isolated foci form in the brain which may caseate and rupture into the sub-arachnoid spaces or ventricles to produce the tuberculous meningitis with intense exudative reaction so characteristic in young people. Rich states that this is the process constantly invoked in tuberculous meningitis, and that the meninges are never affected by direct hæmatogenous spread, an idea supported by the recent work of MacGregor and Green.)

Chronic miliary tuberculosis (Fig. 45), chronic proliferative lesions in the lymph glands, skin lesions, and tuberculosis of the special sense organs are other forms of the disease which come into this category of post-primary dissemination.

The special features of " first infection " or childhood type of tuberculosis thus include the inconspicuous nature of the primary lesions, the early

and pronounced affection of the lymphatic system and the tendency to extensive and often fatal infection *via* the blood stream.

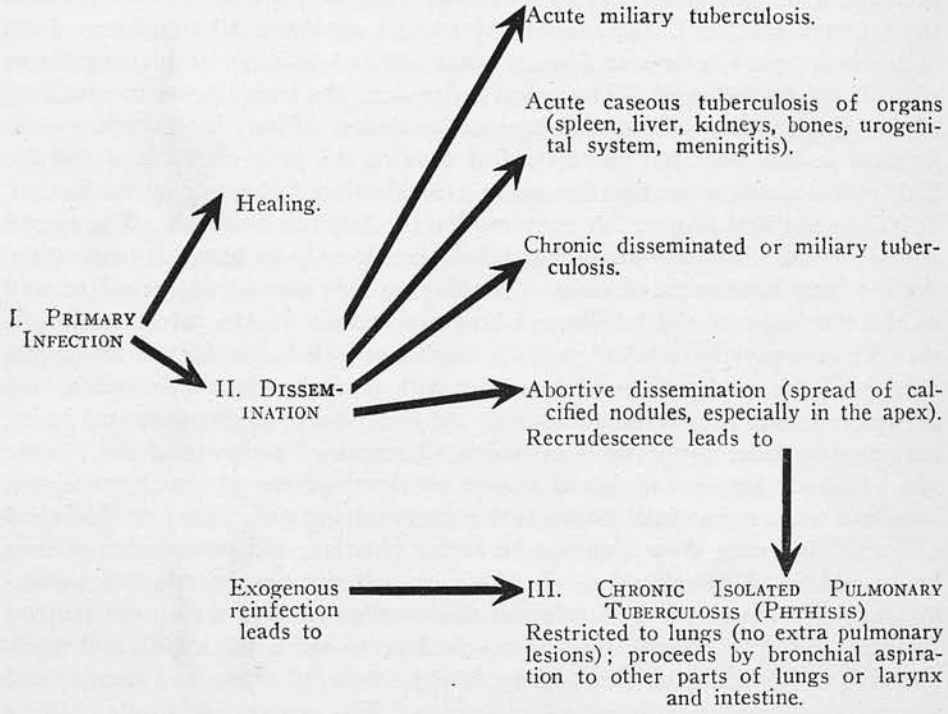
In later life some adults, who have had a previous primary infection, develop tuberculosis which is limited to the lungs. It was once thought that an individual with a primary infection could not be reinfected from without, but opinions have changed, and adult phthisis is now said to be due to a superimposed new or exogenous (re)infection. In any case, the bacilli appear to be arrested in the lung to form a lesion distinct from that of the primary infection. The (re)infection (Puhl's) focus (Figs. 48 and 49) is commonly situated in the apical lobe, and changes of the regional lymph nodes do not occur, or are represented only by microscopic abortive tubercles (contrast with the primary complex). In a series of routine autopsies all transitions from such fresh apical lesions to fibrous scars (healed lesions) or to progressive phthisis can be followed. The apical lesions and the insignificant involvement of the lymph nodes indicate an acquired resistance. Many individuals are reinfected in this way, but relatively few develop the progressive fatal disease. This lesion may also heal by fibrosis and calcification, but rarely by ossification. If it does not heal progressive consumption of the adult develops. The spread of the disease within the lungs then takes place largely by bronchial aspiration, the resultant anatomical changes depending on this manner of spread as well as the virulence of the bacilli and host resistance. If the infective dose is massive or especially virulent, and/or resistance is low, breakdown and liquefaction of the caseous focus may occur with discharge into a bronchus, and cavity formation may result (Fig. 51). At first lobular in character, the lesion may assume lobar proportions in which all stages of progress of the disease can be seen. Erosion of blood vessels or development of small aneurysms may lead to severe or fatal hæmoptysis; the overlying pleura may be thickened (Fig. 52) and may show signs of an active pleurisy, and pneumothorax may be an additional complication. In the more chronic type, productive inflammation predominates, and fibrosis and thick-walled cavities are an outstanding feature (Fig. 52). Bacilli may be coughed up to reach the mouth and nasopharynx and produce tertiary lesions in the tonsils, pharynx and larynx, and if swallowed may cause intestinal ulceration. The process in chronic phthisis is, therefore, mainly localised to the lungs, all other lesions being simply appendicular to the bronchial system. As Pagel says, it seems paradoxical that adult phthisis, which is so often fatal, should be considered an expression of immunity, but the latter indicates simply the restriction of a process, which would be otherwise disseminated, to an organ that is most susceptible. Acute miliary tuberculosis rarely occurs at this stage in contra-distinction to its frequency in the post-primary dissemination phase. In the adult the bacilli, which must escape into the blood stream, even in large numbers, must reach only resistant tissue; many, no doubt, die while others produce abortive tubercles. A heavy enough seeding of the blood stream by viable organisms will always, however, result in acute miliary tuberculosis.

Summary

Tuberculosis in the human can be separated into different phases, all of which are intimately related; primary infection is the initial stage of the evolution of the disease which may heal, remain quiescent or which may pass into the second stage of dissemination which may be acute, chronic or abortive. The third stage, due to reinfection of the adult body, is characterised by the appearance of a chronic isolated pulmonary tuberculosis.

DIAGRAM OF THE EVOLUTION OF THE DIFFERENT STAGES OF TUBERCULOSIS IN MAN

(According to Kayne, Pagel and O'Shaughnessy)



Explanation of Figures

The legends contain additional information to that in the text. Figs. 1-29 illustrate the development and fate of the tubercle; Figs. 1-7 show the series of changes in the formation of the tubercle as seen in the lungs of rabbits following the intravenous injection of BCG, the animals being killed at different intervals. The latter are not necessarily indicative of the periods required for the various cellular changes to develop nor does the lesion develop constantly in this way during the course of the spontaneous disease. Figs. 34-52 illustrate the different phases of the disease in man, and the remainder are from cases in the dog and cat.

(To be continued.)

PLATE I

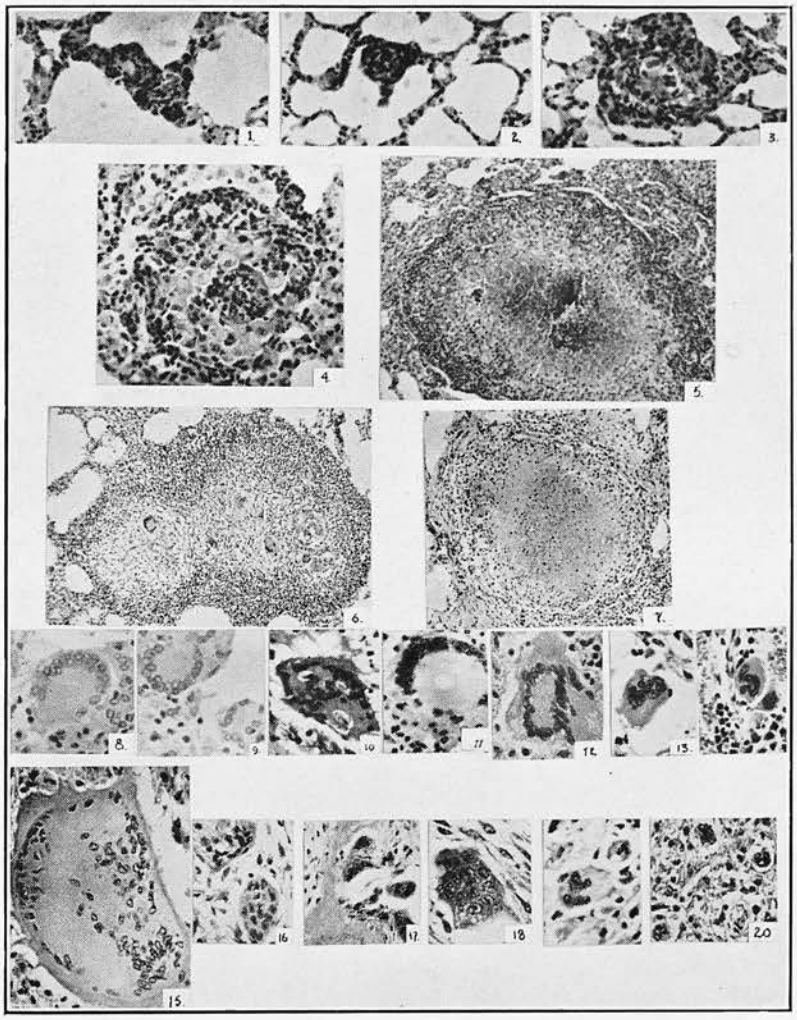


FIG. 1.—Lung. Rabbit. Killed 24 hours after the injection of BCG. Showing the initial intra-alveolar focus of polymorphs and lymphocytes around a clump of bacilli; the latter naturally cannot be seen in this section; no alveolar exudation. H.E.

FIG. 2.—Lung. Rabbit. Killed 48 hours after the injection of BCG. The focus is larger and projects into the alveolus; most of the cells present are lymphocytes. H.E.

FIG. 3.—Lung. Rabbit. Killed 6 days after injection of BCG. The lesion is larger and shows a central area of necrosis around which are large faintly staining cells—early formation of epithelioid cells. H.E.

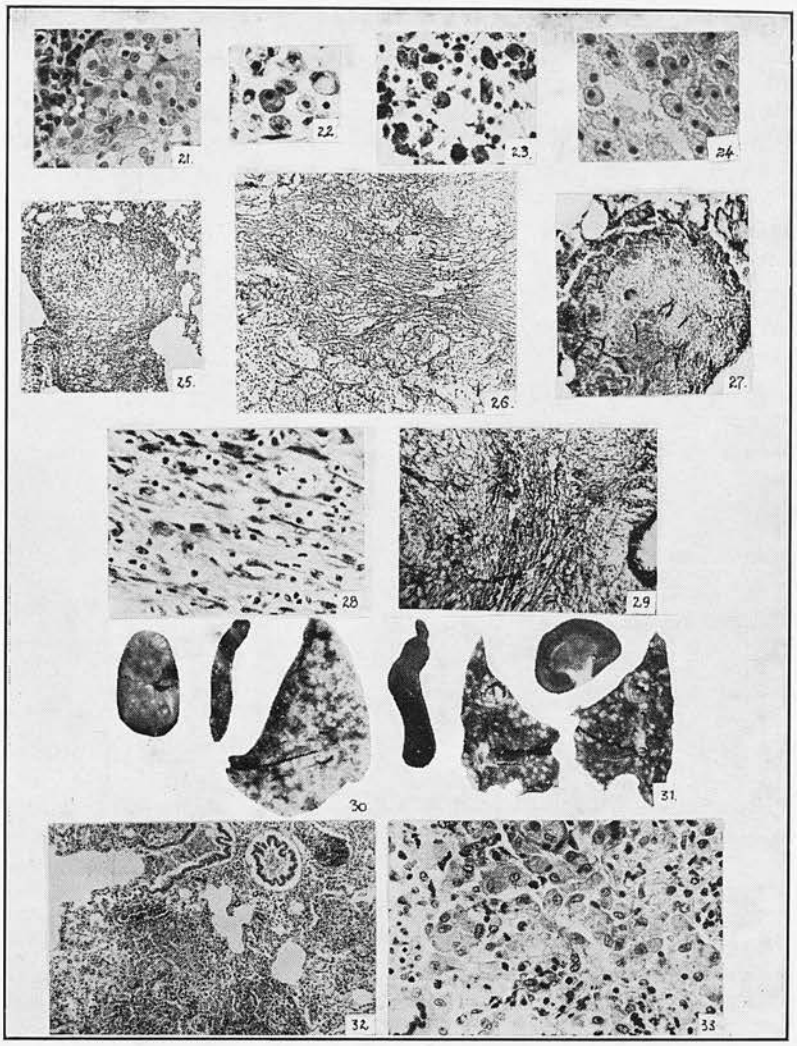
FIG. 4.—Lung. Rabbit. Killed 21 days after injection of BCG. The focus is now a clearly demarcated nodule visible macroscopically and consists of a conglomerate mass of epithelioid cells around a minute central area of cell debris, and a thin peripheral zone of lymphocytes. Many of these tubercles produced by an avirulent strain such as BCG probably do not progress beyond this stage but resolve completely. H.E.

