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A CONTRIBUTION to the PROBLEM of CANCER RESEARCH

Based Upon

The RESULTS of an EXPERIMENTAL INVESTIGATION

of

INFECTIVE SARCOMA OF THE DOG.

A THESIS presented for the DEGREE of M.D.

by

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P R E F A C E.

The experimental investigations which form the basis of this thesis were commenced by me in December 1904. Prior to that date a certain amount of work had been done by various investigators upon the disease which occurs in dogs and is known as Infective Sarcoma. Since then, however, a very considerable amount of attention has been directed to the study of this disease, on the grounds that it might contribute towards the elucidation of the nature and Etiology of Cancer.

As the research has proceeded, the field of investigation has been very much extended so as to reveal facts, not only of interest from the bearings which they possess upon the Problem of Cancer; but, also points of importance in connection with the General Pathology of Inflammation, - more especially as it affects the Kidney, - and Immunity.

I N T R O D U C T I O N.

In recent years it has been clearly realised by all scientists who have concerned themselves with the study of Cancer, that the only source from which further information could be hoped to be obtained was by the experimental method of investigation. The difficulty of conducting such investigations, however, lies in the fact that in the vast majority of cases where portions of cancer have been inoculated into animals of the same or different species, the result has been negative, and further research has been arrested.

The investigation of a disease, therefore, in which inoculation occurred readily and which, clinically and histologically, presented so many points of similarity to Sarcoma as it occurs in man, appeared to be work which offered very fair prospects of results of value.

The disease known as Infective Sarcoma is one which occurs in dogs. It is not uncommon in its occurrence and appears to have a world-wide distribution. In this country it is frequently met with; on the/

the Continent of Europe and in the United States of America it is stated to be also prevalent. According to C.G.Seligmann[1] it is also to be found in the native dogs from the Coast villages of the Central Division of British New Guinea, where, according to the Natives it was endemic before the advent of the white man.

Infective Sarcoma is a contagious veneral disease, the transmission of which appears undoubtedly to occur during the act of coition. Thereafter, there develops on the genital tract of the animal infected, soft, fleshy, highly vascular tumours, which, on examination, have to the naked eye, an appearance similar to that of a rapidly growing Sarcoma. On microscopical examination the tumour is found to have a structure identical with that of an alveolar Sarcoma.

The remarkable readiness by which this tumour is conveyed by contagion has long been known to dog fanciers, as the development of the disease renders the animal useless for stud purposes.

The investigations of Bellingham Smith and Washbourne[3] have demonstrated this point very clearly upon a scientific basis. Their attention was directed to the disease by the owner of a breed of prize dogs./

dogs. Unknown to him one of his dogs had, situated on its penis, behind the corona a small tumour which, on later examination, was found to be of the nature of an infective Sarcoma. Of twelve bitches of various ages, served by this dog eleven became infected with the disease. The younger were less severely affected than the older, the three oldest being so severely affected that they had to be killed. Each bitch was operated upon, the growth being excised, the customary treatment as recommended by the veterinary profession. The tumours, however, recurred in all of the animals, and a permanent cure was only obtained in four, after a second operation. The four remaining animals were, a year and three quarters after, still suffering from the disease when the results of the investigations were published.

Further proof of the contagious nature of the disease was given by the additional fact that another dog contracted the disease also after serving one of the afore-mentioned eleven bitches, when it was suffering from the disease. This animal in its turn served two healthy bitches and passed on the disease to one of them.

The spontaneous transference of the disease occurred per coitum in another of their cases, despite/

despite the fact that every precaution was taken to prevent infection, - the Penis of the healthy animal being frequently washed with antiseptic lotions.

THE/

THE SPORADIC TUMOUR.

In this investigation the material was originally obtained from two sources - one, a Bull Bitch, the property of Dr.Beard of Edinburgh University, the other a wire-haired fox-terrier which was kindly presented to me by Dr.Bashford, Director of the Imperial Cancer Research Laboratories, London.

CLINICAL EXAMINATION.

In the case of the Bull Bitch, it had contracted the disease from a source that could not be determined, but it was known to have suffered from it for more than a year. It was of mature years and, when I first saw it, the animal was obviously ill. It was languid, cowed, and considerably emaciated. The vulvar orifice protruded, and definite lobulated swellings were visible on either side of it, projecting underneath the skin surface adjacent to the vagina. On examination the vagina was found to be occupied by several confluent fleshy swellings, which bled/

bled readily when touched. Some of these were covered by an ulcerating surface; others were felt underneath the vaginal mucosa, and finally on deep palpation or better still, on bimanual examination, the nodules visible from without were found to extend from the vaginal to the skin surface.

At this examination small portions of the tumour were removed by means of scissors from the vaginal cavity, and at once fixed in Heidenhain's Saturated Corrosive sublimate solution.

A fortnight later further portions of the tumours were removed under a general anaesthetic for inoculation experiments to be commenced. During the operation, however, the animal unfortunately succumbed to an overdose of chloroform.

MORBID ANATOMY.

At the post-mortem examination held, the appearances found at the examination during life were confirmed. Confluent tumour masses occupied the vaginal cavity, and of these which were not ulcerated on their surface some were freely moveable under the mucous membrane whereas others were covered with epithelium which was firmly adherent to the subjacent tumours.

On/

On section the tumours had a light pink colour and were of a uniform fleshy consistence. On its deep aspect the growth was found to have invaded the adjacent muscle. Certain of the outlying nodules possessed a distinct capsule, however, and could be enucleated with ease from the adjacent tissue. It was also noted that the disease did not extend into the uterine cavity, and no evidence was present to the naked eye of secondary foci, in the regional lymphatic glands. On a complete and carefully conducted post-mortem examination no secondary deposits could be made out in any part of the body.

This state of affairs coincided with that described as usually being present in such cases. The sporadic tumour usually remains local; death in the untreated case being brought about by ulceration of the tumour and the associated cachexia, or by direct extension of the neoplasm and the production of pressure effects, such as hydronephrosis from involvement of the urethra or ureters. Secondary foci of the disease have, however, been found in various internal organs and infection of the regional lymphatic glands is said to be not uncommon. Personally, however, I have never found secondary infection of the lymphatic glands present either in the animals in which the sporadic tumours occurred or in the numerous/

numerous cases where tumours were produced by experimental inoculation, although I have carefully examined for such in all cases. In many, however, I have found the glands much enlarged, and showing active lymphocyte formation and proliferation of the endothelial cells of the lymph sinuses.

In one case, as will be mentioned later, secondary foci of the disease were present in the Liver, Intestine, and Suprarenal Gland of an animal which died seven weeks after the successful inoculation of a portion of the tumour into the subcutaneous tissue of its flank.

HISTOLOGY/

HISTOLOGY OF THE TUMOUR.

Microscopical examination of portions of the tumour removed from the vagina during life showed (Plate vi Fig.1 and Fig.2) situated in the subcutaneous tissue underneath healthy vaginal epithelium, a mass of new growth which possessed a delicate stroma, forming spaces containing cells arranged usually in a loose irregular manner. At other parts the cells were arranged in a more regular manner, lining the walls of the spaces. On further examination the stroma is found to contain minute capillaries. There is occasionally present within the tumour mass portions of fibrous tissue included from the surrounding tissue during its development. The tumour cells (Plate vii Fig. 1) are virtually uniform in size, shape and structure. They measure from 9 - 11 m. and are polyhedral. They have a large cell body which contains a large nucleus, usually eccentrically placed and having within it a distinct nucleolus. Mitotic figures are very abundant. These are usually regular in type, irregular forms of Karyokenesis are, however, occasionally seen. Tripolar Mitosis as described by Bashford/

Bashford[4] has also been noticed. I have never been able to detect the presence of heterotype mitoses in any of the specimens examined.

The tumour cells can be seen to be invading the surrounding tissue, infiltrating and destroying the adjacent fibrous tissue and muscle. At other parts, where the tumour mass is more definitely encapsulated, the capsule is seen to be made up of delicate fibrous tissue.

With Eosin and Methylene Blue the tumour cells stain in a characteristic manner. The Cell Body stains a light blue and is finely granular. The nucleus stains a darker blue colour and has an open chromatin network. With Pyronin Resorcin and Methyl Green the finer structure of the tumour cell is especially well brought out. The cells are polygonal in shape and their membrane is seen to be faint in outline. The cytoplasm is stained a light salmon colour. The nucleus, which is large, stains of a dark slate blue colour and contains darker granules of a similar shade in addition to a single true nucleolus which stains a bright rose red with the Pyronin. The centrosome apparatus cannot be made out readily. Throughout the tumour, cells, which might appear at first sight to be tumour cells, are seen. Here/

Here and there throughout the field, usually close to the capillary stroma, a small round cell, is occasionally seen. It possesses a scanty cell body and has a large nucleus which stains a deep blue with Methylene Blue and Eosin, the cell body being also strongly basophilic. This cell is readily recognised as being identical with the small lymphocyte of the blood and so-called wandering cell. Other cells are seen which are similar in appearance to large lymphocytes. A third variety is present, which is almost identical in its structure with the tumour cell. This, however, can be recognised by differential stains to be of a different nature. With Eosin and Methylene Blue these cells stain, both as regards their cytoplasm and nucleus, much more deeply than do the tumour cells. The nucleus does not possess the same open granular network. With Pyronin Resorcin and Methyl Green they stain in a manner that is quite characteristic and differentiates them at once from tumour cells. Their cytoplasm is stained of a bright rose red colour throughout and is not so granular as is that of the tumour cell. In every case there is contained in its centre, in contact with the nucleus which is eccentric a minute clear spot, the centrosome apparatus. The Nucleus of this cell stains/

stains a dark slate blue colour, as does that of the small lymphocyte-like cell. It contains numerous fine closely set chromatin granules. In many cases larger granules are seen which have the appearance of being the true nucleolus. These are, however, seen to be Karyosomes, false nucleoli. The plasmosomes are seen in most as minute bright rose red staining points which lie in the centre of the nucleus.

These latter cells are identical with those described by Maximow (5) as Polyblasts. There would thus appear to be present, scattered in an irregular manner throughout the tumour, cells few in number and which show all transitions from the small and large lymphocyte to fully formed Polyblasts, and occasionally to Plasma cells.

Microscopical Examination of the border of the tumour shows two definite appearances. Towards the vaginal surface (Plate vi Fig.I.) the tumour cells can be made out extending into the adjacent connective tissue. They are invading and destroying it. It is here extremely difficult to recognise whether or not formation of additional tumour cells is occurring from the tissues of the host infiltrated. Just beyond the outer border of the tumour, cells are however seen which might be mistaken for tumour cells. These/

These are especially abundant in the neighbourhood of the blood vessels, and on examination are found to be extravascular accumulations of cells which are mainly of the lymphocyte type with the presence of an occasional clasmatocyte and Plasma cell. Various transition forms from the former to fully formed Polyblasts are present.

On the deep aspect of the tumours (Plate viii. Fig.2) the tumour cells can also be made out to be infiltrating and destroying the surrounding fibrous tissue and muscle.

Another appearance is, however, noticed on the deep aspect of some of the outlying nodules. Here the tumour is definitely encapsulated by a membrane of delicate fibrous tissue. The connective tissue cells forming this capsule are seen to be young Fibroblasts, which have an elongated spindle-shaped cell body, and possess a longitudinal striation which is most evident when stained with Haematoxylin and Iron Alum and Picrofuschin. The delicate rose-red striae at the periphery of the cell are, by the addition of the latter stain, rendered clearly visible. The nucleus, which is oval in contour, has a single distinct nucleolus. The staining reaction of these cells is similar to those of the tumour with

which as will be seen later, they are identical in nature. At the encapsulated border the tumour is seen to be infiltrated with numerous Polyblasts; an appearance in marked contrast to that seen at the extending margin of the new growth where they are few in number. These cells are, as already mentioned, rarely found in the tumour mass itself.

The second aminal from which the stock of material used in my experimental work was obtained, was that presented to me by Dr Bashford. It was a wire-haired fox terrier and had situated underneath its foreskin and growing from the corona of the Penis a cauliflower like mass of new growth, of a soft fleshy consistence, which bled readily when touched. Its size was such as to render protrusion of the organ impossible except under an anaesthetic. Histologically this tumour presented an appearance identical with that obtained from the Bull Bitch.

From its histological appearance this tumour has been generally regarded as a sarcoma, with that opinion I agree. It, in my opinion presents during life and reveals on examination after death, appearances which to the naked eye and microscope are/

are identical with that of an alveolar sarcoma.

Stricker,(9) who has done a very large amount of work on this disease considers it to be a lympho sarcoma. Certain other observers have, however, put a different interpretation upon the appearances presented. This may in part be explained by the fact pointed out by the above observer, that two other infective inflammatory diseases occur on the genitals of dogs which closely resemble the true infective lympho sarcoma.

One is where pointed condylomata of the genital occur after long contimued irritation. Histologically these consist of hypertrophic epithelium supported by new vascular connective tissue infiltrated with round cells. The other is where lymphoid hyperplasia occurs in the subcutaneous tissue associated with an infective follicular vaginitis.

It would not, however, appear to be the case that any of the dissimilar opinions which I will proceed to quote arose from this cause.

In the case of the work done by me I was fortunate in possessing in the dog sent to me from the Imperial Cancer Research Laboratories, material belonging to a stock which had been recognised by Stricker as being of the type of true infective lympho/

lympho sarcoma, and which had been recognised by Dr Rose Bradford as similar to that with which Smith and Washbourne conducted their investigations. It was identical in every respect with the tumour used in the earlier part of my work.

(13)
Nowinsky (1877) is said to have been the first to give a detailed account of the morbid anatomy and histology of this disease, and to successfully inoculate it into other animals of the same species by transplanting portions of the primary tumour into the subcutaneous tissue. The structure of the primary tumour, and those of the second generation, was according to him that of a myxo sarcoma.

(2)
Bellinghame Smith and Washbourne (1898) were the first in this country to submit the disease to a careful examination. In their opinion the tumours were certainly sarcomatous in structure. They succeeded in one of their cases in obtaining secondary nodules in the internal organs, so that according to them, "The tumours do not differ in structure or 'malignancy' except perhaps in degree from the sarcomata met with in the human subject."

(6)
Wehr in 1889 conducted an experimental investigation of this disease. He succeeded in reproducing the disease by inoculating portions of the tumour into the subcutaneous tissue of other dogs and

in/

in certain of his cases obtained deposits in various internal organs. The primary tumour was in his opinion a carcinoma and the secondary deposits were of a carcinomatous structure.

Duplay and Cazin[8] (1894) were of the opinion that the structure of the tumour resembled inflammatory rather than carcinomatous tissues. They also were successful in transferring the disease to other dogs by subcutaneous inoculations.

Geissler[7] (1895) inoculated successfully from a cauliflower-like tumour which occurred on the prepuce of a dog and which he considered to be a carcinoma. He communicated the results of his investigations to the German Surgical Congress, and in the discussion which followed the general concensus of opinion was not in his favour.

Hansemann's[16] opinion was that it was not a carcinoma. The exact nature of the condition present he was not, however, prepared to give an opinion upon without further opportunity of careful examination of the specimens.

Similar tumours, which he had examined in dogs, he never found to be carcinomatous. They showed a mixture of granulation tissue and sarcomatous-like tissue.

Israël[16] agreed with Hansemann that the preparations/

preparations of these tumours exhibited were not carcinomatous in nature.

Wehr (16) was of the opinion that the lesion present was, like that in the cases described previously by him, a primary epithelial carcinoma.

Powell White (14) (1902) studied the disease in two animals. In his opinion the histological structure was identical with that of a lymphosarcoma.

Stricker (9) (1904) successfully inoculated 31 dogs by subcutaneous and intraperitoneal implantation. The sporadic tumour he considered to be a lymphosarcoma. In his two subsequent communications (1905) (11) (1906) (12) he reports further series of successful inoculations with similar lymphosarcomata.

Bashford, Murray, and Cramer (4) (1905) who specially investigated the histogenesis of the tumour nodule formed after subcutaneous inoculation were of the opinion that the tumour resembled malignant new growths in its histological features. The transformation of connective tissue cells into tumour cells at the extending border of the nodule which they figure, as occurring, militated in their opinion, however, against the view that the growth was in reality a true sarcoma. On this ground and on others/

others I will have occasion to refer to later, they would prefer to describe the disease as an infective granuloma.

McFadyean (19) (1905) in a foot note to a paper by Dunstan on Infective Sarcoma in the Bull Dog said, "The Classification of these tumours with sarcomata is not justifiable in view of the fact that they represent the lesion of an infective disease and have a histology closely resembling that of Granulation Tissue."

Beebe and Ewing (15) (1906) in a paper describing their investigations into the histogenesis of the tumours formed after subcutaneous inoculation, express the opinion that the general histological characters of the disease suggest to them the diagnosis of alveolar sarcoma or endothelioma.

S E R I A L/

SERIAL INOCULATION.