FIG. 5.—Lung. Rabbit. Killed 200 days after injection of BCG. Showing a large well-developed tubercle with extensive necrosis and caseation; the zones of lymphocytes and epithelioid cells are distinct, but no giant cells are present. H.E.

FIG. 6 & 7.—Tuberculosis. Compare the previous figures with these tubercles from (6) miliary tuberculosis, lung, child, in which the lesion is more proliferative in type without central caseation but with giant cells, epithelioid cells and lymphocytes, and (7) miliary tuberculosis, lung, horse, in which central caseation is marked.

FIGS. 8-20.—Giant cells. These show the variations to be found in the form and size of the tuberculous giant cells and the number and distribution of the nuclei; some conform to the so-called "typical" large, round giant-cell (Langhans) with the nuclei arranged in a concentric, horse-shoe fashion around the periphery, others are more atypical but are just as commonly found. 8—From tuberculous meningitis, calf; 9—from primary lung infection, calf; 10—from an intimal lesion, longitudinal sinus, monkey; 11—from udder, tuberculosis cow; 12—from miliary tuberculosis, lung, horse; 15—tuberculosis, tongue, human. Compare these giant cells with those found as: 13—A foreign body giant cell in a chronic inflammatory lesion; 14—a megakaryocyte, normal bone marrow; 16—an osteoclast concerned with normal bone resorption, rib, 1-day-old pig; 17—osteoclasts in excessive numbers, osteodystrophia fibrosa, pig; 18—a giant cell of the osteoclastoma or giant cell tumour of bone, man; 19—giant cells in a case of osteogenic sarcoma, man; 20—bi- and tri-nucleated giant cells of Hodgkin's disease, man (Dorothy Reed or Sternberg cells). H.E.

PLATE II



FIGS. 21-24.—These figs. show (21) epithelioid cells in a tubercle compared with other cells of the same nature arising in different situations and in response to stimuli other than the tubercle bacilli; (22) Alveolar phagocytes, lung, man (often called "heart failure cells"); (23) Macrophages in the sinus of a lymph node showing erythrophagocytosis from a node regional to a hæmorrhagic area in a suspected swine fever case; frequently erroneously termed hæmorrhagic lymph-adenitis; (24) Compound granular corpuscles (gemästete Glia) in an area of demyelination in the brain, Schilder's disease, man; the cells are large and round with eccentric nuclei, and contain much lipoid derived from the broken-down myelin.

FATE OF THE TUBERCLE

(For caseation see FIGS. 5 and 7, and for calcification and ossification see FIG. 38.)

FIG. 25.—Fibrosis. Lung, rabbit injected with BCG. Showing early fibrillary formation in a tubercle demonstrated by a silver impregnation reticulum stain; the fibrils form a delicate basket work at the periphery of the tubercle; these may persist even in advanced necrotic areas. Wilder's method.

FIG. 26.—Fibrosis. Lung, showing a more advanced state of fibril formation which form a closely anastomosing network. Wilder's method.

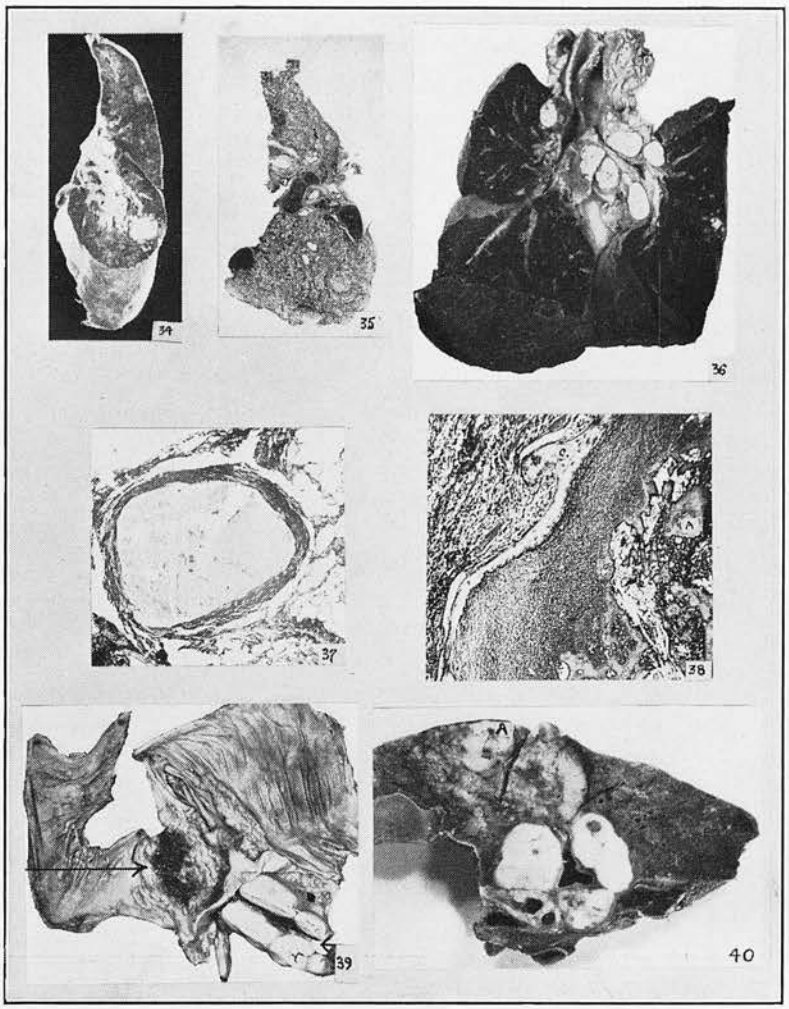
FIG. 27.—Tubercle, lung, child, stained for elastic fibres to show their persistence even in caseous areas. Weigert's Elastic stain.

FIGS. 28 & 29.—Fibrosis. Advanced healed fibrotic lesion, calf; showing the numerous fibroblasts in the midst of dense collagenous tissue, the latter being more clearly brought out in the silver impregnation. (FIG. 29.)

FIGS. 30 & 31.—*Reinfection.*—These figures show the effect of experimental reinfection in the rabbit. (30) Control rabbit injected intravenously with virulent tubercle bacilli; died after 50 days with acute miliary tuberculosis; the multiple nodular lesions in the lungs, spleen and kidney are illustrated. (31) Experimental rabbit injected with a virulent strain 70 days after previous avirulent infection with BCG, thus producing an *isolated* pulmonary tuberculosis with early cavitation in the infra-clavicular area; the spleen and kidneys are free from lesions. This process is comparable with chronic isolated pulmonary tuberculosis of man.

FIGS. 32 & 33.—Lung, rabbit. This animal was reinjected with BCG 21 days after the initial inoculation and died within 24 hours, after showing acute respiratory distress. This demonstrates the occasional high degree of allergy developed in some animals after one infection. Such an animal responds to the second injection by an intense pneumonic exudation of large phagocytic cells and fluid which may be sufficient to cause death. The effects are limited to the lungs. (32) Low power showing the extent of the alveolar exudation, and (33) high magnification showing the epithelioid-like cells in the alveoli.

PLATE III



TUBERCULOSIS IN MAN

(FIGS. 34-52 for comparison with the disease in the dog and cat.) Primary Infection in Children.

FIG. 34.—Primary lung complex. Primary (Ghon) focus in lower lobes; a caseous sub-pleural nodule with satellite tubercles around; the line of lymph spread infection is marked by a row of tubercles passing towards the root; two intra-pulmonary root glands are enlarged and caseous.

FIG. 35.—Early primary lung complex. Large section of the lung showing a primary focus in the lower lobe with early caseous enlargement of the root glands; small tubercles in the lymphatics form rows passing between the primary focus and glands.

FIG. 36.—Primary focus in left lower lobe (<—) as a small caseous sub-pleural nodule with satellite tubercles; gross caseous enlargement of root glands and inferior and superior tracheo-bronchial glands on left side and of one right superior tracheo-bronchial gland.

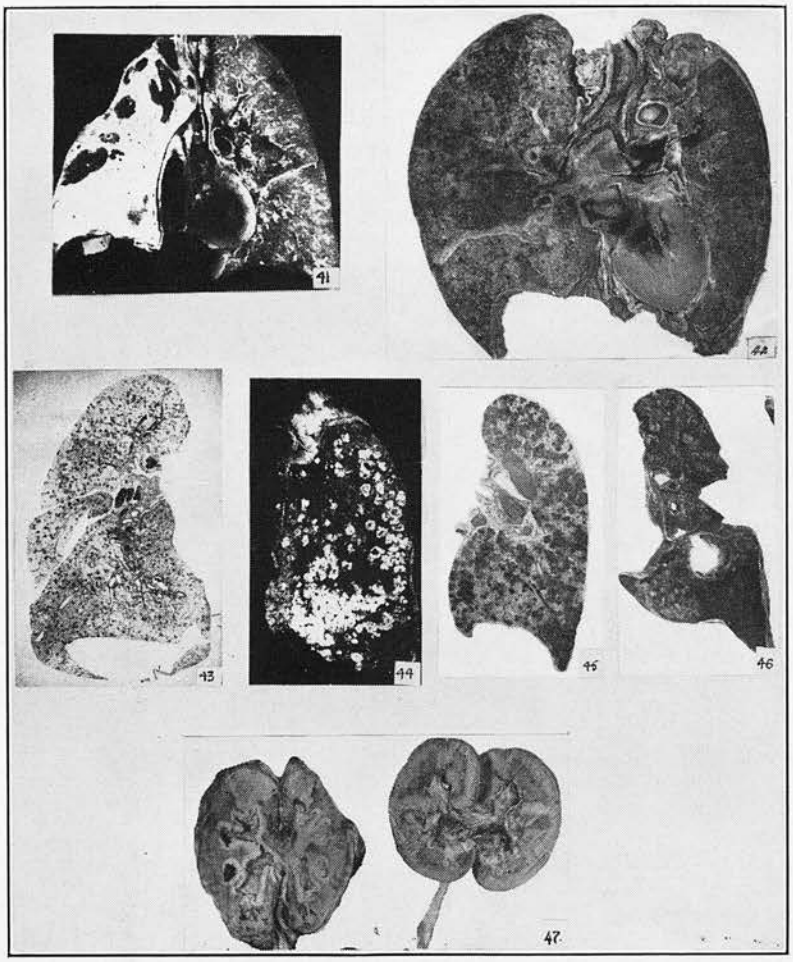
FIG. 37.—Primary focus showing the histology. A sub-pleural (pleura on the left) lobular, caseous, pneumonic area with complete encapsulation; the lung tissue adjacent shows no changes. If healing takes place, this focus becomes calcified and even ossified. (See next figure.)

FIG. 38.—Primary lung focus, showing a focus at a much later stage of development in which there is a calcified centre (A), a fibrotic capsule (B), and ossification of the latter (C). This is the fate of all obsolete primary pulmonary lesions in the human, but does not appear to occur in animals.

FIG. 39.—Primary abdominal complex, showing a primary ulcer at the end of the ileum with enlarged caseous regional lymph nodes.

FIG. 40.—Fresh primary complex with extensive caseous enlargement of the regional hilar glands; the Ghon focus (A) shows localised extension due to bronchial spread; for convenience, the photograph has been placed with the apex to the right.

PLATE IV



POST-PRIMARY EXTENSION AND SPREAD

FIG. 41.—Lobar caseous pneumonia; unilateral extension of the infection by aspiration; early thin-walled cavity formation.

FIG. 42.—Acute miliary tuberculosis. The left lung is collapsed by pleurisy and is free from tubercles; the right side is studded by very minute tubercles, evenly scattered throughout; this involvement is but part of a general dissemination throughout the body, and in this case was universal.

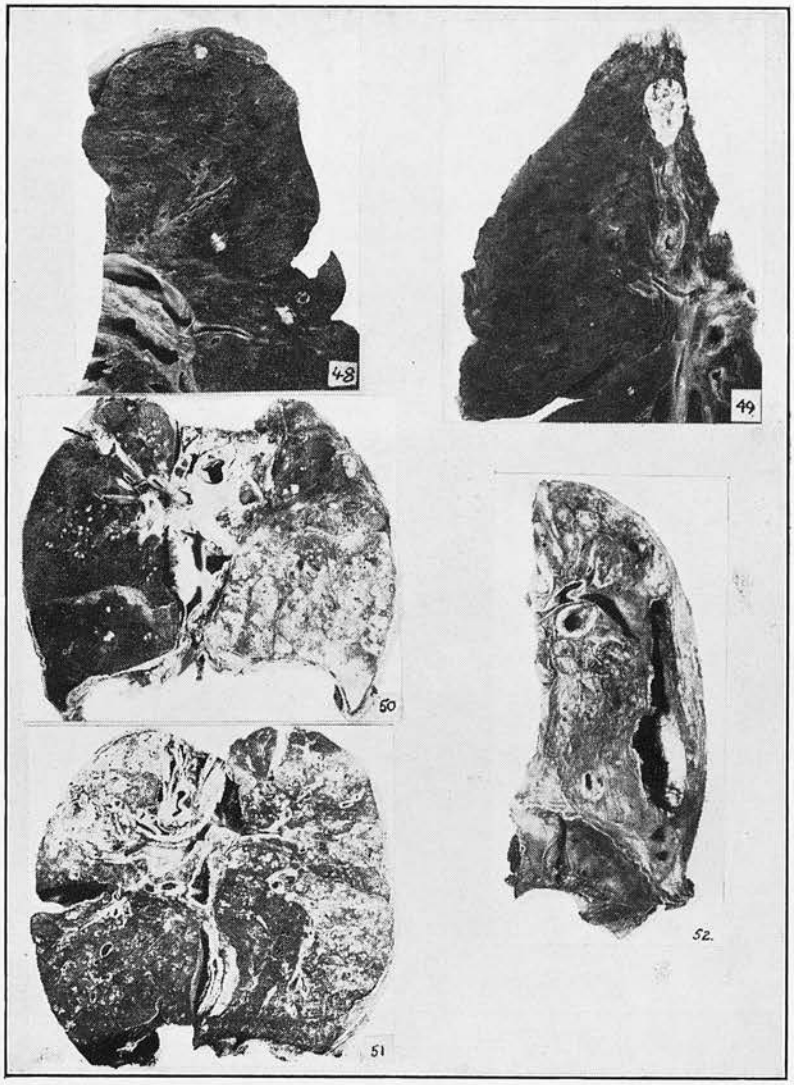
FIG. 43.—Acute miliary tuberculosis. Large lung section. This shows the process more clearly; the characteristic lesions are minute tubercles seeded uniformly throughout the lung, in which caseation has hardly begun; again simply part of general dissemination throughout the body; the primary focus for this dissemination need not be pulmonary.

FIG. 44.—Lobular type of caseous pneumonia; the extension of the infection has arisen by the breaking down of a cavity and resultant aspiration.

FIG. 45.—Subacute or chronic miliary tuberculosis; this occurs when infection of the blood stream has not been severe enough to cause early death; the miliary lesions have thus time to develop more fully. The lungs are studded with numerous caseous foci, many of them being confluent; the lesions vary in size from a pin-head to half a centimetre. Other organs in the body may be similarly involved, but cavity formation is rare.

FIG. 46.—Thin-walled cavities. This picture shows the type of cavity which is found in the lungs when the latter are involved in a more chronic process at this stage of evolution of the disease. They appear as if they were "punched out," and have thin walls, and should be compared with FIGS. 52 and 59.

FIG. 47.—Chronic urogenital tuberculosis. Hæmatogenous spread following in the course of post-primary dissemination, causing a chronic caseating pyelo-nephritis, pyelitis and ureteritis. This is given as an example of the spread which occurs at this stage; other organs may be similarly infected.



CHRONIC ISOLATED PULMONARY TUBERCULOSIS OF THE ADULT DUE TO REINFECTION

FIG. 48.—Typical calcified reinfection (Puhl's) focus near the lung apex. These lesions may heal and give rise to the typical apical scars; if not, they form a nidus for all future developments and extensions of the disease to produce the chronic phthisis; the associated lymph node does not show any change.

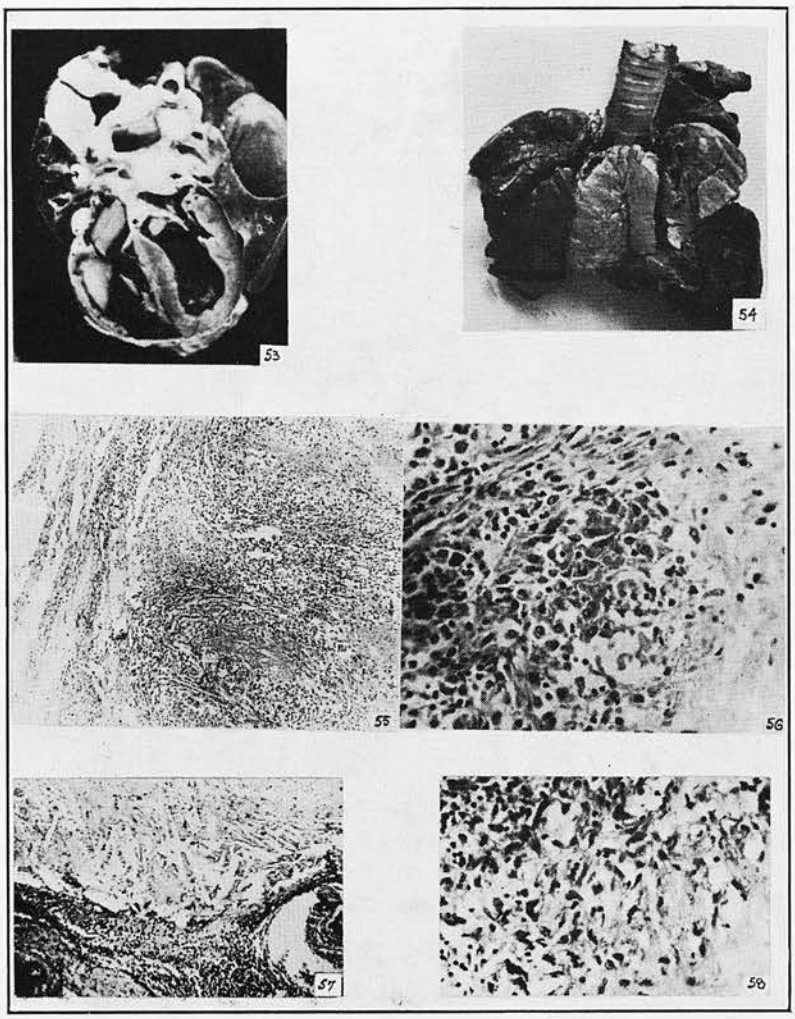
FIG. 49.—Old, calcified, reinfection focus at the apex, with no involvement of the regional node.

FIG. 50.—Reinfection focus in right upper lobe; confluent, caseous pneumonia in left lower lobe, due to aspiration infection from a cavity which is not shown; the lesions in parts show the typical clover-leaf form ("acino-nodular").

FIG. 51.—Early cavitation of the focus in the right upper lobe with aspiration infection and the production of acino-nodular lesions.

FIG. 52.—Chronic phthisis. Showing extensive cavitation in the left lower lobe with thick fibrotic walls and thickening of the pleura. Compare with thin-walled cavities in FIG. 46.

PLATE VI



TUBERCULOSIS IN THE DOG

FIG. 53.—Primary pulmonary infection. Transverse section through lungs and heart just in front of the bifurcation of the trachea. Showing a fully developed primary complex as a small hazel-nut-sized focus in the hilar region of the right apical lobe of the lung(<—); enlargement of the lymph nodes of the same side (A) and slight enlargement of the hilar glands on the other (B). This case also showed a few nodules scattered over the pleura and pericardium (C). Compare with the figs. showing similar lesions in children. Tuberculin negative. Terrier. M. 4 yrs.

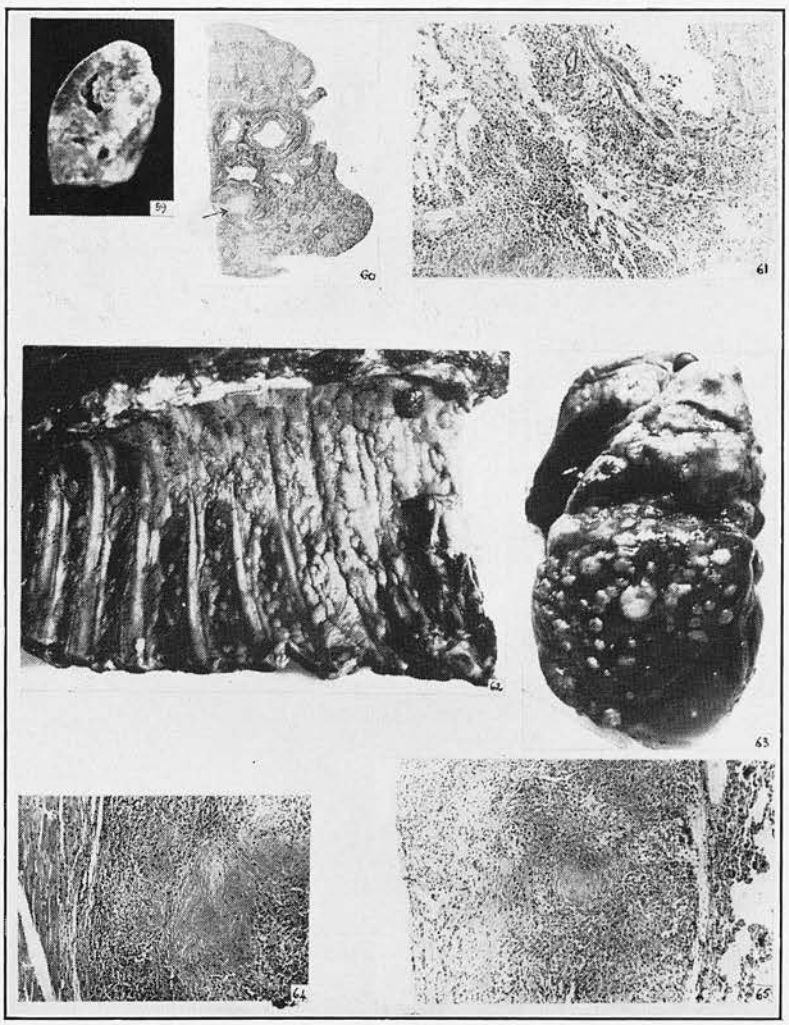
FIG. 54.—Primary pulmonary infection. Showing a primary complex in a more advanced condition, with massive caseous enlargement of the bifurcation (A) and right hilar nodes (B). The lung lesion was a large walnut-sized focus present in the dorsal border of the right cardiac lobe (C), which was partially excised for histological purposes; this focus is fused with the enlarged lymph glands; the left hilar node was free from lesions; no generalisation; bacilli numerous in smears. Bull terrier. M. 2 yrs.

FIG. 55.—Primary infection. Showing the lung lesions separated without any encapsulation from partially collapsed lung on the left. Irregular confluent foci of necrosis surrounded by a cellular infiltration consisting mainly of macrophages, lymphocytes and fibroblasts, the latter arranged in a trabecular manner. No typical tubercle formation; no giant cells or calcification. Same case as FIG. 54.

FIG. 56.—Primary pulmonary infection, showing a higher magnification of the cellular infiltration in which macrophages, fibroblasts and lymphocytes can be clearly distinguished. Same case as FIG. 55.

FIGS. 57 & 58.—Primary abdominal infection. Incomplete primary complex. Mesenteric lymph node from a dog which was killed because of carcinoma testis. The node was not enlarged and showed a small bean-sized caseous focus in the centre; there were no other tuberculous lesions in the body. Bacilli were present in smears but not recovered by culture; the lesion might thus be regarded as quiescent or obsolete. Histologically, the lesion consisted of a central necrotic area with a few calcific deposits, but without any definite fibrotic capsule; between the normal gland structure and necrosis there is remarkably little reaction; a few macrophages and fibroblasts lie amidst collagenous strands; no giant cells. FIG. 57.—Low magnification of the lesions with normal lymph gland below. FIG. 58.—High magnification of an area at the edge of the lesion.