SECOND GENERATION.

In order to determine the facility with which tumour masses could be reproduced in other animals of the same species and to confirm such work as had already been done in this direction, portions of the original sporadic tumour were excised and implanted into the subcutaneous tissue of the abdomen of dogs. The removal of the tumour was conducted with the rigid aseptic precautions of a surgical operation, in order to avoid the risk of an accidental infection. The tumour masses, having been cut down upon from the outer skin surface, and removed, were at once divided into minute fragments the size of a pea, and implanted directly through skin incisions into pockets made underneath the panniculus carnosus of the new host. Two animals were inoculated.

EXPERIMENT/

EXPERIMENT NO.I.

A young wire haired fox terrier, bitch, (weight 4629 grms.)

Six minute portions of the tumour were implanted into the subcutaneous tissue of the abdomen and inner aspect of the left thigh. The wounds all healed by first intention.

On the day following the inoculation the portions of tumour introduced were easily palpable underneath the skin, the wounds appeared healthy and caused the animal no discomfort. On the third day the nodules were still palpable, the wounds were united and showed no evidence of suppuration. At the end of a week, the nodules could only be palpated with difficulty and appeared to be disappearing. A fortnight after the date of inoculation, the nodules introduced could no longer be palpated so that it appeared as if the result was to be negative. During the third week, however, minute nodules were able to be felt at the sites of inoculation. From now onwards, these gradually increased in size, and minute,/

minute, pea-like tumours could be felt leading from them along the line of the channel in the subcutaneous tissue through which the portions introduced were thrust into the pockets in which they were placed. These tumours still continued gradually to increase in size, so that at the end of 38 days four confluent fleshy masses could be felt, which were each approximately the size of a hen's egg. They were irregular in contour, lobulated in outline and, from their situation appeared to have grown from all the six portions of tissue introduced. They were painless to the touch, firm in consistence and the skin was freely moveable over them. The tumours reached their maximum limits of growth two months from the date of inoculation when they felt somewhat larger in size than a hen's egg. From this date they commenced gradually to disappear, at first very slowly, later more rapidly until, 81 days from the date of inoculation, they had all entirely disappeared except one, which felt the size of a split pea. This was adherent to the skin over it which was ulcerated. This nodule was excised, and showed changes which will be referred to later. The weight of the animal remained remarkably constant throughout/

throughout. There was a very slight loss of weight for the first 5 days. This, however, was soon made up, and when the tumours had disappeared, the animal was found to have lost 128 grammes in weight.

EXPERIMENT/

EXPERIMENT NO. 2.

A smooth haired fox terrier dog.

It was inoculated in a similar manner with six pieces of tissue.

In this animal, however, the disease ran a somewhat different course.

The wounds healed readily, and the nodules introduced were palpable for four days after introduction; thereafter a period intervened during which nothing could be felt, but on the 10th day, nodules could be distinctly made out at the sites of inoculation. These gradually increased in size, and on the 38th day had reached the size of bantams' eggs. They possessed an irregular contour and firm consistence, and were freely moveable on the surrounding tissues, as was the case in the former experiment.

The effect upon the general health of this animal was much more severe than was the case in the other dog.

The animal for the first month, gradually emaciated until, at the end of three weeks it had lost $\frac{1}{6}$ of its body weight and now almost entirely refused/

refused to take its food. Thereafter, however, it improved considerably in health. It looked brighter, took its food better and put on weight steadily.

Coincident with this, the tumours were gradually increasing in size. On the 43rd day, however, it again relapsed and gradually sank, and on the 47th day, as it was on the point of death, it was killed. The tumours, which had gradually increased in size up to this period, were now the size of hen's eggs and formed six separate masses.

The weight of the animal at the time of death was $\frac{1}{8}$ less than before treatment.

At the post mortem examination, the nodules were found situated in the subcutaneous tissue of the flank and abdomen. They appeared to possess a distinct capsule, and could be shelled out readily from the surrounding tissue.

On section they had a uniform firm hepatic-like consistence and showed no evidence of necrosis to the naked eye.

The regional lymphatic glands were only slightly enlarged.

Abdomen showed no evidence of peritonitis.

The Liver was enlarged, and showed scattered over its surface several elevations which, at its anterior/

anterior margin were wedge shaped. These were firmer in consistence than the surrounding liver substances, and had a dark mottled appearance, the appearance resembling that seen in a recent infarction in the spleen, but with a general light coloured mottling throughout it.

On section this appearance was further revealed. The lighter opaque grey strands which ran through the darker russet coloured substances, were seen to follow the lines of the portal tracts and to have associated with them definite minute nodules at certain parts.

At the transverse fissure of the Liver, the Hepatic Artery and Portal vein were apparently healthy, and no evidence of thrombosis was seen.

The Heart and Lungs showed no definite gross lesion.

The spleen was slightly enlarged, and firm in consistence.

The Kidneys were somewhat bulky, but showed no obvious lesion to the naked eye.

The suprarenals were enlarged, especially the left, which, on section, showed at the upper pole a small opaque grey area situated underneath the/

the capsule and invading the cortex.

On laying open the intestines a small "plaque" was found in the middle of the small intestine at its mesenteric attachment. This was firmly adherent to the coats of the bowel, was the size of a florin, firm in consistence and of a light grey colour.

MICROSCOPICAL EXAMINATION.

The tumour showed (Plate viii. Fig.2) a histological structure which was in the main identical with that which was found in the first generation. The tumour cells were identical in every respect with those already described. The stroma was, however, seen to be much more scanty, so that in certain areas the alveolar appearance was absent, and the histological picture was more akin to that seen in a large celled sarcoma. The incapsulation which to the naked eye appeared to exist, was not found to be present. At the border of the growth (Plate ix. Fig. 1.) the tumour cells could be seen to be infiltrating and destroying the surrounding fibrous tissue. The destruction/

destruction of the fibrous tissue was evident at the outer margin of the tumour mass, where (Plate ix. Fig.2) the fibrillary intercellular substance was fragmented and disintegrated. The connective tissue cells of the host were here swollen up, increased in number and appeared healthy, and although no actual mitotic figures could be seen the appearance present very strongly suggested that additional tumour cells were being formed from them.

Proliferation of the endothelial cells of the capillaries of the host was also apparent, and it appeared to be certainly evident that the fine capillary stroma of the tumour was being formed from these. An occasional small haemorrhage could be made out in the substance of the tumour mass, and at certain areas (Plate x. Figs.1 and 2) vessels were seen which had outside them accumulations of cells, of the nature of lymphocytes and polyblasts. These were situated at parts where minute foci of necrotic tumour cells were present and in some of them evidences of phagocytosis could be detected. Inside the lumen of some of these vessels, polyblasts and large mononuclear/

mononuclear leucocytes could be seen which contained ingested foreign particles

THE LIVER. On microscopical examination of the dark mottled areas, it was noticed that the liver cells were at parts entirely destroyed and replaced by small cells. In the Portal Tracts (Plate xi. Fig.1) cells were seen surrounding the vessels, and in many of the sections the Portal vein was the site of a recent thrombosis. These cells were present in large numbers and infiltrated the adjacent liver tissue. The great majority were young connective tissue cells (Plate xi. Fig.2) identical with tumour cells and many were undergoing mitosis. Young capillaries were interspersed throughout, and here and there a leucocyte was seen.

The appearances present in the Portal vein varied. Some of the larger trunks contained recent thrombi. In others the greater part of the lumen was occupied by cells similar to those outside. The contour of these vessels was altered, the new formed cells being arranged around the wall and extending through it into the Portal space. In some of the smallest vessels the lumen was seen to/

to be entirely blocked by them and in one vessel of moderate size what looked like a portion of new growth was seen in the centre of its lumen.

The periphery of the dark coloured zones showed liver cells in various stages of degeneration.

The Intestine showed the nodule (Plate xii. Figs.1 and 2) to be made up of small cells which were mainly young connective tissue cells, and in many of them mitosis was apparent. Young capillaries ramified throughout the mass and the cells composing it could be seen to be invading the adjacent adipose tissue and infiltrating the muscular coat of the bowel to which it was adherent.

In the centre of the mass a vein and artery were present. The vein showed in a lesser degree the change present in the smaller veins of the Portal tract. At one part a distinct papilliform process projected into the lumen of the vessel, the endothelial lining of which was ruptured and cells were present inside its lumen two of which are undergoing mitosis and were identical with those of the tumour.

THE LEFT SUPRARENAL GLAND showed, (Plate xiii. Figs.1 and 2) situated on its outer surface and/

and invading the cortex, a nodule which had a histological structure very similar to that of the intestinal nodule. Here however the arrangement more closely conformed to that of the primary tumour. The tumour cells extended between the parenchymatous cells of the stratum glomerulosum which were compressed and degenerated.

THE KIDNEYS, showed accumulations of small cells throughout its substance - these were especially seen in relationship to the interlobular vessels.

The detailed examination of the organ will however be described under the changes induced in the Kidney by Infective Sarcoma and its toxin.

THE LUNGS, on microscopical examination showed at certain parts an appearance very similar to what was present in the Kidney. Especially in the neighbourhood of the Bronchi accumulations of small cells which are somewhat difficult to differentiate, were present. These were mainly of the nature of Polyblasts. Section of the Pulmonary Artery showed Red Blood cells, polymorpho nuclear leucocytes/

leucocytes and numerous lymphocytes which appeared to be increased in number.

THE SPLEEN showed lymphoid hyperplasia; no evidence of focal necrosis could be detected.

LYMPHATIC GLANDS, showed accumulations of Polyblasts in the lymphatic sinuses. These cells stained in the characteristic manner with Pyronin Resorcin and methyl Green. They were also seen in the periphery of the cortical lymph follicle; transition stages from lymphocytes being readily made out.

The results of the examination of the histological structure, show:-

The structure of the tumours formed at the sites of inoculation was similar to that of the tissue introduced.

The changes present in the liver intestine and suprarenal, consisted in the localised formation of masses of new growth, which possessed in the main a structure similar to that of the primary focus.

The appearance in the portal tracts had, however, many points of resemblance to an inflammatory reaction such as is found in an acute interstitial hepatitis, The localisation of the disease/

disease, the great preponderance of the connective tissue cell, and the very active cell division in these unaccompanied by the presence of mature fibroblasts is more in favour of new growth. It would appear probable that the change in the intestine occurred prior to that in the liver, and that it spread from the former to the latter, by way of the portal circulation possibly by actual cellular metastasis.

The changes in the other organs were such as to suggest the action of a toxin upon them.

THIRD/

THIRD GENERATION.

EXPERIMENT.3.

Young fully grown wire-haired fox terrier dog. (weight 15 lbs. 3 oz.). (6903 grms.)

Six minute portions of the tumour from Experiment 2. were inoculated into the subcutaneous of the flank.

The wounds healed by first intention. The effect upon the general health of the animal appeared to be very slight. After an initial transient slight loss of weight, the animal regained its former weight and was slightly heavier when killed. The nodules were palpable for three days, and then disappeared to reappear on the eighth day, after which they grew rapidly, ultimately reaching when the animal was killed on the eighty-fifth day after inoculation, the size of golf balls. As can be seen from the accompanying photograph (Plate xiv Fig 1) the animal was at the time, stout and healthy. At the post mortem, four separate masses were found three being large and non-adherent. From two of these, rows of small nodules extended from the wound to/

to the tumour mass, the fourth nodule was smaller and adherent to the cicatrix of the wound over it. The tumours were found to have, both to the naked eye, and on microscopical examination, (Plate xiv. Fig 2) a structure identical to that of the previous ones. Plate xv. Fig 1)

The organs of the body showed little change. The spleen was enlarged and firm. The lymphatic glands, cervical and inguinal, were also enlarged and firm in consistence. They revealed, however, no evidence of secondary infection, nor could such be detected in any of the other organs of the body.

FOURTH GENERATION.

EXPERIMENT 5.

A large young smooth-haired fox terrier
10,510 grms.
(weight 23 lbs.2 oz.) was inoculated with seven pieces of larger size.

All the wounds healed without suppuration, a certain amount of acute inflammatory reaction occured, however, with two of them. On the third day, the nodules were all palpable, and the animal
567 grms.
had lost one and a quarter pounds in weight. On the/

the eighth day the inflammatory reaction had subsided, the nodules were still palpable, and felt about the same size as when introduced. On the thirteenth day they felt smaller. From this date they commenced to enlarge until in two months' time, they had reached the size of ducks' eggs. They then gradually diminished in size and finally disappeared in ninety-two days from the date of inoculation.

The animal's weight at the time of death, 9657 grms. was 21 lbs. 4 oz. At the post mortem examination no trace of where the nodules had been could be detected. The most evident change in the organs was that present in the kidney, which will be referred to later.

When the growth was at its summit, one of the nodules was excised. It had an appearance and structure similar to that found in the primary growth.

FOLLOWING GENERATIONS.

The tumour was carried to the eighth generation, when owing to the vacation occurring it/

it was allowed to die out. Its characters appeared the same in all cases. In no case did an animal, inoculated for the first time, fail to develop tumours, except two to be mentioned later. The age of the animal made little difference to the facility of successful inoculation. Successes were achieved with young growing tumours, those which had reached maturity, and ones which had undergone almost complete resorption.

Two young puppies were inoculated with portions of a nodule the size of a pea which was all that remained of a large rapidly disappearing mass. The result in both cases was negative.

There was no attenuation of the virulence from generation to generation. The tumours on the contrary grew more readily and more rapidly in the later generations.

The larger the piece introduced the larger appeared to be the tumour which developed.

SECOND/

SECOND SERIES OF INOCULATIONS.

From the wire haired fox terrier dog presented to me by Dr.Bashford successful inoculations were carried out into two animals. The tumour mass was as already mentioned, situated underneath the foreskin and grew from the corona glandis and body of the Penis, and consequently, owing to its situation it was more difficult than in the previous case to assure sterility of the portions excised. These were washed thoroughly with sterilised normal saline solution before being placed in the tissues of the new host.

Slight suppuration occurred in a wound of one of the animals inoculated, and a nodule failed to develop here; the other pieces all led to the development of tumour masses except a portion introduced into the General Peritoneal Cavity. In both animals which were inoculated with this - the first generation of the new tumour - the portions introduced could at first be felt underneath the skin, but then disappeared to reappear in the third week. They reached the summit of their growth

two and a half months after inoculation and then commenced to disappear. One animal was then killed and in the other the tumours were allowed to disappear which they did in ninety-one days.

SUCCEEDING GENERATIONS

The tumour was successfully cultivated through eight generations and is still growing.

The characters shown by it were very similar to that seen in the former series. (Plate xv. Fig.2) The nodules in the latter cases however took longer to appear and persisted longer.

In none of the animals killed were secondary foci of the disease detected in internal organs.

All the tumours formed disappeared spontaneously or appeared to be doing so when the animal was killed.

INOCULATION INTO ANIMALS IN WHICH SPONTANEOUS RECOVERY HAD OCCURRED.

Six dogs (two from the first series and four/

four from the second) were inoculated. In each case the portion introduced disappeared in less than two weeks and no subsequent tumour formed. This immunity was found to be present, from one to eleven months after the date of recovery. Five animals were inoculated with portions of the tumour from the same strain as that which they had themselves possessed. Two experiments were done on the same animal. (Experiment No.I.) It was inoculated with a portion of the tumour of the first strain, similar to that from which it had itself recovered. Ten months later it was inoculated with a portion of strain No. 2. and proved also immune to it.

INOCULATION INTO ANIMALS OF DIFFERENT SPECIES.

Rabbits, Rats and Guinea Pigs were inoculated with portions of the tumour without success. In all cases the portion introduced underwent gradual absorption and disappeared.

INOCULATION INTO FOXES.

Two fox cubs were inoculated with portions of the tumour from the fourth generation of the first series. In both cases tumours gradually developed, increased until they finally reached the size of pigeons' eggs when the animals died considerably emaciated.

These results confirm many of the points brought out by previous investigators, but the percentage of successful inoculations is higher than has been the case with them. This, however, is to be explained by, the method of direct implantation adopted in place of using a trochar, and, the care necessary where the stock of animals must necessarily be few.

The inoculations into the foxes were carried out to demonstrate whether the tumour which failed to grow in animals of different species to the dog would grow in one which was closely allied with that animal. At the time when the successful result was obtained by me no published record of such existed. Two months later, however, Stricker published his second paper in which he described the development of tumours after inoculation/

inoculation into two foxes.

CAUSAL VIRUS.

Cultivations were made with a negative result from all of the tumours of the first series to endeavour to find the parasite, if such should exist. All the common and many of the rarer media used in bacteriological work were tried without success. Neither an aerobic nor anaerobic micro-organism could be isolated, except an occasional germ which was obviously a contamination. Dr.Ford Robertson kindly examined for me portions of the tumour by his special staining methods without being able to detect inside the cells the presence of those bodies which he along with myself figured as occurring in human carcinomata and which possess a certain morphological resemblance to Plasmodiophara Brassica.