PLATE VII



POST-PRIMARY DISSEMINATION

FIG. 59.—Primary pulmonary infection with extension by aspiration throughout the lung, producing bronchitis, caseous pneumonia, and bronchiectatic cavitation; the irregular cavity is not comparable to the thin-walled "punched-out" cavities of childhood tuberculosis. Naked-eye photo of transverse section through one lobe of the lung.

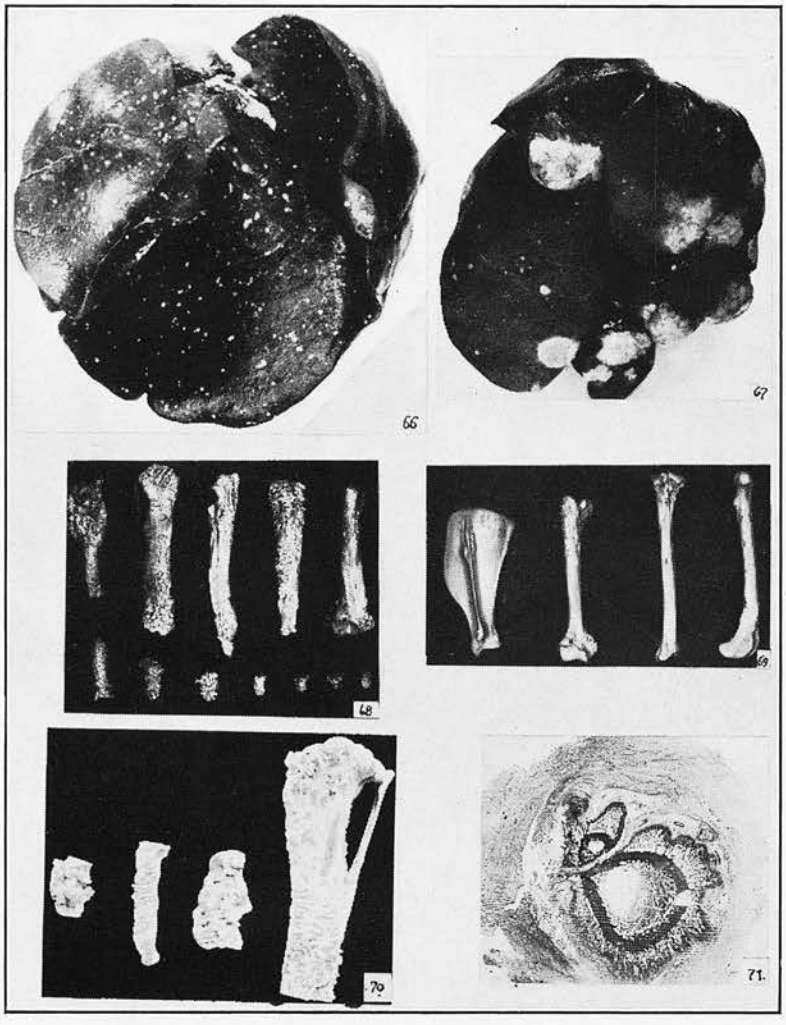
FIG. 60.—Large section from same case as FIG. 59, showing the multiple caseous foci mostly in relation to bronchioles and part of the cavity (—>).

FIG. 61.—Same case as FIGS. 59 & 60, showing the peribronchial infiltration and the edge of a caseous area at bottom left of photo; the cellular reaction is similar to that seen in FIG. 56.

FIGS. 62 & 63.—Pleurisy and pericarditis. This shows the appearance of the nodular lesions which occur on the pleura and may involve the pericardium (FIG. 63) and other thoracic structures such as aorta, but the changes may be more diffuse; sero-sanguinous transudate may be so abundant as to cause compression atelectasis.

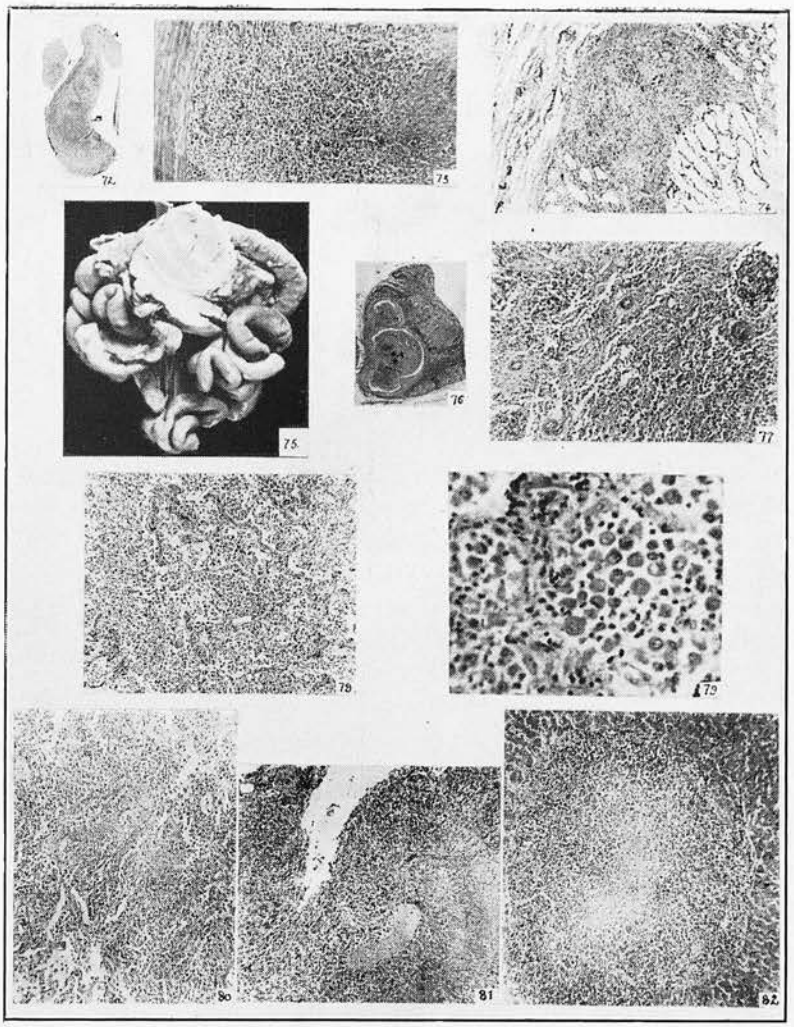
FIGS. 64 & 65.—Pleurisy. Showing the lesions present in the costal pleura (64) and the lungs (65). The histological picture is similar to that seen in the primary lung lesion, although it may be more granulomatous. This is from a case of primary pulmonary infection which showed similar pleural involvement to that seen in FIG. 62.

PLATE VIII



FIGS. 66 & 67.—Liver. Subacute miliary tuberculosis. Showing types of dissemination; FIG. 66.—The whole organ is evenly and diffusely scattered by small tubercles, while in FIG. 67 the lesions form isolated large nodular masses. Both would show similar histological picture to that seen in other canine lesions.

FIGS. 68-71.—Acropachia or hypertrophic osteo-periostitis. FIG. 68.—The limb bones and digits from a case of pulmonary tuberculosis; all the bones are covered by an exuberant periosteal overgrowth. FIG. 70.—Another case showing similar lesions in the tibia, tarsal and metatarsal bones, but all bones in the leg (right) which were examined were similarly affected; this is from the same case as FIG. 59, in which the probable duration of the disease was about 1 yr. FIG. 71.—Photograph of whole transverse section (slightly enlarged) of the tibia from same case as FIG. 70, showing the formation of excessive spongy bone arising from the periosteum to form another irregular layer of compact bone; note also the massive paraosteal fibrosis. FIG. 69.—Early lesions of limb bones of dog with carcinoma involving head of pancreas with secondary extension to the liver. No tuberculosis.



TUBERCULOSIS OF THE CAT

FIGS. 72 & 73.—Primary abdominal infection—complete complex. A single isolated 1 cm. ulcer was present in the mucosa of the terminal part of the ileum with gross caseous enlargement of the regional mesenteric lymph nodes; dissemination to the lungs with the production of caseous pneumonia, isolated foci in the spleen and liver. This section shows normal mucosa at (A) and ulceration of the thickened mucosa at (B) extending deep into the muscle; the cellular infiltration at the base of the ulcer (FIG. 73) adjacent to the muscle consists mainly of large macrophages and lymphocytes; there are no giant cells. The serosa was fused with a small lymph node (C). The histology is identical to that seen in the canine lesions; bacilli were numerous in smears and the lesion was obviously a progressive one.

FIG. 74.—Primary abdominal infection—complete complex; this shows a small fibrous scar in the mucosa of the ileum in a region which was fused to a large caseous mesenteric node such as is seen in FIG. 75. This might be regarded as a healed minute primary intestinal focus even although spread of the infection had occurred to the regional nodes and to the lungs.

FIGS. 75 & 77.—Primary abdominal infection—incomplete complex; showing a massively enlarged caseous mesenteric lymph node; in this case there was no ulceration visible macroscopically in the intestine; isolated foci were present in the lungs, liver, spleen and kidney. FIG. 77.—The lesion shows a similar picture to that seen in canine tuberculosis, the cellular reaction consisting mainly of macrophages and lymphocytes; no giant cells or calcification.

FIG. 76.—Primary abdominal infection—"quiescent incomplete complex." The only lesion present in this animal was a small caseous area in a mesenteric lymph node similar to the dog case, FIG. 57. This section shows a caseous area on the left (A), sharply demarcated from normal lymph node by a thick well-developed fibrotic capsule (<—); bacilli were present in smears but could not be recovered culturally or by guinea pig inoculation.

FIGS. 78 & 79.—Tuberculous pneumonia. Early generalisation to the lungs from a primary abdominal infection with the production of an acute pneumonic process involving whole lobes; the lungs were enlarged, voluminous and consolidated; the alveoli are filled with a cellular exudate consisting mainly of macrophages (alveolar phagocytes), lymphocytes and a few polymorphs; these cells are seen in greater detail in the high magnification (FIG. 79).

FIGS. 80 & 81.—Subacute miliary tuberculosis. Lungs; low magnification of a caseous area (FIG. 80), an area adjacent to a bronchiole and with the same type of cellular reaction as seen above. (FIG. 81). Much exudation of large macrophages in the adjacent lung alveoli.

FIG. 82.—Subacute miliary tuberculosis in the liver; the naked-eye picture was similar to FIG. 66. Low magnification of a nodule showing central necrosis and cellular reaction as seen in other canine and feline lesions.

(Reprint not available. Copy of paper accepted by J. Path. Bact.)

FAMILIAL CEREBELLAR HYPOPLASIA AND DEGENERATION IN HEREFORD CALVES.

by

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The condition described in this paper appears to affect cattle of the Hereford breed only. This breed, which has probably been in existance for over 300 years, is remarkably pure. The Herd Book has been closed since 1884 and registration is now limited to the progeny of sires and dams entered at that time. The annual export of most of the outstanding sires of the herd to other countries has led to the retention in England of a relatively small number for breeding purposes. Further, the restriction of the choice of sires has resulted in a high degree of both inbreeding and line-breeding. Although attention is now drawn for the first time to the occurrence of this disease in English cattle, there is little doubt that it has been known to farmers for many years but has hitherto escaped investigation.

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Material from five pure-bred Hereford calves suffering from the condition has been examined histologically. They came from three different and widely separated farms. Calf I from the first farm was one of three cases, all females, which were born in 1936, showed similar symptoms and were the offspring of cows sired by the same bull, and part of a herd of fifteen animals. After disposal of the bull no further cases occurred nor did the condition occur in the progeny of other cattle outcrossed with Shorthorns. Our examination was confined to a few pieces preserved from the brain. Calves 2 and 3 were two of three calves (2 females and 1 male) affected at a second farm in 1939. On this farm two affected calves (both females) had been observed in 1938. One of the calves in 1938 and one in 1939 were the progeny of the same cows. A similar disease had been seen ten years earlier and had been eliminated by disposal of the bull. Calves 4 (male) and 5 (female) came from a third farm. The two cows concerned in a herd of 20 had had normal progeny previously by the bull responsible for the diseased calves.

Information was received concerning the occurrence of the disease on a fourth farm. The herd consisted of 25 cows of which 20 were pure-bred Herefords, and affected progeny again occurred only in the latter. Five affected calves were born in 1938 and six in 1939, all being females except one.