Portions of the tumour were mixed with sterile normal saline solution pounded up in a mortar with sterilised sand and then filtered through a Berkefeld Filter. The fluid obtained was introduced into the subcutaneous tissues of a number/

number of dogs. In none of them did tumours develop either at the site of inoculation or in any part of the body. One of the animals (Experiment No.4) died on the sixth week with most important changes especially seen in its kidney, which fact formed the basis of a further series of experiments the results of which will be detailed under the changes induced in these organs.

Films from the tumours were also examined for the presence of spirochetae as possible etiological factors, but none were found.

RESULTS/

RESULTS OBTAINED BY OTHER INVESTIGATORS.

The site of implantation appears to have but little influence in determining the success of the inoculation. Stricker has worked out this point with a colossal thoroughness. His results are briefly as follows:-

Tumours developed in sixty dogs after inoculation of the subcutaneous tissue.

Sixty-one dogs and two foxes were successfully inoculated by peritoneal implantation. In these there was produced either an isolated nodule always situated in the great omentum, or a general dissemination of the disease like a general peritoneal sarcomatosis. He has also inoculated the tumour with success into the thoracic cavity, the Scrotum, the medullary cavity of tibia, the eye, the tissue of the vagina, and the subdural space. Of three animals fed with the tumour one which appears to have been at the same time inoculated into the gum developed general sarcomatosis of the cervical lymph glands, hard and soft palate, and peritoneum, submucous and subserous nodules being present also in/

in the wall of the stomach.

He also worked out the Thermic Mechanical and Chemical Resistance of the Tumour.

Thermic cold exposure for 24 hours to a temperature of -11° C. diminished but did not destroy the vitality of the tumour, retention for 30 days at -11° C. completely destroyed its vitality.

HEAT.

Tumour tissue which had been kept for two hours at a temperature of 50° C. was successfully inoculated.

MECHANICAL.

Rubbing down in a mortar if conducted with moderate violence did not prevent successful inoculation.

FILTRATION.

As also found by me the filtrate from tumours rubbed down in a mortar was inert.

Centrifugal action gave a similar result.

CHEMICAL RESISTANCE.

Glycerine possessed no destructive property on the tumour cells.

CHARCOAL.

Powdered charcoal was found by Stricker to give the unexpected result of entirely checking the growth of the tumour cells.

Like /

Like other observers Stricker failed to find any parasite, extra or intra cellular.

The only observer who has found a parasite which he claims to be the pathogenic organism of the disease is San Felice (20) who has isolated a Blastomyces.

Plimmer (21) failed to find the cell inclusion, which he described in human carcinoma.

Beebe and Ewing (15) found a large spiral organism like the Spirocheta Refringens in one ulcerating tumour. They do not suggest, however, that it was at all likely to have been the cause of the tumour formation.

HISTOGENESES/

HISTOGENESES OF THE TUMOUR PRODUCED AFTER INOCULATION AND ITS LIFE HISTORY.

At the very outset of my investigations into the nature of this disease, it became apparent that in order to determine the exact relationship which it bore to cancer as we know it, it would be necessary to study the life history of the tumour from its inception to its final disappearance.

In the case of the Adeno Carcinoma that occurs in mice, and which was originally described and fully investigated by Jensen, this has been done in a very complete manner by Jensen, Bashford, Borrel, Ehrlich, Gaylard, Clowes and others, and through the courtesy of Professor Jensen I have also been able to carry out inoculation experiments with his tumour and study its life history.

Briefly the main facts concerning this tumour upon which the experimental investigation of cancer has been virtually entirely based in recent years, are as follows:- The tumour is one which occurs in the subcutaneous tissue of mice. The discovery/

discovery of a sporadic tumour is an event of rare occurrence but when it is found it can, in contradistinction to all other known malignant epithelial new growths be readily inoculated into animals of the same species.

Clinically and pathologically it is accepted by virtually all competent observers to be in all respects homologous with carcinoma as it occurs in man. It is looked upon by some as an adeno carcinoma of the skin , by others as a mammary tumour.

No evidence of spontaneous infection has been absolutely proven to occur although Gaylord (25) reports the occurrence of upwards of sixty sporadic tumours during three years, in animals kept in the same cage, the stock of animals being entirely renewed at least on one occasion, and Borrel (28) has succeeded in tracing twenty sporadic tumours to animals all of which came from the same cage.

The percentage of successful inoculations varies. It is usually low at first, in succeeding generations this, however, rises and may reach as high as 90%. After introduction in a successful case there soon develops a tumour which grows rapidly, occasionally produces metastatic foci and usually ultimately leads to the death of the animal. In a certain percentage of cases spontaneous recovery occurs/

occurs which renders the animal immune to subsequent inoculation.[29] Ehrlich and Clowes[30] who have studied the problem of this immunity have found that they can render mice which have not been inoculated immune against the disease by various methods. Ehrlich has also found that the nature of the tumours may be altered by repeated inoculations. In tumours of a rapidly growing strain he has been able by this means to make an adeno carcinoma become a typical sarcoma, a change which he attributes to an irritative effect exerted by the cancer cells upon the cells of the stroma which causes these mesodermic cells whose proliferative activity has been so greatly augmented by repeated inoculations to grow luxuriantly and independently like cancer cells.

The parenchyma of the tumour which develops after inoculation was proven by Jensen[31] to arise from the cells introduced and this fact is now accepted by all.

The fate of the stroma introduced is, however, not considered to be the same by all.

Bashford[4] says, "The stroma introduced at inoculation undergoes degeneration and is replaced by supporting structures derived entirely from the tissue of the host."

Apolant/

Apolant and Ehrlich (23) working with tumours similar in structure but mostly of a more virulent strain agree with the well-known observation that "frequently in cancer transplantations the connective tissue stroma inoculated with it undergoes atrophy but the mesodermic cells of the tumours formed are derived from those of the sporadic growth". With this the majority agree.

Fisch (26), in reviewing the progress of work on Cancer Research during the year that is past supports Bormann (32) when he says, "One of the main questions in the cancer problem is to the impartial mind settled. Tumours grow from the beginning through the multiplication of their own substance, destroy surrounding tissue in various ways, but never change it to tumour tissue."

At the time when my investigations commenced, no record existed of the process by which the tumour in the dog originated, grew and finally disappeared.

Since then, however, the results have been published of a certain amount of work done on this subject. Bashford, Stricker, and Beebe and Ewing/

Ewing have investigated the histo genesis of the tumour and the second of these observers has studied the changes which are associated with its disappearance.

Bashford is of the opinion that "the establishment of a new tumour is accompanied by a transformation of the connective tissue cells at the site of inoculation into elements indistinguishable from those of the primary growth. The tumour cells introduced degenerate very rapidly, a few only retaining their vitality for a period of not more than three or four days". Briefly he considers it entirely an infective process (24) "which is identical to that which occurs after inoculation with isolated pathogenic organisms, the cells of an infective granuloma introduced at the time of inoculation being merely vehicles - living substances for the platinum loop for the conveyance of the infective agent".

Stricker believes that the process is one where a grafting or implantation occurs similar to that which takes place with Jensen's Adenocarcinoma in mice. According to him the tissues of the host at no time and in no place take part in the formation of the tumour.

Beebe and Ewing hold that the tumour develops/

develops from the cells introduced. That some tissue cells may be included in the tumour or transformed into tumour cells, they are not prepared to deny although there was no evidence of such a process in their specimens.

That these diametrically opposed views should exist is not difficult to understand when one remembers that we are here dealing with a structure, the cell of which is identical with certain young connective tissue cells which are formed in the process of repair which occurs round any foreign body. The special features of the parenchyma cell of Jensen's tumour on the other hand renders the investigation of this point comparatively simple.

In order to determine the accurate mode of origin and growth of the tumour, my investigations have been carried out as follows.

1. The tissues of dogs, killed when in apparent good health have been examined.
2. Portions of a growing Infective Sarcoma were excised and then inoculated in the way already described. These were then removed $6\frac{3}{4}$ hours, 18 hours,/

hours, 24 hours, 3 days, 8 days, 10 days, 21 days at the summit of the growth during its declension and just prior to its final disappearance. The companion portion to that introduced was in the cases of the earlier periods kept at body temperature outside the body for a similar length of time and then examined. In order as far as possible to obtain a consecutive series of changes and to avoid fallacy the nodules were excised under an anæsthetic from the same dogs at different periods. In studying the changes in the earlier hours it was, however, found necessary to kill the animal and fix the parts "in situ"

3. The reaction which occurred when portions of pith were introduced into the subcutaneous tissue was investigated.

4. Portions of a sarcoma, obtained after amputation from the limb of a dog, were introduced into subcutaneous tissue and the reaction which they induced was studied.

5. Portions of the Infective Sarcoma tumour were/

were introduced into dogs which had acquired immunity and then excised at intervals and examined.

THE SUBCUTANEOUS TISSUE OF THE HEALTHY DOG

showed at the site used for implantation delicate areolar tissue united superficially to the Panniculus Carnosus and below to the deep fascia.

The usual histological structure was seen, wandering cells were virtually absent and neither could the extravascular accumulations of adventitia cells described by Marchand (33) nor the lymphoid nodes described by Beattie (34) as occuring in the peritoneum, be detected. The connective tissue cells were few, showed no evidence of direct or indirect cell division, and were never swollen.

THE/

THE HISTOGENESIS OF THE TUMOUR.

As already mentioned it was found necessary to destroy the animal when the changes which occurred in the earlier hours were being studied. The skin, subcutaneous tissue, and deep fascia, were then removed in one layer, fixed for a few hours in saturated corrosive sublimate solution and then sectioned to allow the fixative to penetrate. By such means an excellent opportunity of examining the relationships of the growth could be obtained. Certain fallacies had to be avoided, however.

In the carrying through of the specimen, owing to the shrinkage of the tissues which occurred, retraction of the nodule and the tissues of the host took place at some parts. This was liable to leave fragments of the tissue of the nodule adherent to the tissue of the host, and on the other hand loose tags of areolar tissue were apt to remain fixed to the outer side of the nodule introduced, and might be mistaken for portions of the capsule of the tumour introduced at the time of inoculation. In order to make/

make this point clear, in every case where such a fallacy was liable to occur, the nodule was sectioned completely, and serial sections of its entire bulk examined. This point, as will be seen later is of especial importance, as the areas from which the tumours especially grow are in the majority of cases ones where a process of immediate union between the tissues of the host and nodule are present with no fibrinous zone to demark their boundaries.

Control inoculations, were, of course made in every case into other dogs. Tumours developed in every one of these.

HISTOLOGICAL/

HISTOLOGICAL TECHNIQUE.

Saturated corrosive sublimate solution was found to be the best fixative, and for staining purposes, the following reagents were used in every case.

Haematoxylin and Eosin.	H.E.
Haematoxylin and Picrofuschin.	H.Pf.
Haematoxylin and Iron Alum.	H.I.A.
Haematoxylin Iron Alum Picrofuschin.	H.I.A.Pf.
Eosin and Methylene Blue.	E.MB..
Pyronin Resorcin and Methyl Green.	P.R.MG.

and such special stains as Geimsa's etc. as the occasion required.

HISTOLOGICAL/

HISTOLOGICAL TERMINOLOGY.

From six hours up to fifteen days certain well marked changes could always be seen at the site of implantation.

1. The centre of the nodule introduced hereinafter called "the necrotic centre" showed in every case evidence of cell degeneration.
2. Further out close to the periphery of the nodule, there was present a zone where leucocytes - at first mainly of the polymorphonuclear variety - abounded, "the zone of leucocytic invasion".
3. Beyond that but still in the substance of the nodule a zone where after twenty four hours large mononuclear phagocytes could be seen removing the necrotic tumour debris - "the boundary zone".
4. Beyond this there was present in every case a zone which was directly continuous with the boundary zone and usually continued into the tissue of the host, where healthy tumour cells could be detected, "the tumour formation area."
5. External to this the tissues of the host showed active/

active reaction, "adjacent tissue of the host."

6. In addition, at some parts, the tissues of the host and the nodule introduced could be seen to be separated by a layer containing fibrin and cells of the inflammatory exudate "the fibrin layer."

EXAMINATION OF NODULES REMOVED AFTER $6\frac{3}{4}$ HOURS.

Skin wounds are already united firmly by the fibrinous exudate.

The subcutaneous tissue shows a certain amount of oedema.

Microscopical Examination shows the nodule introduced lying in the subcutaneous tissue of the host. (plate xvi. fig. 1.) It has retracted somewhat and is only in intimate contact with the tissues of the host at certain areas.

The periphery of the nodule is seen to consist at one part of a portion of fibrous tissue.

The channel through which the nodule was introduced and the cul de sac beyond are occupied by a granular fibrinous exudate containing many cells.

The/

The areolar tissue adjacent to the nodule shows an increased number of small cells in it.

The nodule has a structure and arrangement similar to that of the tumour.

A detailed examination reveals:

1. THE NECROTIC CENTRE. The tumour cells in the centre of the nodule already show distinct alteration. Some of them appear normal but for the nucleus which is stained somewhat more deeply than is customary, others show nuclear fragmentations and cell disintegration, some of them are swollen up and have their cell body distinctly vacuolated; these have a nucleus staining more faintly, smaller in size and with an irregular shrivelled contour, and have somewhat the appearance of large phagocytes with phagocytosis proceeding; they are however degenerating tumour cells.

With E.M.B. and P.R.M.G. the various types of cell degeneration are best seen. With P.R.M.G. the great majority of the tumour cells are seen to be in various stages of cell degeneration. They do not stain well. In the majority the contour and structure of the tumour cell can be made out; the cell body, however/

however, does not stain of the deep salmon colour seen in healthy cells, the nucleus stains feebly light green, is distinctly granular, and its nucleolus stains red, but not the bright red of the standard type.

Other cells are seen with the cell body swollen up and vacuolated, others with the cell membrane broken at parts, others with the nucleus condensed crenated and staining a dark slate colour with its nucleolus faintly visible as a dull reddish brown dot. An occasional cell is seen which appears to have been undergoing mitosis at the time of introduction and is now degenerating. The cell body is stained a faint salmon colour, and the chromosomes appear fused into an irregular mass, stained a dull slate colour. In some cells also the nucleus has undergone Karyorrhexis the chromatin network being represented by dark greenish slate coloured particles, diffused throughout the cytoplasm, and in many the free nucleolus is seen as a red dot.

In others Karyolysis is apparent and only a ghost of the nuclear membrane is present. The endothelial cells of the capillary stroma are/

are degenerating as are also the connective tissue cells of the included fibrous tissue.

2. THE ZONE of LEUCOCYTIC INVASION. Outside the centre and inside the growing border. Leucocytes almost entirely polymorphonuclears, are present here in abundance between the tumour cells. The latter show advanced degeneration, and although karyolysis can be occasionally detected, the prevailing change is one where the nucleus is condensed, with the cell body swollen up and sometimes vacuolated. Some cells consist merely of a nucleus with a fragment of cytoplasm adherent to it. Leucocytes can occasionally be detected inside tumour cells. Throughout this area a granular material is seen which consists of disintegrated cell substance.

3. The BOUNDARY ZONE is not yet differentiated.

4. The TUMOUR FORMATION AREA. Beyond the zone where the leucocytes are abundant, the periphery of the nodule introduced is seen to be made up of tumour tissue which has the customary appearance, with a few leucocytes between the tumour cells; very many of the latter/

latter, however, show with E.M.B. a violet staining of their nuclei which are condensed. Tumour cells undergoing mitosis are seen: they are, however, scanty in number and only in slightly greater abundance than in the centre of the nodule. In the majority of specimens a cleft, which is empty but for an occasional shred of fibrin, exists through the outer part of this area and on the outer side of it the areolar tissue of the host is visible, at other parts a direct continuity already exists into the tissues of the host. At such regions the tumour cells are most abundant, least degenerated, and mitotic figures although rarely met with are most commonly found.

5. Adjacent areolar tissue of the host is at most parts retracted from the nodule and is usually covered with a delicate layer of granular fibrin in which is seen - cells, identical in size structure and staining reaction to those of the tumour and in addition polymorphonuclear leucocytes. An occasional cell is seen which is spindle-shaped, with a nucleus of similar contour which stains with P.R.M.G. slate grey and is finely granular, these appear to be endothelial cells.

In the areloar tissue granular fibrin is adherent/

adherent to the connective tissue strands. There is here obviously a great increase of cells from the normal standard.

These are:

1. A few polymorphonuclear leucocytes.
2. A few large and small lymphocytes and mononuclear leucocytes.
3. An occasional Polyblast.
4. Red Blood Corpuscles.
5. Very rarely an eosinophile leucocyte.
6. Connective Tissue Cells.
7. Endothelial Cells.

The capillaries are much engorged and contain a central mass of red blood cells and a peripheral ring of leucocytes some of which are seen emigrating from the vessel. The endothelial cells of the wall of the vessel are swollen up and much more evident than usual. Actual endothelial buds would appear to be present at some parts. The connective tissue cells of the areolar tissue are swollen up and have with E.M.B. a light blue staining granular nucleus with an open chromatin network and a prominent nucleolus. Their cell body is faintly stained and is spindle-shaped with an irregular contour. This change holds good for/

for the whole of the adjacent areolar tissue but at certain areas these cells are seen to be more abundant and some of them which approximate more closely to the tumour cell in appearance appear as if they had undergone recent division. Actual proof of this is, however, difficult to obtain. Where the intimate union is already established between the nodule and the tissues of the host, the areolar tissue adjacent to the nodule is almost entirely devoid of blood cells of the inflammatory exudate, but contains an increased number of fibroblasts and endothelial cells. These both stain with P.R.M.G. with their cell bodies a light salmon coloured tint, their nuclei light blue and containing bright pyronin red staining nucleoli. They appear very similar; the fibroblast is, however, usually larger and has a more granular nucleus. The connective tissue cells of the areolar tissue here are swollen up, the fibrillary intercellular substance is separated, and fragmented, cells which appear recently to have divided are seen, and in one case out of over a hundred sections examined/

examined, a connective tissue cell undergoing mitosis was made out.

The Channel of Entrance and the cul de sac are occupied by a granular fibrinous material in which the usual cells of the inflammatory exudate with the addition of a few degenerating tumour cells are apparent. The adjacent areolar tissue of the host shows here a reaction which is similar to that outside the tumour nodule but not so extreme in degree. The connective tissue and endothelial cells are swollen up and prominent with the characteristic appearance and staining reactions but there is no evidence of their having been increased in amount.

Control nodule $6\frac{3}{4}$ hours shows cell degeneration present but slight in amount. Mitosis is seen in the tumour cells but is not abundant.

NODULE REMOVED AFTER 18 HOURS.

The wounds are united by firm adhesions.

Microscopical examination shows (Plate xvi. fig. 2) the nodule to be directly incorporated with the adjacent areolar tissue at some parts, retracted at others, and separated by a fibrinous layer at others. Fragmentation of the adjacent areolar tissue is present, tags of it being torn off, and hanging free. Where the nodule introduced is in direct contact with the tissue of the host, these may appear as if they were part of its periphery. A most noticable feature even on casual examination is the pronounced cellular increase in the adjacent tissue of the host.

1. THE NECROTIC CENTRE shows evident cell degeneration pyknosis, karyorrhexis and karyolysis is apparent. Cells are seen which are swollen up and vacuolated. The stroma like the tumour cells is also degenerated, but not so extremely. No leucocytes are present in the centre of the nodule.

2/

2. THE ZONE OF LEUCOCYTIC INVASION is sharply defined. Here where the leucocytes are almost entirely polymorphonuclear the cell degeneration is extreme, and leucocytes are seen inside tumour cells.

3. THE BOUNDARY ZONE is not demarked yet; but lymphocyte-like cells are more abundant in this region.

4. THE ZONE OF TUMOUR FORMATION. There is already formed a layer of tumour cells, (Plate 1.) which are healthy, and many of which are actively dividing. This zone is only seen to be present where the cells of the tumour are directly in contact with the tissue of the host, where, in fact the nodule and host tissue are incorporated into one and no definite boundary line exists. This renders it a matter of considerable difficulty often to tell where one begins and the other terminates. This zone is absent where the outer part of the nodule consists of fibrous tissue and where haemorrhage or abundant exudate separates the two surfaces. Capillaries containing red blood cells and polymorphonuclear leucocytes are present in it and in some endothelial cells can be seen budding/

budding off from their walls. An endothelial cell lining the wall of one capillary, which is apparently a lymphatic, is seen to be in mitosis with a tumour cell adjacent to it undergoing a similar change. This capillary can be traced in serial section to belong to the tissue of the host.

Although the tumour cells here show very abundant mitoses, adjacent to them others are seen which by the condensation and staining of their nucleus appear to be in an early stage of cell degeneration. Polymorphonuclear Leucocytes and lymphocytes are virtually absent from this area.

5. The adjacent areolar tissue of the host can be traced directly into the zone of tumour formation.

As an accurate determination of the changes which are taking place in the tumour formation area is of the utmost importance in order to understand the true nature of the process by which the histogenesis of the tumour is brought about, a detailed description of the appearances presented is warranted.

At/

At what would appear to be the outer margin of the nodule introduced a definite zone is seen, even on the most cursory examination, where a band of tumour cells exists. To the inner side of this, that is, in the boundary zone a different appearance is presented. In this region cells which vary in size, structure, and staining reactions, are seen scattered amidst granular material which consists of disintegrated tumour tissue. The majority of them have a spherical contour and contain a large prominent deeply staining nucleus, which occupies the greater part of the cell. With H.I.A., Pf. the nuclei of the cells stains an almost uniform dark colour. In some of them, however, dark granules can be made out to exist. The cytoplasm stains a light reddish brown colour, is very finely granular, and contains minute clear vacuoles. In a few of these cells foreign particles can be detected inside the cytoplasm.

Plate. 2.

With P.R.M.G. the nuclei stain a dark bluish slate colour. Their chromatin is seen to be condensed and they can be made out even in the very smallest to contain a small bright pyronin/

pyronin red granule - the nucleolus. The cytoplasm is seen in the smallest as a scanty rim of grey coloured material. In the larger it is redder stained, more granular, and contains the clear vacuoles already mentioned. In some of them the cytoplasm is distinctly pyronin red in its staining. Very occasionally one of the cells is seen, which is swollen up, distinctly vacuolated, and containing a horse-shoe shaped nucleus. Inside its cell body particles of tissue debris are distinctly visible.

With E, MB. The nuclei of the cells which are being described stain dark blue their cytoplasm of a similar colour, but in a lighter shade. These cells are small and large lymphocytes and show all transition forms from these to mature Polyblasts.

In addition to these cells there is in this region also present a few polymorphonuclear leucocytes. The tumour cells and tissue of the stroma show here signs of advanced degeneration. Some of them appear as merely a structureless/

structureless faintly staining mass having the approximate contour of the healthy cell. Karyorrhexis, Pyknosis, and Karyolysis are seen to be occurring. Some are seen to consist merely of a shred of cell wall of nucleus, with a fragment of cytoplasm adherent to it, which accounts for the general diffuse granular appearance seen in this region.

On its outer side this zone can be noticed to terminate very abruptly, at the inner margin of the tumour formation area, where leucocytes, lymphocytes and their derivatives are virtually absent. The preponderating cell in that region is one which is large in size, usually polygonal in shape, and from the cell wall of which, delicate processes are seen to project which meet with similar processes from other cells. The nucleus, which is large and is usually eccentrically placed, contains an open chromatin network and a distinct nucleolus.

With H.I.A.P.F. the nuclei of these cells stain a dark slate colour the open chromatin network appears clearly and the nucleolus which/

which is almost always single appears as a distinct black point. The cytoplasm stains a reddish brown colour, but is not vacuolated and is more coarsely granular than is the case with the Polyblast.

With P.R.M.G. (Plate 11.) the nuclei stain a light slate colour. The open chromatin network appears and the nucleolus which is large stands out as a distinct red point. The cytoplasm of the cells which is finely granular stains with this reagent a salmon coloured tint. Many cells are seen to be undergoing mitosis.

These which are undoubtedly tumour cells as we know them, have ramifying throughout them spindle-shaped cells, with a large elongated cell body, a prominent nucleus and distinct nucleolus, and which stain in every respect similar to the tumour cell.

At the outer part of this area the appearance again gradually alters. The appearance characteristic of the tumour is now no longer seen. The cells which are here present vary much more in size and structure and shreds of fibrin are scattered throughout the field

along/

along with fragments of fibrillary intercellular substance.

In this region some cells are seen which have an appearance similar to the cell described as being present between the tumour cells. These have an elongated spindle-shaped cell body, a granular cytoplasm and an elongated oval nucleus, possessing one or more distinct large nucleoli. The nuclei of these, has an open chromatin network, and is coarsely granular. Other cells are here seen which are similar in every respect to the above but with a contour which is more irregular. The cell body of these which is large shows various transition forms from the spindle shape already described to the hexagonal shape of the typical tumour cell. It is usually, however, somewhat larger in size. In this region there is in addition seen polymorphonuclear leucocytes lymphocytes, and an occasional mononuclear leucocyte with a horse-shoe shaped nucleus.

Further out the appearance again changes into what can be recognised definitely to be the tissue of the host. The capillaries in this region are much gorged with blood, and

from/

from the inner lining of them endothelial cells can be seen budding off into their lumen. Examination of the extra vascular tissue shows emigrated polymorphonuclears, an occasional lymphocyte and mononuclear leucocyte. The formation of young fibroblasts and young endothelial cells is seen immediately adjacent to the tumour formation area, all transition forms being present from elongated spindle-shaped fibroblasts to characteristic tumour cells.

6. FIBRIN ZONE. The channel of Extrance is occupied by a fibrinous exudate in which are many large mononuclear leucocytes with a horse shoe-shaped nucleus. These are as abundant as the polymorphonuclears. The adjacent areolar tissue here shows certain of the connective tissue and endothelial cells swollen up and prominently stained, but the reaction here as compared with that present outside the nodule is most strikingly, much less.

NODULE/

NODULE REMOVED AFTER 24 HOURS.

All the wounds are firmly united.

Examination of a typical nodule shows (Plate xvi. fig. 3) the portion of tissue introduced to be almost entirely incorporated with the tissue of the host. As is virtually always the case the nodule introduced contains strands of dense fibrous tissue, and at one part the periphery of it consists of this. The contrast in staining between it and the fibrous tissue of the host is well seen when stained with H.Pf. The partly degenerated fibrous tissue of the nodule stains a dull brownish red, the healthy fibrous tissue of host stains bright red with fuschin. This distinction is evident and the recognition of it is of importance at the zone of tumour formation, as here no line of demarkation exists and tags of the fibrous tissue of host can be seen passing down through that area to the region where numerous necrotic cells are present

At the periphery of the nodule a zone is seen where healthy tumour cells are present. this zone has the appearance of being situated in the outer border of the nodule introduced.

The/

The stroma here stains a dull brownish red except for the thick bands of bright red staining fibrous tissue which as already mentioned dip down from the adjacent tissue of the host into it.

Where the dense fibrous tissue of the nodule introduced is in contact with the tissue of the host, as is always the case, no tumour formation zone is present, and the line of demarcation is easily made out.

On further examination

1. THE NECROTIC CENTRE is seen to be made up of tumour cells in various stages of degeneration similar to that already described. Karyorrhexis, Karyolysis, Pyknosis, and cell vacuolation are seen.
2. THE LEUCOCYTIC INVASION area forms a clearly defined zone below the growing border, the leucocytis being mainly polymorphonuclear.
3. THE BOUNDARY ZONE is not apparent.
4. THE TUMOUR FORMATION AREA (Plate iii.) consists of cells almost every one of which is a tumour cell, the vast majority of these appear healthy and many are undergoing mitosis. Between these, delicate spindle shaped cells ramify. These have delicate processes which meet with similar/

similar processes from other cells and form the spaces in which the tumour cells are contained, and are mainly endothelial cells although some are young fibroblasts.

5. Passing from the area where the tumour cells are abundant into the adjacent areolar tissue of the host a gradual transformation occurs. At first tumour cells are seen which are large in size, and as one proceeds outward all transition forms, can be made out from these to young fibroblasts. The differentiation of young fibroblasts from young endotheloal cells is, however, not always possible. At certain parts which are clearly in the adjacent areolar tissue tumour cells are seen undergoing mitosis. In addition there is present here some polymorphonuclear leucocytes, an occasional lymphocyte, polyblasts, and mononuclear leucocytes.

As in the previous case the reaction of the tissue adjacent to the channel of introduction is much less intense than in that which adjoins the nodule.

6. THE FIBRIN AREA. shows the usual appearance. Below it at one part, where it is scanty, there is/

is noticed in the outer parts of the nodule an occasional cell in mitoses. These are few in number and are situated amidst degenerating tumour cells.

NODULES REMOVED AFTER THREE DAYS.

The nodule is now found on examination to be completely incorporated with the surrounding tissue. The general picture seen is that of a central mass which has undergone complete necrosis bounded by a thick zone of tumour substance which is directly continued into the adjacent areolar tissue. The outer part of this tumour formation area is seen not to have the typical tumour structure; it consists of cells which are larger more irregular and spindle shaped. These can be made out to have been formed from the contiguous connective tissue. Through the growing tumour border young capillaries from the tissue of host are seen to pass down right to the necrotic tissue at its outer parts.

As was found in the previous cases the areas of tumour formation did not extend completely/

completely around the nodule.

1. THE NECROTIC CENTRE. (Plate xvii fig. 1) is now invaded by leucocytes.

2. THE LEUCOCYTIC AREA. The predominating cell here is now not the polymorphonuclear but a mononucleated cell with a horse shoe-shaped nucleus.

3. BOUNDARY ZONE. (Plate xvii fig. 2) is now distinct. It is rendered so by a zone of engorged mononuclear phagocytes which are actively engaged in removing the necrotic tumour debris.

4. This mononuclear phagocyte is usually a large cell with a deeply staining irregularly crenated nucleus, in which the nucleolus cannot be made out. The cell body is faintly stained with an irregular spherical contour inside which foreign particles as for example degenerating red blood cells, can be detected.

5. TUMOUR FORMATION AREA. (Plate iv, v.) shows abundant mitoses to be present, and new formed capillaries ramifying throughout it. Leucocytes are virtually absent from this area.

6. ADJACENT AREOLAR TISSUE. (Plate xviii fig. 1.) shows very active formation of young fibroblasts many of which are seen to be undergoing Karyokinesis. All transition forms from these to/

to tumour cells are able to be made out. Situated deep inside the adjacent areolar tissue of the host typical tumour cells are present which are undergoing division. The blood vessels of this area show evident reaction. They are engorged and have outside them accumulations of cells which are mainly of the lymphocyte type and are going on to form Polyblasts.

EXAMINATION/

EXAMINATION OF NODULE REMOVED AFTER EIGHT DAYS

The skin wounds are now united by firm fibrous adhesions. The nodules are seen to be situated in the subcutaneous tissue and are about the same size as the portions introduced. They are firmly incorporated with the surrounding parts, so that they can be dissected out and the changes present in the adjacent tissues of the host examined without difficulty. The changes which the nodule has undergone and which its presence has induced are found on microscopical examination to correspond very closely to those already described as being present in the earlier stages. The area of tumour formation is, however, so much enlarged that it now assumes the appearance of a definite tumour. The nodules in this case were larger and more fibrous at the time of introduction than was the case in the earlier experiments. The amount of necrotic tissue is correspondingly greater.

THE NECROTIC CENTRE (Plate xix. Fig.1) shows advanced cellular degeneration. The tumour structure can no longer be made out. The whole of the tissue here stains with H and E, a uniform dull red/

red colour. The outline of the tumour cells is seen, but any indication of intracellular structure is, in the majority of cases completely awanting. It is possible in some cases, however, to make out the contour of the nucleus, which stains slightly darker in colour. No healthy or degenerating leucocytes are present in this area. The outline of the tissue of the stroma and included portions of fibrous tissue is faintly visible. The majority of the connective tissue cells have a homogeneous appearance. In some, however, the nucleus can be made out more clearly and the cell degeneration is seen to be in them not so complete. Throughout the centre of the nodule minute darker staining particles are seen which appear like cocci. These are fragments of chromatin situated inside and in other cases outside degenerated tumour cells.
2. THE AREA OF LEUCOCYTES INVASION does not now stand out as a definite zone. In this situation, however, that is, towards the outer part of the necrotic tissue, mononuclear phagocytes are present. Very many of these have their cytoplasm swollen up and vacuolated, and containing foreign particles. The stroma of the necrotic tissue is seen faintly as/

as a skeleton framework from the spaces of which the tumour cells have been almost entirely removed by the action of the phagocytes.

3. Between this area and the tumour formation area in the region spoken of as the "Boundary Zone", a new appearance is now seen for the first time.(Plate xviii. Fig.2). A zone of tissue exists here having an appearance akin to that of granulation tissue, and in which numerous capillaries are visible, formed and in the process of formation. The endothelial cells forming the walls of these are delicate, elongated, spindle-shaped structures, which stain in a manner similar to that already described. The contrast between them and young fibroblasts is here able to be made out distinctly. The former is usually smaller, its cytoplasm is much more homogeneous. Outside the capillaries in many cases young fibroblasts are visible in contact with their wall. They are also seen separate from the vessels. When stained with H.I.A.Pf. their intracellular structure is well brought out. By this stain they appear as large cells which possess a broad elongated contour and somewhat spindle-shape, the nucleus of which is large and oval, has an

open/

open granular network, and contains a large dark nucleolus. The cell body which is granular, has longitudinal dark striae.