CLINICAL.

The same symptoms were present in all the calves observed. The animals seen at birth, and later, appeared stuporose and were unable to rise. They lay on their side with their limbs and neck stiffly extended (Fig.1), and if placed on their feet collapsed immediately, often backwards. Balance was maintained for a little longer if the animal was placed in the abnormal posture shown in Fig.2. Rigidity disappeared at times and then there were convulsive movements which seemed to be attempts to get up. In the more successful of these the calves sometimes adopted th

same method of getting to their feet as a foal; that is, they raised themselves on their forelegs first. Occasionally they might get as far as the sitting position but soon collapsed again. There were no fits. Opisthotonus was sometimes conspicuous and periodically there were tremors of the head. Nystagmus was seen in one case only. The animals readily responded to the call of their dams by bawling and had no difficulty in sucking. In no instance has any clinical improvement been seen although there was apparently no difficulty in keeping them alive by careful attention and bottle feeding. The calves were killed when from 1 to 20 days old.

PATHOLOGICAL EXAMINATION.

The necropsies were done immediately after death and disclosed no abnormality apart from the brain. The material reserved for further examination was fixed and hardened in 14 per cent. saline formaldehyde.

Macroscopic appearance of brain.

Compared with a control specimen (Fig.3.) the cerebellum was abnormally small (Figs. 4 & 5), all the folia showing some degree of wasting. On section the texture was tough and leathery. The brain stem and cerebral hemispheres were normal both superficially and on section.

Microscopic examination of brain.

Methods. Blocks from different parts of the brain were embedded in paraffin and sections were stained with Ehrlich's haematoxylin and eosin, Weigert's iron haematoxylin and van Gieson's mixture, Weigert's fuchselin and neutral red for elastic fibres, phosphotungstic acid haematoxylin, toluidin blue for Nissl bodies and Loyez' haematoxylin method for myelin.

Frozen sections were stained with Scharlach R and haematoxylin, Spielmeyer's method for myelin, Hortega's silver carbonate methods for neurofibrils and for glial fibres and by Penfield's modification for microglia and oligodendroglia.

Pathological changes are confined to the cerebellum and are similar in all the cases examined. The folia everywhere are narrower than in normal controls (Figs. 6 & 7). The degree of wasting varies slightly in different parts of a specimen and between the different cases. It is associated with gross disorganisation of the normal cortical structure (c.f. Figs. 6 & 7). There is no inflammatory or other change in the adjacent leptomeninges. Beneath the pia the foetal granular layer is still present in places but is seldom more than three cells deep. The molecular and granular layers are both abnormally thin. Their boundaries are somewhat blurred through the presence of an excess of cells in the molecular layer and the almost complete absence of Purkinje cells. The cells in the molecular layer are

principally round or oval and are about twice the size of the granule cells (Fig.8.) They are fairly evenly distributed throughout the layer. Each contains a relatively large, eccentric nucleus which, in silver preparations, has a pale grey nucleoplasm and a single large central nucleolus. About the nucleus is a narrow, uneven rim of argentophil cytoplasm which occasionally extends to form from one to three delicate unbranched processes. These cells are rarely bipolar; they are then orientated in either the vertical or horizontal directions. From their appearance they are undoubtedly neuroblasts, but they show little evidence that they are precursors of the granule cells proper and they are considerably larger than the cells of either the foetal granular or inner granular layers. Apart from these neuroblasts the molecular layer contains occasional microglial cells of normal appearance and astrocytes whose processes contribute to a dense vertical gliosis (Fig.9.) The greater part of these glial fibres are, however, derived from Bergmann cells occupying the upper boundaries of the granular layer. Amongst them an occasional Purkinje cell may be found. These are usually shrunken and deformed, their dendrites being few, coarse and angular. In Nissl preparations they often show great pallor, loss of Nissl substance and karyolysis. In one instance a retraction ball was found upon the axon. Rarely neurones of the Purkinje type occupy the molecular layer or the depths of the granular layer. Most often the larger neurones

in the granular layer are, as normally, of the Golgi type (Fig.8.) These often show eccentricity of their nuclei and margination of their Nissl substance. A few neuroblasts, similar to those in the molecular layer, are also present in the granular layer. The granule cells proper are fewer than in normal controls; their nuclei are sometimes shrunken and pyknotic. In this layer also there is an excess of neuroglial fibres.

The white matter is somewhat reduced in amount. Both axis cylinders and myelin sheaths are well preserved except for occasional ballooning and droplet-formation in the latter. The tangential fibres of the cortex are also preserved (Fig.10.)

The central nuclei of the cerebellum appear normal. Comparison with sections from a normal calf 5 to 7 days old showed no differences in the structure of the pons and medulla oblongata. In particular the inferior olives were similar in both.

No abnormality was observed in the spinal cord.

DISCUSSION.

The changes in the cerebellum in these animals might be attributed either to an arrest of development, or to a degenerative process or to both. In support of the former theory is the

relative paucity of cells in the granular layer, the excess of neuroblasts in the molecular layer, the absence of any but trivial destruction either of axis cylinders or of myelin, and the normal appearance of the microglial cells. In favour of a degenerative process is the pronounced gliosis of the cortex and the evidence of degeneration in the surviving Purkinje cells and other neurones. Post-mortem degeneration can be absolutely excluded since the material was fixed within 20 to 30 minutes of the death of the animal. It is generally agreed, following Cajal, that the granule cells are derived from the foetal granular layer of Obersteiner by the inward migration of these cells. The foetal granular layer in the diseased calves is no greater than in the controls, and yet the inner granular layer is not of normal density. This might be attributed equally to destruction of granule cells or to defective formation (hypoplasia) of the foetal granular layer. The neuroblasts in the molecular layer are about twice the size of the granule cells and for the most part lack the bipolar character of these cells during migration. It is unlikely, therefore, that they are intermediate forms. They might be regarded as precursors of the Purkinje cells which are conspicuous by their absence from their normal position. The origin of the Purkinje cells has never been finally settled though it is usually supposed that they have an independent origin from the inner mantle layer. While the occasional presence of recognisable Purkinje cells in the molecular

layer is a normal feature in various animals (Cajal, 1911) and is therefore without special significance in these cases, the histology of the molecular layer suggests that the origin of the Purkinje cells from the external granular layer is at least a possibility.

It might, however, be argued that the Purkinje cells had already reached their proper destination and subsequently disappeared through degeneration. The evidence of degeneration in the few existing Purkinje cells supports this. On the other hand, when many Purkinje cells have been destroyed, it is usually possible to demonstrate the places which they occupied by the empty baskets of climbing fibres which persist at such sites, while destruction of their axones leads, in sections stained for myelin fibres, to the appearance of zones of pallor about the dentate nuclei. Neither of these features was seen in the present cases. It seems probable, therefore, that there is deficient formation of Purkinje cells as well as degeneration of those that have been found. The large neuroblasts in the molecular layer may represent immature Purkinje cells that have been delayed in transit. The possibility that they are morphologically abnormal precursors of the granule cells cannot of course be excluded. There appears sufficient reason to conclude that the pathological changes in the cerebellum in these calves is the result of arrested development in addition to degeneration of the cortex.

A similar condition has been recognised in kittens

(Herringham and Andrewes, 1888, Brouwer, 1934). In both of these reports several kittens of the same litter were affected. While the histological condition described in the earlier paper appears to have been identical with that now described, in Brouwer's cases there was, in addition, an atrophy of the inferior olives and of the oral parts of the nuclei pontis. The clinical features in both batches of kittens were similar to those in the calves but of less severity. Congenital ataxia in a cat six months old was described by Langelaan (1907). The other five of the litter were unaffected. This case resembled the calves in respect of the cerebellar changes and the immunity of the inferior olives but differed from them in that there was degeneration of the spino-cerebellar tracts. One of a litter of congenitally ataxic puppies was reported by Risien Russell (1895). It is of interest that the parents were described as being healthy, prize dogs. Histological examination in one puppy showed a condition of the cerebellar cortex which was probably identical with that in the calves except that the absence of Purkinje cells was not so complete.

An analogous condition almost certainly finds a place in human pathology. Congenital cerebellar ataxia was distinguished clinically from Friedreich's ataxia by Batten (1905) and has more recently been reviewed by Hallervorden (1937). While familial examples have been recorded, the onset of symptoms in such has

usually been in the middle decades (Thorpe, 1935). Pathologically there is a primary degeneration of the Purkinje cells with their axones and a reduction, often uneven, of the molecular and granular layers (Holmes, 1907). Of greater interest is the report by Fraser (1880) of a familial cerebellar ataxia affecting a brother and sister in the second to third year of life and persisting to adult life. Pathological examination of the male showed a diffuse cerebellar atrophy with great reduction of the cortex and loss, through degeneration, of a large number of Purkinje cells. In these familial human cases, therefore, the changes in the cerebellum appear to be purely degenerative and the aplastic features seen in the young domestic animals are lacking. Nevertheless, it is possible that the underlying cause is the same in both and that the observed differences are attributable simply to the onset of degeneration in the young animals at a time when development is incomplete. Scherer (1935) has reported three cases of non-familial cerebellar cortical atrophy in young infants. His first differs from the calves in that the cerebrum also was small and there was demyelination in the neo-cerebellum. The cortical changes in the cerebellum also were patchy. His second and third cases were histologically similar to the calves but bore a closer resemblance to Brouwer's kittens in that the inferior olives and the pons were also atrophied. In this they agreed with the case reported by Holmes.

The aetiology of the human familial cerebellar ataxia is unknown; it would therefore be highly important if it were established that the condition in the calves is due to an hereditary factor. The evidence supporting this possibility is highly suggestive.

Breeders of pedigree stock usually raise only those that are perfectly sound and in consequence when abnormalities appear the animals are discarded with as little comment as possible. Their attempts, however, to fix certain types or characters have occasionally produced structural defects in cattle, the most noteworthy being the so-called "bull-dog" calf, mainly of the Dexter breed. The facts available which support the idea that the disease is hereditary are:-

(1) Defective calves were born when the Hereford bull was bred to Hereford cows but did not occur in Shorthorn crosses.

(2) From this, and because only a relatively small number of diseased cattle were born in any one herd, the character might appear to be recessive.

(3) More females were affected than males. This accords with the common finding that many defective inherited characters have a relationship to sex. (Sewall Wright, 1934).

(4) In two instances replacement of a bull, which appeared to be siring defective progeny, was followed by non-recurrence of

the disease.

Further discussion is beyond the scope of this paper, but it is apparent that the subject merits attention from the genetical aspect.

SUMMARY.

The clinical and histological features of five examples of familial cerebellar hypoplasia, arrested development and degeneration in calves are described. The pathological changes are confined to the cerebellar cortex.

In cattle the condition appears to be restricted to the Hereford breed and is possibly due to an hereditary factor which has emerged as the result of intensive inbreeding and line-breeding.

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NERVOUS AFFECTIONS IN MAN AND ANIMALS

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DR. FINDLAY has dealt with a large group of conditions of the nervous system common to man and animals; there are, however, many others, and it is my intention to deal with only a few examples.

MALFORMATIONS OF THE NERVOUS SYSTEM.

Probably more has been written about malformations of the nervous system in animals than any other condition (*see* Dobberstein, 1936; and Ernst, 1909). Most of the malformations (lethal and sublethal) occurring in man have been described, for example anencephaly, acrania, total and partial cranioschisis, exencephalia, amyelia, encephalocele, meningocele, spina bifida, macro- and micro-encephaly, various hypoplasias and aplasias, micro- and macro-gyria, anophthalmia and micro-ophthalmia; one which has not been recorded is tuberose sclerosis. Such studies as these are of importance from the aspect of comparative embryology.

PIGMENTATIONS.

Melanosis.—It has been stated that melanin pigmentation of the cells of the *substantia nigra* is specific for man, but Scherer (1939) has shown that it occurs in adult anthropoids, though invisible macroscopically. In some animals (cows and sheep) melanin pigmentation in association with the central nervous system is found with a frequency and degree not found in man or the apes. In these animals pigmentation of the dura and/or pia mater is a constant finding, and this has been confirmed by the personal examination of many sheep brains. In the sheep, intense pigmentation may be confined to the pia mater covering the superior frontal gyri of the cerebrum (motor area), while scattered fainter deposits may extend over the peduncles, pons, and medulla. In cattle similar changes may occur in the cerebral meninges and the spinal pia, but the latter is usually regarded as an accompaniment of generalized melanosis, affecting for example the lungs, endocardium, aorta, and liver. Even when intense, it causes no apparent functional derangement. The cause is unknown and can be regarded more as an abnormal increase of chromatophores constantly present in the meninges rather than as a heterotopic phenomenon. As the melanomatous meningioma enters into all type classifications of the meningioma in man, this condition in animals is important from that aspect.

Lipofuscin.—Many normal nerve cells contain lipoid in the form of granules of lipofuscin and in man its presence is regarded as a normal phenomenon of unknown significance associated with advancing age. The same type of cellular inclusions has been observed in senile horses by Kikuchi (1928) and others.

Iron pigmentation—Pallidal siderosis.—Hurst (1934) has shown that in horses iron, in association with calcium, is often found in the vessels of the *globus pallidus*, while Kikuchi (1928) and Holz (1936) have recorded similar lesions ("pseudo-calcification") in the *nucleus dentatus*. The histological appearances were comparable with the human condition, the deposits lying either in the media or adventitia, or both; in Hurst's cases the horses did not show signs of generalized vascular disease, nor could the siderosis be related to the pathological condition responsible for death. Similar appearances were seen in monkeys but not in guinea-pigs, rabbits, mice, or rats, while personal examination of the brains of old dogs has not revealed similar lesions. Hurst concluded that the changes represented a phenomenon of some constancy in higher animals of advancing age.

ATROPHY.

Senile atrophy.—Although some senile changes are well known, the effects of age on the nervous system in animals have not been studied extensively, largely because

few animals are allowed to live to an age comparable to the senile state of old people. Available information (*see* Dobberstein, 1936) indicates that the changes described in man also occur in animals. The brain may be shrunken and less in weight, although this is difficult to assess for there are no data of normal weight standards for animals of different ages; the meninges may be thickened, the convolutions separated by markedly deep and wide sulci, and the brain tissue tough. Specific human senile or presenile entities, such as Alzheimer's and Pick's disease, and conditions such as paralysis agitans and idiopathic tremor, have not been observed in animals.

Pachymeningitis Spinalis Ossificans.

This is a pathological condition which might also come within the range of senile phenomena, and has been described by Kitt, Dexler, Joest, and others (*see* Dobberstein, 1936). In the cervical and lumbar regions particularly, but occasionally in the cranium, the dura mater shows bluish-white plaques which may fuse and reduce the dura to a rigid tube. These plaques are true lamellar bone with Haversian systems, and do not arise from the vertebræ for a normal epidural space is present. The bone lies in the middle of the dura thickness and is covered on both sides by connective tissue; the ætiology and pathogenesis of this condition is still uncertain. There is not sufficient evidence to show whether it is an ossification of an old-standing chronic pachymeningitis or neoplastic in nature. The effect of this dural thickening may, in severe cases, cause a compression myelitis and chronic neuritis of the nerve roots which may in their turn cause paralytic symptoms, protracted muscular spasms, and painful paræsthesias. The closest parallel in the human being seems to be the occurrence of calcified plaques in the arachnoid of old people.

Intracranial Tumours.

Gliomata are regarded as rarities in domesticated animals, but until some thousands of animal brains are examined, no dogmatic statement can be made. The few records in the literature were published before the work of Bailey and Cushing (1926), and refer to the tumour simply as a "gliosarcoma". This only indicates that with hæmatoxylin-eosin sections the tumour was highly cellular with an appearance like a sarcoma in non-cerebral sites. The case recorded by Pallaske (1935) in a dog was an astroblastoma and no histological descriptions are given of ten cerebral tumours in dogs recorded by Milks and Olafson (1936), although the diagnoses included oligodendroglioma, "ganglio-neuroma", medulloblastoma, choroidal papilloma, and "perithelial sarcomas". A few cases of "glioma" have been described in horses and these were classified as "gliosarcoma". There are no records of the occurrence of other tumours of the glioma group such as neuro-epithelioma, *spongioblastoma multiforme* and astrocytoma; an ependymoma of the lateral ventricles of a mouse has been described by Slye, Holmes and Wells (1931).

Similarly the meningioma as such has not been recorded in animals, although several tumour entities have been described as spindle-cell or round-cell sarcoma which might well have been meningioma. The "cholesteatomata" (*see* Holz, 1935; Dobberstein, 1936; and Critchley and Ferguson, 1928) which occur in the horse either as a dural "epidermoid cholesteatoma" or as "plexus cholesteatoma", and which have often been termed by veterinarians as "psammoma", is not a meningioma. The "epidermoid" type, which may compress the brain, contains epidermoid derivatives and has been suggested to be teratoid in nature, while the "plexus cholesteatoma" has been regarded, mainly by French and German authors, as a chronic granulomatous lesion of the choroid plexus with massive deposition of cholesterin crystals. A suprasellar craniopharyngioma in a dog has been described by White (1938) and he refers to the few records of pituitary tumours in animals.

The incidence of metastatic tumours of the brain in animals is also not known but is probably not so common as in man. (Willis (1934) gives the incidence in man as about 5% of all fatal cases of malignant disease.) I have seen in the brains of dogs, metastases of a malignant melanoma of the skin and of a primary carcinoma of the mammary gland.

CANINE ENCEPHALITIS.

Although a so-called nervous form of canine distemper exists clinically it is now generally held that the distemper virus itself is not associated with cerebral manifestations. Infection with the distemper virus may, however, sometimes precede these nervous complications. In 1930 Perdrau and Pugh observed demyelination of the type found in subacute disseminated sclerosis in 4 out of 14 cases of disseminated encephalitis in dogs; other observations included mononuclear infiltration of the meninges and perivascular "cuffing", but there was no uniform distribution of the lesions except for the meningitis of the olfactory lobes; in most cases lesions predominated in the pons, medulla, peduncles, and cerebellum. They concluded that "distemper" was not an essential antecedent of the disease, as it occurred in only 7 of their 14 cases, but the virus played a rôle similar to that which some acute infections play in certain post-infective demyelinating diseases of man. Other papers on the same subject include those by Gallego (1928), Posrednik (1930), De Monbreun (1937), Marinesco *et al.* (1933), Peters and Yamagiwa (1935), and King (1939).

The terms "distemper" or "post-distemper encephalitis" have been loosely applied and with no more proof than that the dog may, some time previously, have suffered from a febrile illness. Scherer and Collet (1939) have recently remarked on this and described pathological entities in the brains of each of three dogs which were clearly distinct from each other; all three processes described by them have at various times been illustrated as part of "post-distemper encephalitis".

Nearly 50 dogs with a "nervous disorder" have been examined personally, and the clinical diagnosis included "fits", "hysteria", "chorea", "epilepsy", and "distemper encephalitis". Some may have been a true "hysteria" or psychopathic state as they showed no obvious brain lesions. The remainder showed varying changes indicative of the disseminated encephalomyelitis with demyelination, described by Perdrau and Pugh (also described in one of Scherer's cases), and by King (1939), while others corresponded to the process of diffuse intracortical vascular proliferation without inflammatory infiltrations and occasional foci of meningitis described in Scherer's first case. (The latter type of lesion is also described by Peters and Yamagiwa in their "distemper encephalitis" and who compare it with Wernicke's encephalopathy.) Whether yet others correspond exactly to the disseminated focal type of sclerotic lesions described in Scherer's second case is uncertain. There is, however, enough pathological evidence to show that more than one entity may occur in the dog which may have been regarded in the past as nervous forms of distemper or as "post-distemper encephalitis", and in which the association of the distemper virus is problematical.

Dunkin and Laidlaw (1926) had been unable to demonstrate lesions similar to those described by Perdrau and Pugh in their experimental cases of distemper which showed nervous manifestations. The brains of dogs with natural distemper infection examined personally have also shown no similar lesions. With Professor Dalling, attempts have been made to isolate distemper virus from the blood, brain, or spleen of cases of "encephalitis" and the results were negative. Inoculations of the distemper virus into the brain of dogs have failed to produce any distinct encephalitis, while attempts to transmit the encephalitis to other dogs have been equally unsuccessful (Dalling, personal communication).

The clinical evidence is also puzzling; in many cases there is a history of a febrile illness ("distemper"?) at periods varying from a week to more than a year before; in some the illness was intermittent. Occasionally, after the initial illness, there was a slowly progressive paraplegia with ultimate blindness. Some showed fits and convulsions passing into a flaccid paralysis, some a spastic gait, others a loss of sensation in the extremities.

The uniform time interval which occurs in man between the onset of some acute exanthematous infections and the onset of nervous symptoms has no parallel in the

dog. The demyelination encephalitis in dogs is therefore not strictly analogous to the post-infective demyelinating diseases of man. As Perdrau pointed out, the variable and lengthy time interval in some canine cases is more comparable to the relation which has been stated to exist between disseminated sclerosis and a previous acute infection, and one case of Scherer's seems to bear a closer resemblance to disseminated sclerosis than the acute disease.

There is more support for the view that there is no direct relation between the distemper virus and the " encephalitis " from the failure to find the inclusion bodies which are said to be typical of distemper.

The pathology of the condition indicates that its ætiology is likely to be linked to a virus, or viruses, not yet identified. Green (*see* Green and Shillinger, 1934) states that fox encephalitis is transmissible to dogs, and that inclusion bodies are found in the ependymal and endothelial cells of the brain and in the liver. These specific inclusion bodies are stated not to be found in natural or experimental canine distemper. An experiment by Dalling and myself supports the contention of Green that the two viruses (fox encephalitis and distemper) are distinct. The former produces meningo-encephalitis in dogs in contrast with the distemper virus ; in experimental fox encephalitis infection in dogs, specific intranuclear inclusion bodies may be found, particularly in the hepatic cells. A search for these inclusion bodies in the liver and other tissues of natural " canine encephalitis " cases was negative.

The disease in the dog occurs very frequently, and probably more often than the post-infective encephalitis in man. The nervous phenomena in the dog, known as " fits ", " hysteria ", " chorea ", " epileptiform convulsions ", are vague in meaning ; the study of these conditions would be valuable not only to clarify their significance but also to understand better the acute and chronic forms of disseminated encephalomyelitis in man.

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NO. 27.

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THROMBO-ANGEITIS OBLITERANS IN A HORSE.

by

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INTRODUCTION.

There appears to be no record of the occurrence of arterial disease in animals analogous to that originally described in man by Buerger (1908) as thrombo-angeitis obliterans. Nieberle and Cohrs (1931) state that the disease does not occur in animals while Holz (1938) uses the term erroneously as a synonym for endarteritis obliterans in describing the vascular changes in equine infectious anaemia. Intermittent claudication is well known to occur in horses, a good description of such cases being given in Hutrya, Marek and Manninger (1938). In enumerating the causes of the clinical entity, these authors do not mention any condition which might be regarded as Buerger's disease. At present the commonest cause of claudication in horses is thought

to be thrombosis of the abdominal aorta and its main branches, secondary to invasion of the walls by larvae of Strongylus vulgaris - a common roundworm of horses. The arterial lesion in these animals might suffer from a disease similar to Buerger's was suggested by Talford (1936) who states that "Thrombosis in the horse may be the result of larval infestation of the arterial coats of arterio-sclerosis and of embolism. These morbid processes have, however, no share in the disease of thrombo-angeitis obliterans in man..... There are reasons to believe that the same disease may appear in the horse, and it would prove of real interest and of vital importance to the surgeon if an equine form of thrombo-angeitis obliterans were disclosed, supported by clinical and histological details".

Incidentally it might be emphasised here that the occurrence of arterio-sclerosis in domesticated animals in the sense of atheroma (athero-sclerosis) has not been clearly established in animals; many of the descriptions appear to be more comparable to medical calcification of man.

Our chance finding of vascular lesions simulating Buerger's disease in a horse free from intra-vascular strongyle infestation is of significance.

Subject: Thoroughbred mare of 9 years.

History: Raced with success till the age of 4 years when she was put to stud and not used again for work of any kind. Apart from being barren during the last two years, the mare had been perfectly well. On November 20th 1939 the mare received, for experimental purposes, a heavy dose of Phenothiazine (as an anthelmintic). She became dull and refused food the following day, seemed to recover on the 22nd but on the 23rd was down, showed marked respiratory distress, a very fast, weak pulse and a temperature of 103. Later the same day, when apparently moribund, the mare was shot.