In addition to these cells, numerous polymorphonuclear leucocytes and lymphocyte-like cells are visible. With the same stain as that used above, these latter cells stand out prominently owing to the deep black homogeneous staining of their nuclei, and darker finely granular cytoplasm.

Young blood vessels can be seen to be growing from this area into the necrotic zone. These are accompanied by, and have an advance guard of, the above described lymphocyte-like cells "the polyblasts". Where these are removing the tumour debris they are swollen up sometimes to twice their original size, due to the enlargement of the cell body which is vacuolated. The nucleus, on the other hand appears if anything somewhat smaller, and is usually crenated.

4. THE TUMOUR FORMATION AREA is now seen to be larger but is not uniformly distributed around the whole of the nodule. It is found at isolated areas, places, where presumably the tumour cells of the nodule introduced were directly in contact with the tissues of the host. Where the tumour formation/

formation area is absent, a broad band of organising granulation tissue extends from the adjacent areolar tissue to the necrotic tissue of the nodule introduced. The histological structure of the tumour formation area is such that cells which in the majority of cases have a uniform size, structure, and staining reaction are seen arranged inside spaces which are formed by delicate capillaries. Inside the spaces the cells are arranged in a compact manner, and the outline of them is not always able to be made out clearly; they can be made out, however, to possess the characteristic polygonal shape, and from many of them, delicate processes pass off to blend with similar processes from other cells. In some the cell body appears blunt and spindle-shaped and has an oval nucleus. The nuclei of all the cells are large with a very evident reticular network in which the dark granules of the net knots are clearly visible. The nucleoli which are usually single are large and evident.

Many of these cells are seen to be undergoing karyokenesis.(Plate xx. Fig. 2)

No intercellular substance can be made out, and in the centre of the growing tumour apart from/

from the cell described no other cell is visible.

The spaces which are formed by delicate drawn out spindle-shaped endothelial cells vary much in size and shape. The structure seen here is typical of that of the rapidly growing tumour.

The absence of lymphocytes and their derivatives from the centre of the tumour is worthy of note.

5. THE ADJACENT TISSUE OF THE HOST is partly areolar and partly dense fibrous tissue. In both cases the tumour cells are seen invading it.(Plate xix. Fig.2). In the latter case, the interfibrillary ground substance is seen to be loosened and disintegrated, and lymphocytes and larger cells, Polyblasts, are present at this part of the tumour in moderate amount. No engorged and vacuolated phagocytes can be made out; a few clusters of spindle-shaped young fibroblasts are here seen at certain areas. Very occasionally one of these is noticed to be undergoing mitosis. It is a very rare occurrence to meet with an area, where the fixed tissue cells at the site of tumour invasion show any evidence of degeneration. Where it is areolar tissue which is being invaded (Plate xxi. Fig. 1), a similar appearance is presented. In the/

the connective tissue strands of it, connective tissue cells are increased in amount, and many of these appear to have been recently formed.

In the adjacent tissue, beyond the tumour border, especially in the neighbourhood of the blood vessels, cells are seen, (Plate xx. Fig.1) which are mainly of the lymphocyte type, and from them transitional forms can be traced to larger cells similar to those described by Maximow as Polyblasts.

EXAMINATION/

EXAMINATION OF NODULE REMOVED AFTER TEN DAYS.

The appearance presented here is very similar to that seen at 8 days. The cells of the tumour in the necrotic centre have undergone complete necrosis.

Young capillaries accompanied by adventitia cells dip down into this necrotic tissue at its periphery. No definite zone of leucocytic invasion exists, but at the border of the necrotic tissue; here and there a mononuclear phagocyte is seen, which is enlarged and contains debris inside its cytoplasm.

The boundary zone is like that in the previous case represented by cells which have the structure of granulation tissue. The tumour formation area is definite and has a structure similar to that described. Few lymphocyte-like cells are present throughout it, but at its outer part where it is invading the surrounding tissue, these are more abundant. Throughout the tumour area many cells are noticed in various stages of indirect division.

The adjacent tissue is seen to be being invaded by tumour cells and young fibroblasts are present/

present in increased numbers; some of these are also undergoing mitosis.

EXAMINATION/

EXAMINATION OF NODULE REMOVED AFTER FIFTEEN DAYS.

The necrotic centre is still present. The boundary zone is represented by a band of granulation tissue which can at some parts be seen to be splitting up the necrotic tissue by large finger-like projections into it. (Plate xxi. Fig.2)

Tumour formation area is large and unicellular, the tumour cell alone being present inside the delicate capillary stroma, in the centre of it.

At its extending margin it is noticed to be flooding the surrounding fibrous tissue with tumour cells, which are extending along the extravascular connective planes, the so-called perivascular lymphatics.(Plate xxii. Fig.1)

The isolation of islands of dense fibrous tissue and their inclusion inside the developing tumour is by this means being brought about. The adjacent tissue of the host shows an increased number of young connective tissue cells, which appear to have been formed by direct division. Mitosis can, however, very occasionally be made out to be occurring in these cells. No evidence of cell destruction is visible at the periphery of the tumour, where it is invading the surrounding tissue.

THE INFERENCE OF THESE EXPERIMENTS.

After 15 days from the date of inoculation the tumour can be made out even to the naked eye to have become definitely extablished. This therefore is a convenient time to break off from considering the description of the appearances seen in the serial experimental inoculations carried out to trace the life cycle of the tumour, and to look back at the changes which we can claim to have proven to occur in associationship with the origin of the new growth.

At the very outset it is clearly shown that palpation of the nodule in no way gives an indication of its progress in tumour development. The apparent retrogression being explained by a more rapid resorption of the necrotic tissue of the portion introduced than new tumour formation at its periphery.

The reaction which the introduction and presence of this foreign body in the tissues of the new host excites follows the lines, as one would naturally expect, of the cardinal changes associated with the inflammatory process.

At six hours the vessels of the surrounding tissue are seen to be much engorged. In many the classical picture is obtained of an axial column of Red Blood Corpuscles with a peripheral ring of leucocytes adherent to the vessel wall, through which leucocytes, mainly polymorphonuclear cells can be made out to be emigrating. These cells are abundant in the tissues of the host outside the vessels and are also seen in the fibrin area, and in the nodule introduced.

The variety and proportion of the cells of the inflammatory exudate correspond closely with that found at this period by Beattie (26) in his study of the cells of the inflammatory exudate present after intraperitoneal injection with Bacillus Coli Communis, polymorphonuclear leucocytes are most abundant, mononuclear cells, lymphocytes and mononuclear leucocytes are, however, also seen in addition to a very occasional eosinophile leucocyte. The situation of these is of interest.

In the adjacent tissue of the host and in the layer of the fibrinous exudate the customary relative proportion is maintained. Deep in the degenerating tissue of the nodule, in the area spoken of as the zone of leucocytic invasion, the polymorphonuclear/

polymorphonuclear leucocytes alone are present, in a manner similar to that obtained by Maximow who found in the centres of the capsules he introduced at first the polymorphonuclear alone present. The mononuclear cells are also at six hours seen to have emigrated into the nodule. They, however, do not penetrate so deeply as is the case with the polymorphonuclear. The presence of the polymorphonuclear is associated with a concomitant cell destruction in this area, although actual phagocytosis of cellular debris by them is not apparent.

The negatively chemiotactic influence of the living tumour cell is well shown at the outer growing border, (the tumour formation area), where leucocytes are virtually absent although through it the white blood corpuscles must have passed to reach the degenerating tissue beneath.

In the later experiments the relative proportions of the various cells seen in the necrotic tissue at different periods, corresponded very closely to that proved by Beattie to be the case in the non-fatal cases of Peritonitis. The polymorphonuclear is most abundant up to three days, it then gradually diminishes and is virtually entirely absent/

absent on the 8th day. It is present but not abundantly in the granulation tissue zone at this period.

Of the other cells of the inflammatory exudate which are present, the lymphocyte and the large mononuclear leucocyte of Ehrlich are seen and the evidence here would bear out, the now generally recognised fact, that both these cells migrate from the blood stream, and act as cellular phagocytes

The latter cell is especially seen in the earlier inoculations from 24 hours to the third day.

The mononuclear cells of the lymphocyte type and their further derivatives, the Polyblasts, would appear to be the most important cells present during the histogenesis, growth, and disappearance of the tumour. The role which they play, will be referred to in detail later. The source of the Polyblasts would appear at first undoubtedly to be almost entirely from lymphocytes which have emigrated from the blood stream, as Marschalko, Maximow, and others maintain.

During the growth of the tumour and the disappearance of it, there will be seen to be, however./

however, evidence in support of the arrival of these cells by the lymphatic vessels, and their emigration from these into the tissue of the nodule.

There is no evidence in the cases examined by me, of the possibility of isolating as a separate entity "the wandering cell" as a special lymphocyte-like cell of the tissue, as Pappenheim (34) and Dominici (35) would claim, from which these cells of the inflammatory exudate could be derived.

After immigration the lymphocytes undergo a gradual process of development, similar to that which can be well seen in the reaction induced in lymphatic glands by certain toxins. The cytoplasm of the cell gradually enlarges, it develops inside it a substance which stains faintly, and later a dark rose colour, with P.R.M.G. The clear spot containing the centrosome apparatus becomes evident, and is seen in contact with the nucleus, which is large placed, eccentric, and contains a dense chromatin network, which stains deeply and in which a small nucleolus can usually be made out distinctly. These cells which correspond to the Polyblasts of Maximow, are seen at 6 hours, present at 24 hours, abundant at 3 days. They are always absent/

absent from within the tumour formation area, except at its extending border. They are present in the granulation tissue zone and always abundant in the adjacent areolar tissue just beyond the extending margin of new growth. The role which they play and their relationship to Plasma cells will be discussed when considering the later stages of the life cycle of the tumour.

Neither the operation of introduction, nor the presence of the foreign body, appears to have caused death of the surrounding cells to any appreciable extent.

At six hours the adjacent connective tissue cells are swollen up and have an appearance similar to that seen prior to cell division. The endothelial cells show a similar change. It is probable that already young fibroblasts have been formed by direct, and in one case by indirect cell division; but this is not capable of absolute proof. The changes which occur around inert foreign bodies correspond closely to those described. If the formation of fibroblasts has occurred, however, this has taken place at an earlier period than is usually found in similar cases.

(27)

Kiener and Duclert in their work upon the formation of the abscess membrane describe similar/

similar enlargement and definition of structure of the adjacent connective tissue cells.

I have never been able to make out the coloured partition at the equatorial plane which they figure as indicative of direct cell division. I have however seen two nuclei separate inside one cell which they describe as occurring during that process.

At 18 hours the formation of fibroblasts and transition forms from these to Tumour Cells can be made out. Young endothelial cells are already present in the outer part of the tumour formation area, and in one case, mitosis is seen in the endothelial cells of a lymphatic capillary, which is directly in contact with the tumour cells of the nodule.

At three days the formations of young fibroblasts and the development from them of tumour cells is seen to be occurring very actively in the tissues of the host at the outer part of the tumour formation area. Mitotic figures in these being here apparent. Young capillaries can also now be made out passing from the tissue of the host into the tumour formation area.

At/

At 8 days the adjacent areolar tissue of the host is seen being invaded by Tumour Cells, the adjacent connective tissue cells are increased in number and show transition forms to Tumour Cells. Mitosis in the former has been seen, but is now not commonly found.

At 15 days a similar change is present.

CHANGES IN NODULE INTRODUCED.

In every case the centre of the nodule shows early and extensive degeneration. After six hours this is found to be present. The chromatin of the nucleus shows already in some cases condensation, in others fragmentation, and in still others solution and diffusion.

A similar piece of tumour kept for a like period in normal saline solution at body temperature does not show this change.

The cell degeneration affects in this area all the component parts of the tissue introduced. The tumour cells are the first to show alteration. The stroma, however, also degenerates. At three days hyaline degeneration is noticed to be present in the fibrous tissue introduced.

PRESENCE/

PRESENCE OF MITOSES. At $6\frac{3}{4}$ hours they are present in the tumour cells in the centre of the nodule. These appear to be degenerating cells, however.

At 18 hours such are absent from the centre of the nodule, but recent mitoses are seen deep in necrotic tissue and on the surface: at this period there is also seen abundant mitosis at the growing border of the Tumour Formation Area.

At one day they are found abundantly in the Tumour Formation Area, but are absent elsewhere, except for an occasional one in one nodule, where a delicate layer of fibrin separates the nodule from the tissue of the host.

At three days they are very abundant in the Tumour Formation Area.

At eight days they are even more abundant. Tripolar mitosis was seen in one section.

CONCLUSIONS/

CONCLUSIONS REGARDING THE HISTOGENESIS OF THE TUMOUR

The Tumour Formation Area is present only where tumour cells of the nodule introduced are directly in contact with the tissue of the host from the first; here tumours form. At other parts granulation tissue forms between the two surfaces. At no period can complete death of the tumour cells of the nodule introduced be made out associated with the creation of a Tumour Formation Area definitely within the tissues of the host adjacent. At 18 and 24 hours and 3 days. there are seen cells which are identical in appearance and are obviously derived from two sources; both undergoing active mitosis. Tumour cells of the nodule introduced. and similar cells from the tissue of the host.

The tumour is thus seen to be formed by certain of the peripheral cells of the nodule introduced, living and actively dividing and at the same time altering the process of repair in the surrounding connective tissue cells.

It induces an intense reaction which so affects the connective tissue cells, that instead of/

of going on to form maturer fibroblasts they remain immature and continue to actively divide, having acquired the character of a sarcoma cell, as which they will live and ultimately die, without ever again developing into mature fibroblasts, and forming fibrous tissues. This virus is one which is only conveyed by direct contagion.

How far each source of the tumour cells is responsible for the ultimate tumour formed, it is impossible to say, and from the nature of the change which occurs can never be accurately determined.

The Capillary stroma of the tumour formed, is created from that of the tissues of the host. At 18 hours endothelial cells in the capillaries of the host can be seen in mitosis, and penetrating the Tumour Formation Area. At 24 hours this is also evident and at 3 days new capillaries are seen to have completely permeated the zone of new tumour formation.

I thus cannot agree with Bashford whose opinion I, at one time, was inclined to homologate, when he said that the tumour formed at the site of inoculation was created entirely by a transformation of the connective tissue cells at the site of inoculation/

inoculation into tumour cells. I fail to perceive, also, how he is able after the facts I have brought out, to state that the tumour cells introduced degenerate very rapidly, a few only retaining their vitality for a period of not more than three or four days.

I also cannot agree with Stricker when he claims for the tumour created an origin entirely from the nodule introduced, and states that the tissues of the host in no way take part in the creating of new tumours.

I agree with Beebe and Ewing when they state that the tumour is formed from the cells of the nodule introduced, but instead of only considering it possible that it may be added to by new cells formed from the tissues of the host, I would claim that its stroma is formed from this source, and that to a very considerable extent this also serves to add to the source of new tumour cells.

THE/

THE LIFE CYCLE OF THE TUMOUR

(contd.)

We have seen how the inoculation of a portion of the tumour into the subcutaneous tissues of another animal produced after 15 days a nodule which had, to the naked eye, and on microscopical examination, characters similar to that of the previous generation. We will now proceed to examine nodules removed at later periods to determine the process by which the growth reaches its maximum size and then undergoes gradual resorption and finally disappears.

EXAMINATION of NODULES removed after TWENTY-TWO DAYS.

The nodules in all the cases examined could be made out to have increased much in size during the third week. They were now usually the size of hazel nuts, and could be removed with ease from the subcutaneous tissue. They appeared as if they were encapsulated and on sections had the uniform/

uniform fleshy consistence characteristic of the tumour. In none of them could evidence of the necrotic tissue of the portion introduced be made out.

The appearance presented on microscopical examination differed in many respects from that previously seen. In a typical case it was as follows:-

The centre of the nodule shows no evidence of the previously described "necrotic centre" or "boundary zone of granulation tissue". It is made up entirely of tumour tissue. This consists of a delicate capillary stroma with an occasional strand of fibrous tissue forming spaces that contain cells, which are characteristic in every respect of the typical tumour cell.

The most striking feature which is noticeable is the number of these which are undergoing mitosis (Plate xxii Fig.2.) Throughout every part of the new growth is this change evident. At some parts the number of dividing cells is so great that as many as ten can be seen in one field in various stages of karyokinesis. Tripolar mitosis is not uncommonly met with.

In contradistinction to what was seen at 15 days, the tumour mass is not now unicellular in nature./

nature. In addition to the tumour cell there is now occasionally seen another cell which is in almost every case small in size and has all the characteristics of the lymphocyte. These are seen arranged in contact with the capillary stroma and at some parts of the tumour, transition forms from these to the larger cell called "the Polyblast" can occasionally be made out. When such is present it is situated away from the stroma inside the tumour space.