Post-mortem findings:

Summary: Pulmonary embolism; thrombosis of termination of abdominal aorta; occlusion of the large arteries of both hind limbs by thrombus and mural thickening; thrombosis and thickening of some veins in the hind limbs; numerous acute, haemorrhagic erosions in fundus of stomach; sub-endocardial haemorrhages in wall of left ventricle and right atrium; congestion of both lungs; areas of mucosal necrosis and ulceration in renal pelves and ureters probably attributable to the toxic action of the drug.

VASCULAR CHANGES : NAKED EYE.

A solid mass of thrombus (20 x 4 x 3 cm. thick) was attached to the dorsal and right lateral wall of the abdominal aorta from immediately posterior to the origin of the renal arteries to the terminal bifurcation. It covered the orifices of the lumbar and right external iliac arteries. Superficially it was pink, soft and easily detached, while deeper it was paler

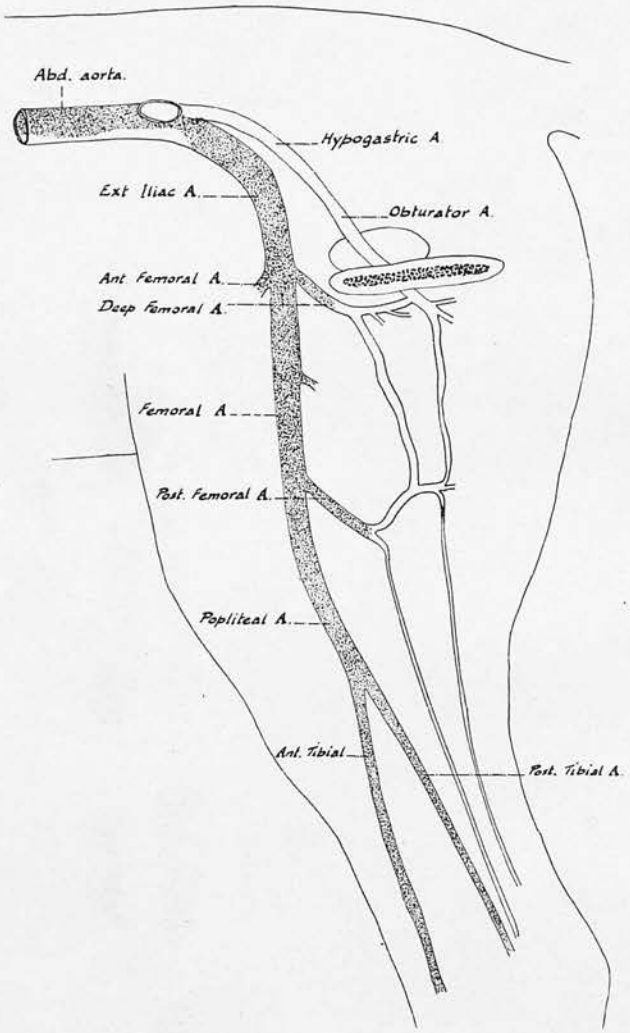
in colour and firm. Plaques of intimal thickening (about 1 cm. thick), covered in part by fresh thrombus, were present at the orifices of the left external iliac and left hypogastric arteries. The hypogastric arteries were otherwise fully patent, but the external iliac arteries were occluded, the right from its origin and the left from a point 3.5 cm. from the aorta.

Numerous transverse sections of the main arteries of the right hind limb revealed widespread obliterative disease. The vessels were completely occluded in segments by solid, greyish-brown tissue perforated by small openings from which could be expressed a little fluid blood. On either side of such segments, the degree of occlusion was less, and indeed, occasionally the vessel was fully patent. Where occlusion was partial, the lumen contained either recent thrombus or fluid blood.

The veins were similarly affected. There was no peri-vascular fibrosis, and no binding of arteries, veins and nerves into bundles.

The heart, the mesenteric and all other arteries appeared to be normal.

Blechmatten proof



VASCULAR CHANGES : MICROSCOPIC.

The mass in the aorta was composed superficially of fresh thrombus; in the deeper layers, blood cells became progressively fewer and lay in a homogenous, brightly eosinophilic ground substance; the base was firmly attached to the wall of the aorta by collagenous fibrous tissue containing much blood pigment and elastic fibres (derived from the inner elastic layers) very slightly encrusted with calcium salts. The media was fibrosed and vascularised. No evidence of verminous infestation was found.

The lumen of the external iliac-anterior tibial arteries of the right hind limb varied greatly in size, without any regular gradation in any direction. In segments it was entirely obliterated by tissue perforated by only a few canalizing vessels. Whereas in others it was fully patent. In the latter the vessel wall was normal. The lumina contained either blood or fresh thrombus.

The obturating mass lay within a stout undulating internal elastic lamina. It consisted of fibrous connective

tissue, partly cellular, partly collagenous, in which are capillaries and larger vessels of varying size. Round these there was sometimes slight infiltration by lymphocytes and plasma cells. Elastic fibres were scanty and almost entirely confined to the walls of the canalizing vessels which also occasionally had a few plain muscle cells in their walls. Blood pigment, both within macrophages and free, was fairly abundant. There was no calcification.

The media was fibrosed and perforated by vessels passing inwards from the adventitia. Small collections of round cells were present round some of these. No adventitial fibrosis was present. The changes in the veins were similar in nature except that the occluding fibrous tissue was more cellular (c.f. Figs. 3 & 5).

There was no evidence of verminous infestation in any of the vessels, and it must be emphasised that the parasitic lesion is an active endarteritis obliterans (Fig. 6.) often accompained by aneurysmal dilatation.

DISCUSSION.

The vascular lesions are definitely those of organised thrombosis, and in the absence of cardiac disease and of arteritis due to Strongylus larvae, the assumption of primary arterial disease of the vessels of the hind limbs seems to be justifiable. The thrombotic process appears to have extended towards the heart and had reached the aorta at the time of death. Absence of periarteritis and periphlebitis is considered unimportant as Turnbull (1936) found little or no evidence of extra-adventitial fibrosis in 17 amputated limbs from human patients with Buerger's disease. Thrombosis of the veins in this horse was probably secondary to slowing of the blood flow consequent upon arterial obstruction. The lesions in the arteries and veins therefore conform very closely to those of thrombo-angeitis obliterans in man.

The sex of the animal should not be a great obstacle to the acceptance of our view regarding the nature of the lesions as the disease, although rare, does occur in women. The results of the experimental work of Suzman, Freed and Prag (1938) suggests that follicular hormone possibly has a protective action against

the development of Buerger's disease. Relative to this is the fact that the mare was barren for four years before death.

During life the mare showed no symptoms of lameness, but this is readily understood as she was not galloped or worked since the age of four. Considering the degree of arterial obstruction observed at autopsy, symptoms of intermittent claudication must inevitable have developed had the animal been severely exerted. In view of the great muscular bulk of the horses hind limbs it is of interest to note how efficient was the collateral circulation provided by the much smaller vascular bed of the hypogastric arteries.

The arterial changes, because of their chronicity, cannot be attributed to the action of phenothiazine although the drug in the dose given may have been toxic and precipitated the illness.

This type of arterial disease must therefore be added to the recognised causes of intermittent claudication of horses.

SUMMARY.

Obliterating arterial disease morphologically identical with thrombo-angeitis abliterarns of man was encounterested ina horse. The importance of this condition, hitherto undescribed in domestic animals, as a possible cause of equine intermittent claudication is indicated.

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Plate XLII

Fig. 2.—Right femoral artery showing organised and canalised thrombus filling the lumen. Orcein. ×5.

Fig. 3.—Right medial dorsal metatarsal vein at two levels. Orcein. ×5.

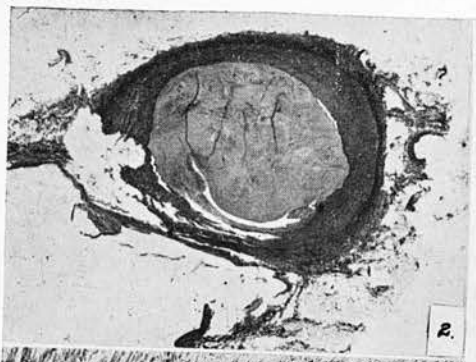
Fig. 4.—Right external iliac artery, showing vascularisation and fibrosis of media (*a*), distinct internal elastic lamina (*b*), and obturating fibrous tissue (*c*) containing small vessels and granules of hæmosiderin. H. and E. ×50.

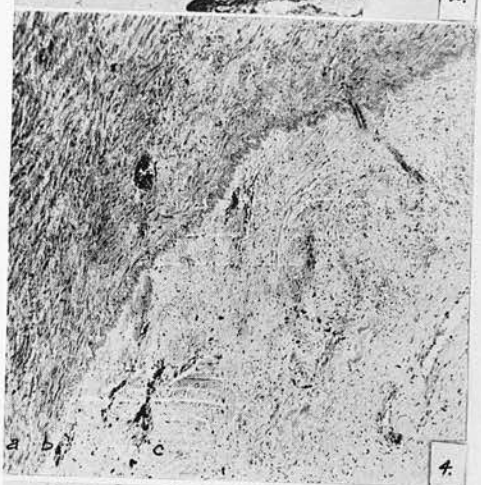
Fig. 5.—Right femoral artery. The canalising vessels here have elastic and muscle fibres in their walls. Orcein. ×50.

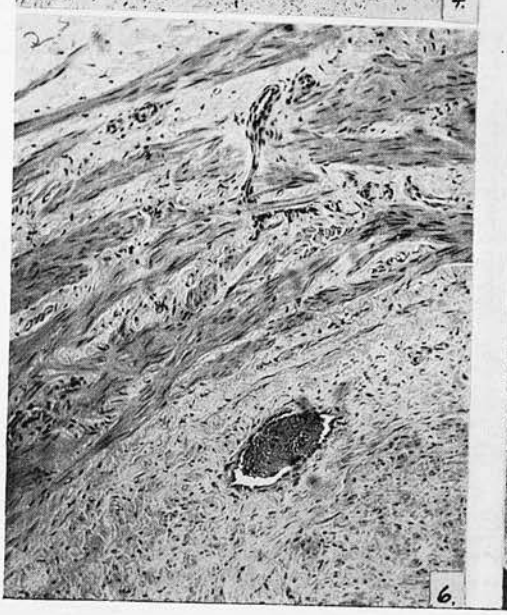
Fig. 6.—Right medial dorsal metatarsal vein. Vascularisation of the media is very clear. The fibrous tissue within the vein is more cellular than that in the arteries and contains a vessel with fresh thrombus. H. and E. ×100.

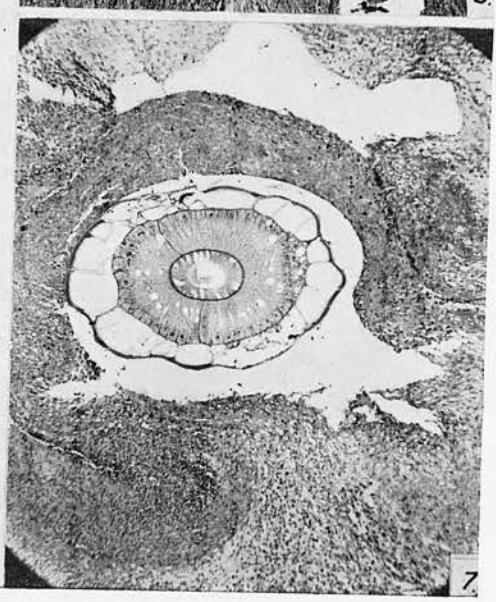
Fig. 7.—Obturating tissue in cranial mesenteric artery from a case of "verminous aneurysm." The larval round worm is surrounded by partly necrotic inflammatory tissue in which eosinophilic polymorphs are numerous. H. and E. ×50.

THROMBO-ANGEITIS OBLITERANS IN A HORSE









LEGENDS TO ILLUSTRATIONS.

1. Right femoral artery showing organised and canalized thrombus filling the lumen. Orcein. X 5.

2. Right medial dorsal metatorsal vein at two levels. Orcein X 5.

3. Right external iliac artery, showing vascularisation and fibrosis of media (a) distinct internal elastic lamina (b) and obturating fibrous tissue (c) containing small vessels and granules of haemosiderin. H.E. X 50.