The lymphocytes can usually be made out to be arranged around blood capillaries, but here and there throughout the tumour what would appear to be lymphatic capillaries (Plate xxiii Fig.1.) are present:- These have inside them a large number of cells, the majority of which are small in size and stain like the small lymphocytes, but some are larger in size swollen up and vacuolated, and in one case what would appear to be degenerating tumour cells are present inside the lumen. Very occasionally an eosinophile leucocyte is seen amidst the tumour cells (Plate xxiii Fig.2.).

At the periphery of the tumour many small blood vessels (Plate xxiv Fig.1.) penetrate it. These contain red blood corpuscles polymorphonuclear leucocytes/

leucocytes, lymphocytes and eosinophile leucocytes. The two latter are always more evident than is customary in health. These blood vessels are rendered more evident by the lines of lymphocytes arranged parallel to and in contact with their outer walls.

At the outer border of the tumour the flooding of the surrounding tissues by the tumour cells is no longer apparent (Plate xxv Fig.1.) An increased number of fibroblasts some of which are in mitosis, (Plate xxiv Fig.2.) are here present. These, however, instead of showing in the majority of cases transition forms to tumour cells are mostly elongated drawn out spindle-shaped cells which are arranged with their long axis parallel to the outer border of the tumour. In this region lymphocytes and their derivatives are found in moderate abundance scattered irregularly throughout the fibroblasts.

An occasional small haemorrhage is seen towards the outer part of the tumour.

EXAMINATION of NODULES removed after seventy-four days.

This period was chosen as for nearly a fortnight the tumours had remained stationary in size and now commenced apparently to undergo gradual resorption.

The/

The nodules could now be seen projecting underneath the skin as large masses, the size of billiard balls. They were usually non-adherent to the skin and moved freely on the subjacent tissues. When excised they were found to be distinctly encapsulated, and on section the tumour had the characteristic appearance.

Microscopical Examination shows an appearance very similar to that described as present in the sporadic tumour. Its stroma is made up mainly of capillaries, but here and there portions of fibrous tissue are included inside the tumour mass.

The arrangement of cells (Plate xxv Fig.2.) inside the spaces is not so compact. The tumour cell is much the most abundant cell unit present, but, in addition to it, there are also here present, other cells which show all transition forms from typical lymphocytes to Polyblasts and an occasional Plasma cell. Mitosis is seen in some of the tumour cells; but this is not anything like so evident as was the case in the previous experiments. The periphery of the nodule is seen to be definitely encapsulated (Plate xxvi Fig.1.) It consists of a laminated border, made up without of denser fibrous tissue and within this layer upon layer of fibrous tissue/

tissue, each representing a more recent stage of development, as it approaches the tumour substance (Plate xxvi Fig.3.) Near the outer border of the tumour the development of the young fibroblast and the formation of fibrous tissue is especially well seen. With H.I.A.PF. the fibroblasts appear as elongated cells with an oval nucleus which contain an open chromatin network and usually two or three distinct dark nucleoli. The cell body is drawn out and has dark striae running parallel to its long axis. At the outer border of the cell, in many rose red striae are seen, and outside the cell an intercellular fibrillary substance is present which stains in a similar manner. In this laminated border between the fribrous strands other cells are present, - an occasional lymphocyte is seen but the majority are larger cells which with P.R.M.G.have their cell body stained rose red and containing a large clear vacuole close up to the eccentrically placed nucleus, which is condensed and contains a distinct bright red nucleolus. Most of these have a spherical or polygonal contour, some, however, can be seen which have their cell body drawn out and are more granular, and would appear to be settling down as sensile adventitia cells. Transition forms from them to typical/

typical fibroblasts forming fibrous tissue cannot, however, be detected.

In addition to these cells a degenerating tumour cell is occasionally seen here.

Throughout the tumours, especially where the dense fibrous bands are present, (Plate xxvii Fig.1.) a similar appearance is seen. Young fibroblasts which are developing fibrous tissue, and have between their strands, cells like those described before, are seen. In the tumour substance adjacent to them lymphocytes and polyblasts are abundant, and tumour cells are few in number, such as are present being in almost every case degenerated, some appearing merely as ghost-like outlines, others showing that form of karyorrhexis in which the fragmented chromatin is arranged around the periphery of the nucleus. Polyblasts are very frequently found arranged in contact with these cells, and phagocytosis of tumour debris by the former is not infrequently noticed.

The tumour shows, in addition to the cells, already described, numerous eosinophile leucocytes at certain parts. With E.MB. the bright red granules of these stand out distinctly, as large circular particles. In some cases these cells are seen/

seen ruptured and similar granules are evident, between the tumour cell, inside some of them, and inside some of the Polyblasts.

As several haemorrhages were present, especially underneath the capsule, these granules were examined for the presence of an iron reaction with HCL. K FeC N. This was found to be absent.
4 6 6

EXAMINATION of NODULES removed at one hundred and eighteen days.

Several of the nodules had already in this case disappeared and such as were palpable could be made out as small minute structures the size of millet seeds, situated in the subcutaneous tissue, (Plate xxvii Fig.2.)

Microscopical Examination shows here:- a minute nodule of tissue having to low power examination somewhat the appearance of tumour structure, situated in the centre of a thick laminated (concentrically arranged) capsule of fibrous tissue.

More detailed examination shows the centre of the nodule to be made up of cells, (Plate xxviii & Plate V.A. Fig.1.) almost every one of which is not a tumour cell. Here and there, lymphocyte-like cells are present; but the preponderating cell is one which is/

is large and stains with E.MB. a deep blue colour. The nucleus stains darkly. Its cell body stains a similar dark blue colour and contains in every case, a clear vacuole on contact with its nucleus. With P.R.MG. these cells stain an intense bright red colour, so much so, as from their abundance to impart a bright red colour to the whole field, when seen with the low power of the microscope. The red staining is confined to the cell protoplasm in which the clear space already described is very evident. The nucleus, when it is not obscured by the superimposition of protoplasmic substance, stains a dark slate-blue colour, and contains inside its coarse closely set chromatin network a bright red coloured nucleolus.

Careful examination shows (Plate xxix Fig. 1.) an occasional tumour cell to be present amidst these cells. In no case can a tumour cell be made out which shows evidence of cell division and all of them that are visible, show evidences of advanced degeneration. Many are only detected as faintly stained structures possessing the general contour of the standard type, others are visible which stain faintly and are vacuolated.

Examination/

Examination of the laminated border of the nodule shows (Plate xxix Fig.2.) it to consist of layer upon layer of dense fibrous tissue which towards its inner surface (Plate xxx Fig.1.) is of more recent formation. Between the fibrous strands cells are present which stain bright red with P.R.M.G. and, as in the previous case certain of these are seen drawn out to form sessile adventitia cells. In addition there is also here present a very occasional degenerating tumour cell.

No haemorrhage is present in the nodule and no coarsely granular eosinophile cells can be seen.

EXAMINATION of the site of a NODULE which had disappeared after ONE HUNDRED and EIGHTEEN DAYS.

To the naked eye nothing could be seen indicating where the nodule had previously been.

Sections of the area, however, showed on microscopical examination (Plate xxx Fig.2.) the site of the tumour to be almost unrecognisable. Wavy bands of dense fibrous tissue could, however, be made out between which at certain parts lymphocytes and their derivatives polyblasts could occasionally/

occasionally be seen. These polyblasts presented the appearance of either the typical mononucleated phagocyte or had their protoplasm laden with debris. As in the previous cases the settling down of certain of these cells to form sessile adventitia cells was apparent.

THE/

THE INFERENCE OF THESE EXPERIMENTS.

Consideration of the afore mentioned facts shows certain constant characteristics to be associated with the later stages of the life cycle of the tumour. At 22 days the growth of the tumour which had previously been to a large extent due to the formation of new tumour cells from the adjacent connective tissue cells is now seen to be virtually entirely accounted for by the active division of those already formed. The process of formation of additional tumour cells from the adjacent tissue continues. The vast majority of the young fibroblasts now formed, however, proceed to the formation of fibrous tissue, by which means the encapsulation of the tumour is to be brought about. In this region lymphocytes and their derivatives are present and these cells mostly of the true lymphocyte type, are also seen as extravascular accumulations throughout the tumour stroma, but are virtually absent from amidst its cells.

At seventy-four days the growth of the nodule/

nodule is found to be entirely due to the division of its own tumour cells, and this is not proceeding so actively. The tumour is now definitely encapsulated. The capsule is formed from the adjacent tissue of the host which now instead of forming new tumour cells to help to extend the border of the new growth is forming cells which are creating layer upon layer of fibrous tissue which is gradually encroaching upon and diminishing the size of the nodule The polyblast is in this region abundant and can now be made out in some cases to be settling down to form a sessile adventitia cell.

This change is also seen inside the tumour mass where fragments of fibrous tissue exist. The formation of fibroblasts from them is here also occurring and the creation of additional fibrous tissue by this means is apparent.

Throughout the tumour the lymphocyte and the polyblast are now evident. These are now much more frequently of the nature of mature mononuclear cells "Polyblasts" than was previously the case and they are always present where new formation of fibrous tissue is proceeding and tumour cell degeneration is occurring.

The eosinophile leucocyte was present in excess/

excess in two cases at this time. This was not, however, a constant phenomenon in all the cases examined.

The tumour mass at this period as is so often found during its decline shows an occasional haemorrhage.

The blood vessels entering show an apparent increased number of lymphocytes. Channels that would appear to be lymphatics exist, some of which contain large numbers of lymphocytes of the standard undifferentiated type, while others contain in addition their derivatives polyblasts and possibly degenerating tumour cells.

At 118 days; the disappearing nodule contains tumour cells all of which are degenerating. The capsule has encroached almost completely upon the nodule. The lymphocyte is present but it is mainly its derivatives the polyblasts and more especially the Plasma cell which is flooding the tumour area. Phagocytosis of the tumour cells is evident but not to any great degree. Tumour cells which are structurally intact in every respect but show signs of obvious degeneration by their staining reactions. are present in many places.

A nodule which had disappeared at this

period /

period is found to be represented by dense fibrous tissue from which the polyblasts have almost disappeared some can however be seen, remaining as adventitia cells.

CONCLUSIONS/

CONCLUSIONS REGARDING THE GROWTH AND DISAPPEARANCE OF THE TUMOUR.

The formation of additional tumour cells from the surrounding tissues continues until the reaction of the tissues of the host is such that it is able to resist the action of virus, encapsulation now occurs.

The growth of the tumour gradually abates and with this, the lymphocyte is found, mainly now developed into the polyblast, throughout the tumour substance. Where such are present in numbers tumour cell degeneration is advanced.

The final disappearance of the tumour is associated with a flooding of the tumour formation area with lymphocyte polyblasts and plasma cells.

With the disappearance of the tumour the lymphocyte-tide recedes leaving an occasional cell as a fixed connective tissue cell.

The only published records which exist of the/

the later atages of the life cycle of the tumour are

1. The brief note by Bellingham Smith and Washbourne who say that "the tumours formed disappear by ulceration"
2. The opinion expressed by Stricker who after describing the haemorrhages so frequently met with in the disappearing tumours attributed to them the disappearance of the growth.

The facts of the life cycle as stated by me were found to exist in one animal at the various dates mentioned from three days onwards. Similar changes were also found to be occurring in several other animals which were examined.

DISAPPEARANCE OF NODULE BY ULCERATION.

Examination of nodule removed 81 days after inoculation.

The nodule was a minute pea-like structure over which the skin was ulcerated.

Examination showed (Plate xxxi Fig.1.) the ulceration present to be a mere coincidence dependent on a previous involvement of the skin.

Microscopically/

Microscopically the ulcer was found to consist of normal granulation tissues.

The subjacent nodule was found to be undergoing gradual absorption. Tumour cells which were mostly degenerated but one of which was undergoing karyokenesis were present (Plate xxxi Fig.2). Polyblasts were present in greater abundance in the nodule than in the ulcer. Some polymorphonuclear leucocytes were seen in both areas.

EXAMINATION/

EXAMINATION OF THE BLOOD OF THE DOG DURING THE GROWTH OF THE TUMOUR.

It was obvious when the lymphocyte and its derivatives were found to play so important a part in the life cycle of the tumour that a careful examination of the blood at stated intervals would give facts worthy of note. This was done.

The blood of eight animals was examined.

No. I. Sporadic Tumour No. 2.

The tumours present on the Penis had been excised three months previously and had not recurred.

The average of lymphocytes and mononucleated cells was found to be slightly higher than is the case in the normal dog. Its blood was examined on four occasions during two months and practically no variation occurred.

No. 2. (Animal No. 29) A large brown bull terrier.

Its blood was examined on the day prior to the inoculation and daily for one month. At the end of which time the tumours had grown to masses as large/

large as pigeons' eggs. Examination of the chart appended shows how an initial leucocytosis occurred which rapidly subsided. A marked relative diminution of the Polymorphonuclear leucocytes with a corresponding increase of the monunucleated cells and also of the Eosinophiles took place.

The examination was discontinued at the end of this period owing to the animal having contracted mange.

No. 3. (Fox cub. No. 2.)

This animal had at the time of the first examination small tumours developed. It showed a very marked diminution of polymorphonuclears and increase of lymphocytes.

Its blood was examined on 10 separate occasions but owing to an eosinophilia due to intestinal round worms, the records were in the later cases invalidated.

The other animals showed a similar change to a lesser degree. For full particulars c.f. appendix.

From the results obtained it would appear that the growth of the tumour is associated with an increase in the number of lymphocytes in the circulating blood.

THE/

THE LIFE HISTORY OF THE NODULE IN THE IMMUNE DOG.

We have already seen how in six of the animals in which spontaneous recovery had occurred, no tumour formed on subsequent inoculation. This result is in accordance with those obtained by Smith and Washbourne and Stricker. The former with four such animals, and the latter with twenty, failed in every case to produce a tumour. Bashford[4], however, says, "A small proportion of growths, after having attained a certain size diminish in volume and ultimately disappear; such recovery has not been followed by an immunity to subsequent inoculation, and animals with large tumours have been successfully reinoculated." With the latter part of the sentence everyone will agree, but as regards his statement about the absence of immunity, I am afraid no one will.

In order to study the changes which occurred after inoculation into immune animals, portions of the tumour were introduced and excised after one day, three days, seven days, and ten days. The only/

only weak link in the chain of evidence obtained was in the three days experiment. In this case the animal was killed and consequently the disappearance of the control nodules could not be here observed as in all the other cases.

EXAMINATION OF NODULES REMOVED AFTER ONE DAY.

The nodules were found to have an appearance very similar to that seen in the previous cases. The centre of the portion of tissue introduced showed evident degeneration. The periphery of it was invaded by leucocytes, almost all of which were polymorphonuclear. The surrounding tissue of the host showed at certain parts the signs of immediate union, and in it the blood vessels were much dilated. The connective tissue cells and the endothelial cells were swollen up, and what would appear to be evidence of the formation of fibroblasts was also seen. In this region there was also seen fibrin, polymorphonuclear leucocytes, a few lymphocytes and mononuclear leucocytes, the latter acting as cellular phagocytes.

EXAMINATION NODULE REMOVED AFTER THREE DAYS.

An appearance very similar to that seen at three days in the former cases was present. The necrotic centre and zone of leucocytic invasions were seen. At one part where the tissues of host and the nodule were intimately incorporated, a band of tissue existed. It had the structure of the tumour, and in it tumour cells were seen which appeared healthy and a few of which were undergoing mitosis. At its outer border this area gradually merged into the adjacent tissue of the host through a zone which contained fibrin, leucocytes, and cells identical to those of the tumour. The blood vessels showed distinct reaction, endothelial cells were seen budding off from their walls, and mitosis could be made out to be occurring in some of these. Formation of young fibroblasts was also very apparent. The great majority of these were forming elongated spindle-shaped cells, but occasional transition forms from these to tumour cells could be made out.

EXAMINATION/

EXAMINATION OF NODULE REMOVED AFTER SEVEN DAYS.

All the zones described previously were seen and on microscopical examination the following changes were found.

1. The necrotic centre is seen.
2. The zone of leucocytic invasion can also be made out.
3. To its outer side in the boundary zone a large number of cells are present which are swollen up and vacuolated. The nuclei of these are usually somewhat crenated; but are sometimes horse-shoe shaped. Inside the cell body of many of them phagocytosed tissue is present. These which might be mistaken for degenerating tumour cells are large monunuclear phagocytes.
4. The Growing border is represented by a zone of tumour tissue, which has the structure of normal tumour substance. The tumour cells are seen to be occasionally degenerating, and it is only after a prolonged search that evidence of mitosis is found. Throughout the/

the tumour formation area lymphocytes are present. Into the tumour formation area young blood vessels containing many lymphocytes are seen dipping, and developing, fibroblasts are also entering it.

5. The surrounding tissue of the host is made up of layer upon layer of fibroblasts forming fibrous tissue, and lymphocytes and Polyblasts are very abundant. The adjacent blood vessels show many of these, arranged as extravascular accumulations.