4. Right femoral artery. The canalizing vessels here have elastic and muscle fibres in their walls. Orcein. X 50.

5. Right medial dorsal metatarsal vein. Vascularization of the media is very clear. The fibrous tissue within the vein is more cellular than that in the arteries and contains a vessel with fresh thrombus. H.E. X 10.

6. Obturating tissue in cranial mesenteric artery from a case of verminous "aneurysm". The roundworm larva is surrounded by partly necrotic inflammatory tissue in which eosinophilic polymorphs are numerous. H.E. X 50.

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PATHOLOGY — ONE

MULTIPLE MALIGNANT AND BENIGN NEOPLASMS IN A DOG WITH SECONDARY TUMOUR OBSTRUCTION OF THE PORTAL VEIN WITHOUT ASCITES.

By J. R. M. Innes and J. W. Whittick,

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Multiple primary tumour formations are admittedly not infrequent in man (Willis, 1934) and domestic animals (Cohrs, 1927). Yet this instance of co-existent sarcoma, carcinoma and a benign neoplasm in a dog is considered worthy of description because of the absence of ascites in spite of marked obstruction of the portal vein by secondary sarcoma. In addition, the minute size of the other primary (carcinoma of the eyelid) emphasises the care necessary in anatomical diagnosis, as the metastasis from this tumour in cervical nodes, might easily have passed for an example of paradoxical metastasis due to transpulmonary passage of tumour cells.

Subject.—Sealyham dog of 8 to 9 years with vague history of ill-health for a year; the animal was killed by HCN subsequent to a diagnosis of an inoperable abdominal tumour.

Anatomical Findings.—These can be conveniently given under four separate headings relating to the three tumour types and other findings.

(*a*) Large irregular primary sarcoma (9 × 8 × 5 cm.) in the sublumbar and subsacral retroperitoneal region intimately adherent to, but not invading, the vertebræ; ventral displacement of the pelvic organs and lateral displacement of the abdominal aorta and caudal vena cava; enclosure and compression of the left ureter with consequent hydronephrosis; cranial contiguity spread of the growth to the lesser omentum with invasion and obstruction of the portal vein; discrete umbilicated metastatic nodule (7·5 × 7 × 5 cm.) in the left lateral lobe of the liver; single metastatic nodule (5 × 4 × 3 cm.) in the left inguinal region discontinuous with the primary. no ascites. no splenomegaly and no œsophageal or rectal varices; no pulmonary metastases.

(*b*) Minute (0·4 × 0·2 cm.) tumour on the conjunctival surface near the margin of the right upper eyelid with metastases in the two right superior cervical lymph nodes.

(*c*) Incidental findings included œdema and congestion of the lungs; slight prostatic enlargement; a few flecks of fat necrosis in the pancreas; the brain appeared normal.

Microscopical Findings.

(*a*) *Retroperitoneal tumour.*—The primary growth consisted of sheets of cells closely packed in an indeterminate manner and supported by a fine argyrophil reticulum. The tumour was not highly vascular and, though there were numerous areas of necrosis, hæmorrhage was not conspicuous. The cells were mainly spindle-shaped, short fusiform or large and round in type, while bi- and tri-nucleated giant cells were not infrequent (Fig. 1); lymphocytes were scanty. Mitotic figures were exceedingly numerous.

The appearances were in many ways similar to those of highly cellular periosteal sarcomata, and in view of the intimate attachment to the vertebræ, the possibility of such an origin must be considered. There was no other indication of the exact site of origin and the alternative is to regard it as a mixed or polymorphic cell sarcoma arising from retroperitoneal connective tissue.

The structure of the single large liver metastasis was identical with that of the primary (Fig. 2). In addition, numerous branches of the portal vein in parts of the liver, remote from the secondary, were found to contain non-adherent tumour emboli (Fig. 3).

The obstructed portal vein was encircled and filled by tumour cells which in the original lumen were very loosely arranged and interspersed with masses of red blood corpuscles. The wall was greatly thickened and distorted by the invasion of the tumour cells, but in spite of the breaks in continuity, the wall could be followed by the remains of the elastic laminæ (Fig. 4).

The inguinal nodule was similar in appearance to the retro-peritoneal growth. No microscopic metastases were present in the sections of the lungs examined.

The interest of this tumour lies mainly in the degree of portal obstruction without the development of ascites or any obvious sign of collateral circulation. Markowitz (1937) has emphasised that, although the ascites of portal cirrhosis has long been explained on the basis of back pressure in the portal vein, ligation of the vein does not cause ascites; the latter is but a stage in the failure of liver function. This case is, therefore, of importance as an observation on a spontaneous condition which supports experimental findings. Apart from the metastases there was no liver damage.

(*b*) *Tumour of the right upper eyelid.*—The nodule was composed of solid masses of epithelial cells which invaded the dermis (Figs. 5 and 6). In places the cells were typically squamous in character while in others they were highly vacuolated and identical with sebaceous gland epithelium. The epithelium above the tumour was ulcerated. The proximity of the tumour to the position of Meibomian glands, the undoubted sebaceous character of many of the tumour cell masses and the absence of typical keratinised cell nests indicated that the neoplasm was an "adenoid carcinoma" arising from the Meibomian glands. *Adenoma sebaceum* or steatadenoma are well recognised growths in man (see Ewing, 1928, Sutton, 1935), and several examples have been seen also in dogs, but malignant forms have not been clearly identified in either man or animals. The squamoid transformation seen in this case is not unusual in tumours arising from the dermal appendages.

In the affected right cranial cervical lymph nodes the architecture was largely obliterated by invading tumour cells though they were

less differentiated (Fig. 7). The sebaceous character of the epithelium was recognisable in only a few areas. The appearances were, however, totally different from those of the retroperitoneal sarcoma. Tumour emboli filled the afferent lymphatics.

(*c*) *Testicular tumours.*—These were discrete nodules (up to 1 cm. in diameter) situated in the substance of both organs. They were composed of solid masses of large polyhedral foamy-like cells reminiscent of the hypernephroma. The tumours were not encapsulated but the seminiferous tubules round the periphery were atrophied. Such nodules are exceedingly common in dogs and in the past have been referred to as "interstitial cell tumours," although it is highly uncertain that they can be regarded as true neoplasms. They are more in the nature of nodular overgrowths comparable to the common hyperplasias of normal cells found in a number of organs of senile dogs such as the spleen (often termed lymphomata), the thyroid, suprarenal and liver where they form "adenoid" nodules.

Summary.

Two malignant tumours are described in a dog. One was a polymorphic or mixed cell sarcoma arising from either the periosteum of the lumbo-sacral vertebræ or the retroperitoneal connective tissue with metastasis to the liver and contiguity spread to the lesser omentum. Occlusion of the portal vein by the tumour was not accompanied by ascites, which is a valuable corollary to the experimental observation that back pressure from the portal vein is not solely responsible for the development of ascites. The other, a tumour of the eyelid, was a malignant form of *adenoma sebaceum* arising from the Meibomian glands with metastatic deposits in the cervical lymph nodes.

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Fig. 1.

Fig. 1. Primary retroperitoneal, mixed-cell sarcoma. H.E. × 300.

Fig. 2.

Fig. 2. Isolated secondary sarcoma nodule in liver with compressed and atrophied liver cells on the left. H.E. × 200.

Fig. 3.

Fig. 3. Portal vein within the liver filled with partly necrotic sarcoma tumour embolus. H.E. × 80.

Fig. 4.

Fig. 4. Cross-section of lesser omentum showing (*a*) bile duct, (*b*) hepatic artery and (*c*) portal vein surrounded and filled by secondary sarcoma. Wilder's and van Gieson. × 2.

Fig. 5.

Fig. 5. Right eyelid with ulcerated tumour nodule close to the lid margin. To the left is the skin surface and on the right is conjunctiva. H.E. × 15.

Fig. 6.

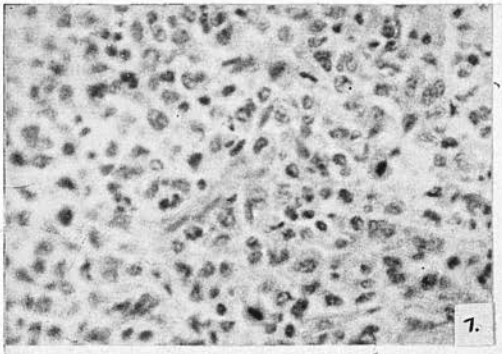
Fig. 6. Malignant adenoma sebaceum of Meibomian glands showing squamous and sebaceous cell constituents and absence of definite cell nests. H.E. × 80.

Fig. 7.

Fig. 7. Right superior cervical lymph node containing early metastases from the eyelid carcinoma ; the epithelial cells are not so clearly differentiated as in the primary although both squamoid and sebaceous characters can be made out in places. H.E. × 80.

Fig. 8.

Fig. 8. Testes showing the presence of discrete "interstitial cell tumours." Natural size.



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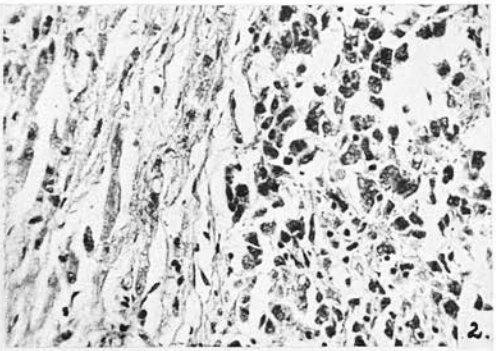

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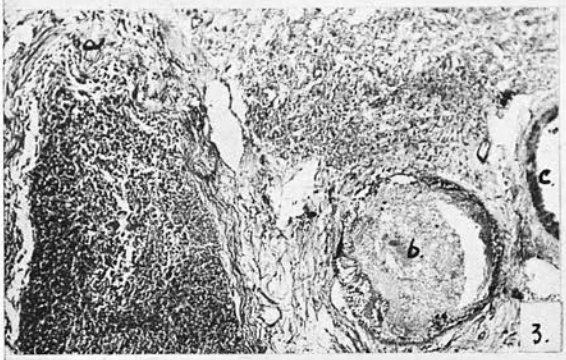

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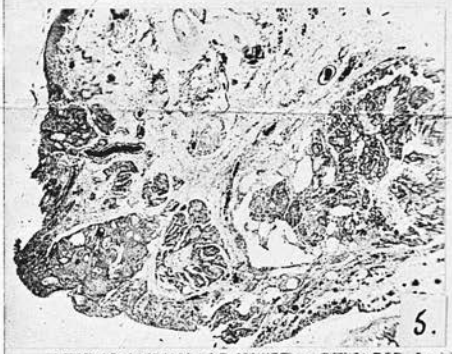

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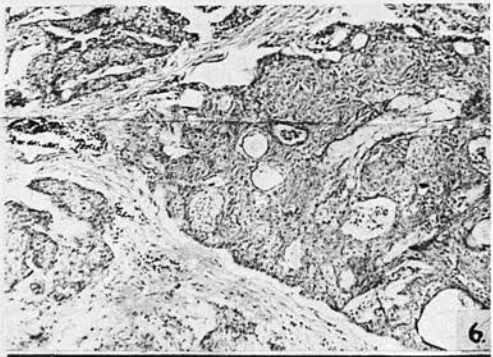

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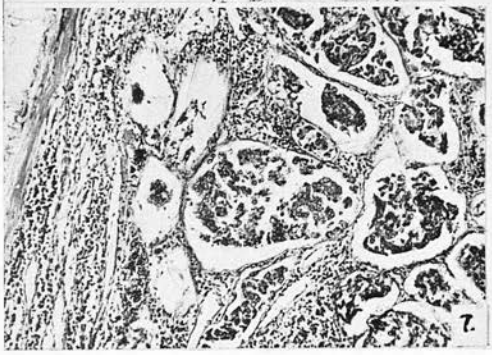

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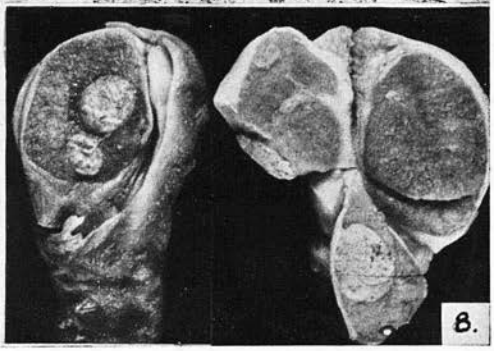

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