EXAMINATION OF NODULES REMOVED AFTER 10 DAYS.

1. The tumour cells and stroma are seen to have undergone complete necrosis.
2. The leucocytic invasion is not great, only an occasional polymorphonuclear leucocyte is seen Lymphocytes are sometimes present.
3. Boundary Zone is made up of some large mononuclear phagocytes and many polyblasts.
4. Tumour Formation Area. Here cells are present which have somewhat the structure and arrangement of the tumour. This area is, however, infiltrated with small lymphocyte-like cells and pierced by young capillaries and drawn out fibroblasts, which are going on to form fibrous/

fibrous tissue. Careful examination of it shows the preponderating cell to be of the lymphocyte type: all transitions from the small typical lymphocyte to Polyblasts being evident. Degenerating Tumour cells can occasionally be made out amidst these cells.

5. The surrounding tissue shows a laminated border made up of organising granulation tissue. The fibroblasts are proceeding to form fibrous tissue. The contrast between them and the degenerating tumour cell is well brought out. Throughout this laminated border Polyblasts are present in great abundance.

In another animal in which a nodule was excised after ten days an appearance (Plate vii fig. 2.) somewhat similar was present. In this case an interesting but unintentional complication arose in the presence of shreds of cotton fibre which were introduced with the nodule; these having been carried from the sterilised gauze swab, on which the excised tumour was cut up. Their presence caused many multinucleated giant cells in addition to Polyblasts to be seen in the disappear-

ing/

disappearing nodule. These appeared to have been formed from fused cells derived from lymphocytes.

CONCLUSIONS REGARDING THE DISAPPEARANCE OF THE NODULE IN THE IMMUNE DOG.

The process by which immunity is established is, from the appearances described, thus seen to be one which may aptly be compared to the complete life cycle of the tumour which develops in an animal which is not immunised; instead of occupying four months, it is in this case finished in less than a fortnight.

The nodule introduced lives.

The cells of it may divide.

It is almost at once flooded with cells of the lymphocyte type from which polyblasts develop, which, when abundant, are always associated with degenerating and disintegrating tumour cells. The surrounding tissue of the host proceeds at once to invade and encapsule the nodule. This is the only record which exists of the changes which occur in such animals.

COMPARISON OF THIS PROCESS WITH THE CHANGES WHICH OCCUR AFTER THE INTRODUCTION OF A FIBRO SARCOMA.

A rapidly growing fibrosarcoma was obtained from a greyhound, a limb of which had been amputated owing to the presence of this disease.

Portions of it were introduced into a fox terrier. All of these failed to develop tumours. One nodule was excised after ten days. It showed when examined:-

A central area of necrosis.

A zone of peripheral invasion by cells mainly of the lymphocyte type. Outside this a zone of laminated granulation tissue, in which fibroblasts forming fibrous tissue with some lymphocyte like cells and polyblasts were situated. The latter were, however, not so abundant as in the immune dog, inoculated with infective Sarcoma.

A nodule of pith excised after ten days showed a very similar appearance, (Plate xxxii fig.2)

DISCUSSION/

DISCUSSION of the GENERAL LIFE HISTORY of the TUMOURS formed after INOCULATION, and a COMPARISON of it with the PROCESS of REPAIR.

The Histogenesis of the Tumour has already been shown to be caused by a grafting of tumour cells, of the portion introduced, upon the tissues of the new host, associated with a concomitant formation of similar cells from the latter source. This change has been seen to occur only in those situations where direct contact is present. The tumour cells created in the tissue of the host are formed by anaplasia from the Fibroblasts present in the natural process or repair round any foreign body. When we compare the changes in the fixed tissue cells adjacent to the nodule introduced to that seen in response to certain other irritants, we see in both cases a rapid response on the part of the connective tissue, and endothelial cells, which varies in rapidity of appearance and degree according to the intensity of the virus. In the reaction of the fixed tissue cells to a definite infective process such as after a micro-organismal infection, there can be seen after twenty-four hours, according to Maximow (36), a swelling/

swelling of the protoplasm of the connective tissue cells, especially those in the neighbourhood of the blood vessels, an alteration in the contour of these wherein the thin flat processes become thick and sharp and their nuclei enlarge, and occasionally mitotic figures. In the course of the first few days Fibroblasts are formed in great numbers which according to his experience was not the case in an aseptic inflammation until three to four days had elapsed.

Anaplasia of these fibroblasts is of frequent occurrence after acute infection and has been noticed to be especially well seen in the dog during the stage of four to five days, and also what would appear to be the undoubted formation of fibroblasts from active proliferation of endothelial cells. This has also been seen by us during the histogenesis of the tumour and in animals which were immune, and would go to support the work of Baumgarten that blood vessel endothelium can produce connective tissue.

The differences which are noticed in the changes associated with the histogenesis of the tumour are:-

The formation of the Fibroblast would appear to take place earlier although, as we have already/

already mentioned Kiener and Duclert (27) describe their formation after a micro-organismal infection as early as six hours. The anaplasia occurs earlier and instead of being a transient reaction it persists, and by the active division of the cells thus formed a mass is helped to be created, having the histological structure of a tumour from the centre of which during its creation and growth the blood borne cells of the inflammatory exudate can be considered as being virtually entirely absent. The transient anaplasia of the Fibroblasts associated with an intense micro-organismal infection passes off, and is followed by organisation, during which there is interspersed throughout them, other cells of the inflammatory exudate, especially those mononucleated cells called Polyblasts which we consider to be virtually entirely derived from altered lymphocytes.

The role of the lymphocyte and its further development, the Polyblast in the process of repair, and histogenesis of the tumour would appear to be in many respects similar. In the acute inflammatory process mononuclear cells similar to them are present in the peritoneal inflammatory exudate, according to Beattie (26), in increased amount in eighteen to thirty hours/

hours. These cells are generally recognised to act as phagocytes to cellular debris and to a lesser extent to living micro-organisms. It is also a well recognised fact that in certain infections, such as with the tubercle bacillus, they are especially abundant and subserve almost entirely the function usually discharged by the polymorphonuclear leucocyte in acute infections. (5) Maximow, in his work on Inflammation, produced by the introduction of aseptic foreign bodies, found an early active emigration of lymphocytes from the blood vessels took place, and a cytological development of these leading to the formation of similar large mononucleated cells which arranged themselves around the foreign body, in contact with it, and throughout the fibroblasts formed round it later.

(37) Schwarz got a similar result and noticed how, during the course of the first two hours, after the injection of the foreign body into the tissues, there appeared a very plentiful and undoubted emigration of lymphocytes from the blood, and the development from these of large mononucleated cells.

(38) ZIEGLER K. in his work on the skin and subcutaneous tissue of human beings saw a similar emigration of lymphocytes from blood and lymph vessels/

vessels.

The function of these in acute inflammation is, in certain respects, obvious. Where tissue debris has to be removed, they can be seen with particles of it ingested inside their protoplasm. Where the infection is less intense, they appear to be largely responsible for limiting the spread of it, as for example in the production of giant cells and the giant cell system in a tuberculous lesion.

In the process of repair round an aseptic foreign body, they are present at an early period, but gradually diminish in numbers until only those remain which have become incorporated in the fixed tissue cells of the part, as sessile adventitia cells known as clasmatocytes. This is brought about by a gradual transformation of the cells characterised by an alteration in their size and an increased granularity of their cytoplasm due to the presence of a substance the nature of which is unknown, but which according to Ranvier (39), who first named them, is a secretion of the cell which may play a part in the nutrition of the tissues. These cells, in his opinion are uni-cellular glands like the leucocytes and their secretion is necessary for tissue metabolism.

JUSTI (40) has noticed especially in tubercu-

lous/

tuberculous infection, cells similar to those described as polyblasts, and he thinks that they are probably the carriers of a substance for the nutrition of the rapidly growing cells.

In the histogenesis, growth and gradual disappearance of the tumour, the lymphocyte and polyblast would appear to play a definitely important part.

They are from the first found in the nodule only in the areas where necrotic cells exist and would appear undoubtedly to be responsible for their removal. In these regions at a later stage, they are associated with mature fibroblasts, and definite granulation tissue. With the increased growth of the tumour and its invasion of this region, they disappear from it and definite fibrous tissue formation never occurs here.

From the earliest period it was always a noteworthy fact that in those areas where healthy tumour cells were found the mononucleated cell was always virtually absent.

In the tissues of the host they were present from the first, and at the extending border of the growth, they were always to be found.

The examination of the blood would appear

to/

to establish the fact that the disease is one which is characterised by an increase of the total number of mononucleated cells, especially of the lymphocyte type in the circulation. Similar cells are also seen, forming extravascular accumulations to blood vessels in the tissues beyond the border of the extending growth.

As the tumour gradually forms, these cells become evident in increased numbers inside the vessels entering it, and outside them throughout its substance, where they form lines of extravascular undifferentiated lymphocyte-like cells.

At this period they are especially evident at the outer border of the growth where the fibroblast is now seen to be proceeding to form fibrous tissue, and taking part in the creation of a capsule to the tumour.

The gradual absorption of the growth is one which is most evident at the outer margin where mononucleated cells abound, and their presence here is associated with a gradual degeneration of the tumour cells adjacent to them and the formation of new fibrous-tissue strands.

Throughout the tumour during its resorption the mononucleated cells become gradually more evi-

dent/

evident and more of them are of the polyblast type.

The disappearance of the growth is accompanied by a great increase in the number of these throughout it, and at this period the number present in the adjacent tissue may be so great as to form distinct lymphoid masses. Those, however, which are directly in relationship to the degenerating tumour cells are now almost all of the larger mononucleated type, and very many of them have their protoplasm rich in the material which stains so intensely and which is the noticeable feature of the so called Plasma cells. This material stains in a manner similar to that found in the clasmatocyte and was looked upon there as an intra-cellular secretion. Phagocytosis of the tumour cells by Polyblasts can very occasionally be made out; but this is very exceptional; the process would undoubtedly appear to be one where the tumour cell is degenerated by cytolysis prior to its removal by the phagocytes.

There is a certain amount of evidence in favour of the contention that the destruction of the tumour cells is due to extracellular digestion of them by the polyblasts. This never occurs in their absence. It is always associated with their presence/

presence and where they abound such of the tumour cells as are not obviously degenerating are never seen undergoing mitosis.

Finally successful inoculation has followed the introduction of a tiny nodule the size of a lentil which was all that remained of a tumour, but this, however, contained a minute portion of tumour tissue uninvaded by polyblasts, whereas on the other hand failure took place in another case where at a slightly later date, the nodule was completely invaded and the tumour cells destroyed. These experiments would appear to support the contention that the cause of cell destruction lies in the invading polyblast and not in the serum of the circulating blood bathing the tumour. The other explanation possible of such a gradually advancing process of cell death is, that the serum contains the cytolysin which is so scanty in amount that it is used up by the peripheral tumour cells and the polyblast can only advance against those which are devitalised. The death of the cell cannot be attributed to the loss of its own inherent vitality in view of subsequent immunity produced and the facility of inoculation which they show in the non-immunised dog.

The part which the mononucleated cell

plays/

plays in the prevention of tumour growth in the immune animal has already been shown.

The tumour grafts as before but is at once invaded by lymphocytes which show a cytological development into polyblasts. The growth is arrested. The cells of it are destroyed and disappear.

THE/

THE ETIOLOGY OF THE TUMOUR.

The process by which tumours arise in the natural course of the disease has not been worked out in detail. It is, of course, as we have already seen, well known that the infection is conveyed during the act of coition from another animal, with tumours upon its genital organs. This is followed after some weeks by the appearance of minute vascular nodules, which develop into tumours that persist much longer, and much less seldom disappear than is the case in those produced by artificial inoculation. The Causal Virus is, as already said, unknown.

Those who claim that the tumours formed after inoculation are created entirely from the portion introduced are logically compelled to acknowledge that a similar process must occur with natural infection, which would mean that not only was the sporadic tumour made up of cells which belonged to tissues of the previous generation, but that all the tumours at present in existence were made up of cells derived from some animal which had in the remote/

remote ages acquired this disease in a manner which is incapable of explanation, but one which had conferred on the cells formed, a power of unlimited growth and proliferation.

Although I am not prepared to deny that such is possible, and that the power of apparently unlimited propagation which the cancer cells of Jensen's Tumour possesses, is evidence in its favour. I would maintain that if this be the natural mode of infection, it stands alone as a unique mode of the propagation of disease, which is without analogy in the domains of pathology. When we consider the other possible explanations of the mode of origin of the tumours, we at once realise that the virus is one, which is so intimately bound up with the life of the cell, that it cannot be disassociated from it by any means known at present, and consequently it would appear that only by the passage of a virus from a living cell to a living cell can the infection be explained. This process must be much akin to that which takes place in the propagation of the disease by artificial inoculation, with this difference that, in the latter case the favourable surroundings allow many of the cells introduced to live and divide, and add to the tumour mass which they have/

have originated and help to form parts. This would explain how, in this case the tumours form much more frequently, grow much more rapidly, become so much larger and also possibly the much higher percentage of spontaneous recoveries.

If we accept the hypothesis that the process of natural and artificial infection correspond with this difference that, in the latter case the process is complicated by certain of the cells of the previous generation living. We may obtain a fair insight into the nature and mode of infection by studying the inception and growth of the tumour.

If such will be accepted, I would submit that the deductions which follow concerning the etiology of the disease from the facts which we have already established, are:-

(1) The tumour is one, which is formed by the reaction of the tissues to a virus which especially affects the fixed tissue cells, leads to the production of fibroblasts in excess, and these by anaplasia become converted into tumour cells, which continue to actively divide and exert a negatively chemiotactic influence on the mononucleated cells at the site of tumour formation.

(2)/

(2) The response on the part of the body of the host consists in the production of lymphoid cells in excess. The arrest of the growth is due to the creation by the host of a substance which is probably borne in the mononucleated cell, and which at first arrests the further formation of new tumour cells from the fibroblasts of adjacent tissue, and allows these to proceed to carry out their natural function of fibrous tissue formation, and later is responsible for the gradual absorption and disappearance of the tumour, by a destruction of its cells with a concomitant formation of fibrous tissue.

The mere absence of the mononucleated cell from the Zone of Tumour formation might explain the continued cell division and the formation of the tumour in a manner similar to that advanced by Ribbert in explanation of the creation of true neoplasms, the formation of which he explains by an isolation of certain of the cells of the body and a loss of restraint normally exerted upon them by their fellows.

The fibroblast in one case may, owing to the absence of the mononucleated cell have been removed from a restraining influence which is naturally present, regulating its development and limiting its growth.

The/

The Causal Factor may, on the other hand be an intracellular virus, which stimulates the cell to active division and repels the mononucleated cell, which only returns when it is the bearer or the follower of a substance which is capable of inhibiting the growth, and destroying the life of the tumour cell.

THE/

THE CHANGES PRODUCED IN THE KIDNEY BY INFECTIVE SARCOMA AND THE ACTION OF ITS TOXIN.

When describing the lesions which were present in the organs of the first animal inoculated, and which died with secondary foci of the disease in certain parts of its body, I have already mentioned how the manifestations of a definite pathological process were detected in its kidneys. The important relationship which this inflammatory change bore to the disease was further emphasised, when a similar change, in a more acute form, was detected in the kidneys of another dog which died seven weeks after the introduction of a filtrate obtained from the tumour; but so far, none of those who have investigated this disease have described any change produced by it in the kidneys. In order, therefore, to determine how far these could be attributed to the presence of the tumours and the introduction of a filtrate obtained from them, investigation was conducted as follows.

The kidneys of six dogs which had been killed outright/

outright when in apparent good health, were examined. These animals, which were used in the course of an investigation conducted in another place, and which, I was informed, were obtained from the same source of supply as mine were, showed no evidence of recent or former inflammatory change in their kidneys. Thereafter the kidneys of twenty-two animals were examined. Fifteen of these were dogs which had been inoculated with portions of the tumour.

Five were animals into which the filtrate obtained from the tumour had been introduced. One dog which had recovered from the sporadic tumour was examined, and finally the kidneys of one of the foxes, which had developed tumours, were also investigated.

In all of these animals, with two exceptions, definite evidence of an inflammatory change was present, the apparent age of which corresponded closely with the period, during which the virus had been exerting its action.

The changes which were found varied in degree, but in all of these, the appearances closely corresponded to, and were the manifestations of the same pathological process, with such differences as one would naturally expect to find from the severity of the/

the reaction produced and its duration.

We have thus been able, in this disease, to study by experimental investigation, the pathogenesis of subacute and chronic kidney inflammation, more especially as it affects the interstitial tissue. The opportunity thus afforded, is of especial value, as the experience of those who have worked at the Pathology of Bright's Disease has been, that although subacute and chronic Nephritis are commonly met with in the post mortem room, and are usually attributed to the action of a toxin carried to the organ, from its source of production in some other part of the body, the difficulty has, so far, been to obtain one which would reproduce in animals similar changes, with a degree of certainty that would enable the earlier stages especially, to be studied in a satisfactory manner and by this means to determine the exact evolution of the process. The experience of most investigators has been, that most of the Toxins which they have used have produced an acute inflammatory reaction, the incidence of which was mainly borne by the Glomeruli and secreting tubules, and either led to the death of the animals from acute nephritis, or passing off, left in some cases, no evidence of interstitial change on the other, such as

was/

was to be accounted for, by either the initial damage to the epithelium of the secreting tubules or by an interference to the blood supply of the part, produced by the occlusion of the lumen of the glomerular vessels. The value of the discovery is thus of wider significance than the mere demonstration, that Infective Sarcoma is a disease, which would appear to be responsible for the production of Interstitial Nephritis, if my contention is sustained that the production of this after inoculation or the introduction of the filtrate can be considered as occurring with fair regularity, in that we thus have, here, a valuable means of investigating a disease which, although common in mankind, is one in which, so far, no very satisfactory method exists of reproducing it in animals, for experimental investigation.

To establish the basis of my contention I will, before passing on to consider common characteristics of the lesions produced in the Kidney, first describe the morbid anatomy and histological structure of these organs in the two animals in which the disease was first reproduced by inoculation, and then detail the changes found in those of the animal into which the filtrate obtained from the tumours, formed/

formed in one of these animals, was injected; with the result that that dog succumbed in seven weeks to its action.

THE EXAMINATION OF THE KIDNEYS OF THE DOGS WITH THE TUMOUR OF THE SECOND GENERATION.

KIDNEYS FROM ANIMAL (EXPERIMENT NO. 2.)

This animal died forty-seven days after inoculation, with portions of the original sporadic tumour, and at the post mortem examination there was found in addition to the primary growths, secondary foci of the disease in the Liver, Intestine and Suprarenal Gland. The Kidneys showed no similar obvious change.

THE MORBID ANATOMY.

The Right was somewhat bulky. Its capsule stripped readily, exposing a surface which was smooth and of a pale somewhat mottled appearance. On section it was of the usual consistence, and its cut surface also appeared pale in colour. The cortex was/

was somewhat swollen, and the vascular marking of it was not distinctly visible. Opaque strands could be seen especially at the boundary zone, extending as faint lines into the cortex.

The Renal Pelvis showed no evidence of disease, and the larger vascular trunks did not appear thickened. No evidence of amyloid degeneration was present to the naked eye or revealed on testing with Iodine solution.

THE HISTOLOGICAL STRUCTURE.

The most noticeable feature on microscopical examination, is the large number of small cells with a more or less spherical contour and deeply staining spherical nuclei, throughout the interstitial tissue of the organ. These are especially seen at the boundary zone, as large extravascular accumulations and can also be made out, following the lines of the (Plate XXXIII, Fig.1) interlobular vessels throughout the cortex, and forming around them, here and there, distinct extravascular accumulations. These cells are also seen, especially between the adjacent tubules and glomeruli, in the labyrinth of the cortex. This appearance is not constant for the whole of the kidney, but is confined to definite zones which radiate through the cortex; but/

but only occasionally reach to the capsule, where they form similar accumulations of cells. It can also be seen, to a slight extent, in the medulla, at certain points; on the other hand, at the base of the pyramids, in the boundary zone, they are in such abundance outside the walls of the vessels, (Plate XXXVII, Fig.2) especially the branches of the Renal Artery, as sometimes to appear to be burying these channels in the midst of a mass of dark staining cells, and from these regions they are noticed extending out and surrounding the adjacent tubules and glomeruli, compressing them and appearing to be responsible for the localised fatty degeneration, noticed in some of the former. The lumen of these they do not invade. Where they were compressing the Glomeruli, Bowmans Capsule at its outer part is swollen up, and shows fatty degeneration occurring in certain minute fibrillary strands composing it. Occasionally the endothelium lining of that membrane shows proliferation of the cells forming it. The effect produced upon the portion of the Glomerulus inside the capsule is, in most cases, very slight.

The Blood Vessels showed no evidence of disease of their walls.

The Glomeruli. These in most cases, when examined/

examined, showed little alteration. Those which show the most definite change are situated in the zones where the small cells are abundant. The afferent and efferent arterioles show no evidence of hyaline degeneration of their walls. The glomerular capillaries also reveal no signs of this. There is no increase of the nuclei of the glomerular tuft. The cells of the glomerular epithelium in some are noticed to be undergoing fatty degeneration. The capsular space is in almost every case empty.

Bowmans Capsule is, in some cases, the site of distinct reaction. The endothelial cells lining it, are swollen up, their nuclei much more distinct, and it is evident that recent proliferation of these cells had taken place. The appearance closely corresponds to that seen in the blood vessels in the process of repair, at the stage of twenty-four hours to three days after the introduction of an irritant, such as we have had occasion to refer to in the earlier part of this work.

In the zones where the small cells are most abundant the reaction on the part of Bowmans Capsule is most evident, and here we can make out a periglomerular accumulation of small cells compressing it, but even here, the capsule itself, apart from the proliferation of its endothelial cells, may show

little change. In many cases, however, it is noticed to be swollen, to be distinctly fibrillated, minute spindle-shaped cells with delicate spindle shaped nuclei, being arranged around it, in a concentric manner, and in some cases as already mentioned what would appear to be fatty degeneration is noticed. (Plate XXXVII, Fig.3)

Tubules. Fatty degeneration has already been seen in certain of the tubules included in the areas of infection, close to the boundary zone. It is also seen at other parts; but is not of widespread distribution. Where it is present, it is in the epithelium lining the secreting tubules, especially in those of the ascending looped tubules of Henle. This degenerative change has a regional distribution, similar to that in the small cells.

Interstitial Tissue. At certain areas, small cells are present in such abundance, as to render difficult the detection of their individual structure, when it is a frozen section which is being examined. The majority of them are small cells of the lymphocyte type, with a deeply staining homogeneous nucleus, others have a nucleus with an opener network which does not stain so densely.

No polymorphonuclear leucocytes are found amidst them. With P.R.M.G. and E.M.B. the various cells/

cells, which go to form the extravascular accumulations and the units surrounding the tubules and glomeruli, can be differentiated. They are found to be mostly Polyblasts and Plasma Cells and some undifferentiated lymphocytes, cells like those described as tumour cells and a few characteristic endothelial cells. Mature spindle-shaped Fibroblasts cannot, however, be detected; although it would appear probable that those described as tumour cells are of connective tissue and probably, also endothelial origin.

The Left Kidney was found on examination to manifest changes of a similar nature.

From the foregoing facts, it would appear, that there was here present a recent, acute, interstitial nephritis, with an associated slight parenchymatous degeneration, mainly affecting the ascending limbs of the Looped Tubules of Henle.

EXAMINATION of the KIDNEY of the DOG

EXPERIMENT No. 1.

This animal it will be remembered, was the first inoculated. In it, the tumours developed and then/

then gradually diminished in size, and finally disappeared eighty-one days after the date of inoculation. Four months later, it was again inoculated, but no tumours formed. A year and a half from the first inoculation, portions of the stock of the second sporadic tumour were introduced into it with a similar result, and the animal was killed ten days later, as these were disappearing, that is, approximately a year and a half from the date of the original experiment.

THE MORBID ANATOMY.

At the post mortem examination, the kidneys were seen to be small, in both, numerous subcapsular cysts existed. The capsules were thickened, somewhat adherent, and when removed revealed an irregular surface, with a morocco leather appearance.

The organs were firm on section and the cortex was atrophied. The vascular markings of it were irregular in their distribution, and it had a general, diffuse, granular appearance throughout. The Pelvis was healthy. The larger vascular trunks were not noticeably thickened.

THE/

THE HISTOLOGICAL STRUCTURE.

On microscopical examination, the capsule is seen to be united to the kidney cortex, under the depressions on its surface, by bands of scar tissue, which pass down to the boundary zone. These have somewhat the appearance and general distribution that was noticed in the previous case. The zones, here, however, in contradistinction to what was found in the former, traverse the cortex to its surface more frequently, and are obviously much more fibrous, having included inside some of them degenerated tubules and obliterated, fibroid, glomeruli.

Blood Vessels. The larger vessels show some thickening of their outer coats, due to an increase of fibrous tissue.

Glomeruli. Those situated in the zones of healthy cortex, appear normal, whereas of such as are included in the zones of scar tissue, some are distinctly fibroid, others show changes present in them to a lesser degree. (Plate XXXVI, Fig.2)

In the latter, the afferent and efferent arterioles show no evidence of disease. In the glomerular tuft, there is no change present in the walls/

walls of the capillaries forming it, and its nuclei are not increased in amount. The Capsular Space is occupied by a granular albuminous exudate, in many cases, into which an occasional Red Blood Cell has escaped.

Bowmans Capsule is, in the majority, distinctly fibrillated by layers of concentrically arranged cells with delicate spindle-shaped nuclei.

The endothelial cells, lining the Capsule, are in some cases, swollen up and distinct. The periglomerular tissue consists, in many, of concentric layers of delicate fibrous tissue, with small deeply staining cells which have deeply staining nuclei between their fibres.

The Tubules show fatty degeneration in the secreting tubules, in relationship to the zones of scar tissue in many cases. In the tubules not included in these, however, fatty degeneration is occasionally seen, whereas, in some others, the swelling of the protoplasm with other appearances, indicate the presence of cloudy swelling. In the collecting tubules in connection with the zones of scar tissue, numerous dark staining, granular casts are situated.

Interstitial Tissue. Bands of fibrous tissue pass from the vessels at the bases of the pyramids/

pyramids to the capsule, which is tacked down by them. These follow the lines of the interlobular arteries and include within them, degenerating tubules and an occasional fibroid glomerulus. Some of these are more cellular, whereas others are more fibrous, and in them at some parts, extravascular accumulations of small cells, with a more or less spherical contour exist, these consist of Lymphocytes, Polyblasts and Plasma cells, with some Fibroblasts and endothelial cells between them. From these regions, they extend between the adjacent tubules, and around the glomeruli, where the two latter cells are much more scanty.

With H.I.A.PF. the fibrous tissue is especially well demonstrated, as it radiates through the cortex and forms periglomerular rings of fibrous tissue, some of which have proceeded further to distinct fibroid glomeruli. It is also seen surrounding the interlobular arteries with a fibrous periarteritis, an appearance very similar to that seen in the last case, but instead of consisting as it did there, of an aggregation of individual cellular units, it is now made up of organised tissues.

The result of the examination would, here appear, to demonstrate the presence of a chronic interstitial nephritis, with the addition of a more recent/

recent infection superadded.

EXAMINATION OF THE KIDNEYS OF THE DOG.

(EXPERIMENT NO. 4.)

This animal was a healthy wire-haired fox terrier, and into it the filtrate obtained from the tumours of the Second Generation which developed in the dog (Experiment No. 2.) was introduced. The process by which this Filtrate was obtained was as follows. The tumours were excised and three nodules the size of small hens' eggs, were introduced into a mortar which had previously been sterilised and sterilised normal saline solution added. They were then teased out with sterilised glass rods and further broken down with the pestle. The emulsion thus obtained, was then passed through a New Berkefeld Filter, which had previously been sterilised for half an hour at 30 lbs. pressure. After an hour had elapsed, 25 c.c. of straw coloured fluid was, by this means, obtained, and this was introduced directly into the subcutaneous tissue of the animal, the whole process being completed in less than two hours from the time at which the tumours were excised.

The/

The amount injected may be, approximately, said to amount to the fluid extract of two thirds of the tumour bulk used.

The effect upon the animal was slight at first. It was kept under especially close observation as the experiment was conducted to see whether or not the tumours formed, might not be due to the presence of an ultra-microscopical parasite, capable, like some others of passing through the pores of a Berkefeld Filter. As has been already stated, no tumours did form. The animal, however, steadily lost weight. Absolutely no local reaction at the site of hypodermic injection could be detected. It continued to lose weight more rapidly towards the end, and finally died, six weeks from the date of the original and only injection.

A complete post mortem examination was at once conducted. The appearance detected in the other organs of the body, consisted solely of the evidence of a doubtful reaction to a possible toxin and no cause of death could be made out until the kidneys came to be examined. They showed:-

Morbid Anatomy. Both Kidneys were of normal size and had under their capsule minute white points, which had somewhat the appearance of miliary tubercles. The/

The report of the examination is as follows:-

The capsule is not adherent and strips readily, exposing a surface which is smooth and studded with minute, almost microscopical points, with an appearance somewhat like miliary tubercle.

On section the cortex is seen to be of normal diameter. The vascular markings appear, if anything, somewhat irregular. The glomeruli are not distinct and no haemorrhages are present. At certain parts minute grey lines are seen, which are following the direction of the interlobular vessels and obscuring them, and can often be made out extending to the minute white specks underneath the capsule.

The Pelvis is healthy and the larger renal vessels are not apparently diseased.

The Histological Structure. The most noticeable feature is, the small cells present in the interstitial tissue throughout the organ. They are seen especially to radiate in zones through the substance of the kidney, following the lines of the blood vessels, and to commence around the vessels, in the boundary zone, and pass to the cortex. Some dip for a short distance, between the tubules in the medulla. The zonular distribution, although seen here, is not so characteristic as in the previous cases, there is

Plate XXXIII. fig. 2.

more/

more diffusion of the cells in this case. They form underneath the capsule of the kidney, at some parts, small distinct dark staining, wedge-shaped areas, made up of deeply staining, somewhat spherical, small cells, amidst which the structure of the kidney cortex can only be made out with difficulty, with its glomeruli and tubules compressed and degenerated, fibroid glomeruli being seldom detected and some appearing fairly healthy, but for the periglomerular, cellular accumulation.

The larger vessels at the Boundary Zone show no evidence of disease of their coats. The fibrous tissue adjacent to the outer coat, is loosened and very cellular, many small deeply staining, spherical-shaped cells being seen between the fibres. The interlobular vessels show perivascular accumulations of small cells which extend into the adjacent kidney substance. These cells surround tubules somewhat atrophied and destroyed, and some glomeruli, which are going on to a fibroid alteration.

GLOMERULI. - most but not all of these show changes.

The Afferent and Efferent Arterioles manifest neither evidence of hyaline degeneration of their walls, nor blocking of their lumen by cellular increase.

The/

The Glomerular Capillaries are filled with Red Blood cells & their endothelial lining shows, in an occasional case, a cell swollen up and loosened; but no pronounced increase of small cells can be made out.

Glomerular Epithelium shows no evident change.

Capsular Space is almost always empty.

Bowmans Capsule. The endothelium lining it, is usually unduly distinct, its cells being swollen up, and increased in numbers. The capsule itself is usually distinctly fibrillated, and is made up of small spindle-shaped cells concentrically arranged, and where this is evident has an associated periglomerular accumulation of small cells. In the zones where small celled increase is in especial abundance, the glomerular changes are most evident, and one may see, - glomeruli similar to those already described, - others with a pronounced small celled increase in the capillaries of the tuft, - the glomerular epithelium degenerating, - the capsular space occupied by a granular material, - Bowmans Capsule with the thickening already seen, but the proliferation of the endothelium lining it more evident, - and cells from without penetrating into the capsule. - Other glomeruli are here noticed in which the glomerular structure has been entirely destroyed, and consist of concentric/

concentric rings of fibrous tissue surrounded by the characteristic small cells, and containing cells with elongated and oval nuclei, and evident cell bodies, which appear to be mainly endothelial cells, derived from the capillaries of the Glomerular Tuft.

The Secreting Tubules show fatty degeneration, especially the ascending limbs of Henle. This is most evident in those supplied by the glomerular unit involved in the cicatrix, but is not entirely confined to these.

The Interstitial Tissue. The most noticeable feature here, is the small cells. It is virtually impossible to detect a polymorphonuclear leucocyte amidst these. Differential staining shows these cells to vary in nature. With E. & M.B. the great majority stain with a dense nucleus eccentrically placed, and a lighter cell body in the centre of which, a clear vacuole is situated in contact with the nucleus. Other cells stain more lightly, and are more granular, have a coarsely granular nucleus with a distinct nucleolus, and, in addition, a larger cell body of more irregular contour. Some other cells are seen, which are elongated and spindle-shaped. These cells are mainly Polyblasts and Plasma Cells, a few lymphocytes, and endothelial and connective tissue cells.

The/

The examination of the kidneys in this case, showed that they were the site of acute Interstitial Nephritis, with the customarily associated slight parenchymatous degeneration.

The complete post mortem examination revealed no other cause of death. The animal appeared in perfect health when first seen. Since then, the only possible toxin which would produce such changes, was that introduced. The anaesthetic for obvious reasons, could in no way have had anything to do with the production of the changes. With these facts before us, we are compelled to accept the cause of death here, as being due to the Toxin obtained by Filtration from Infective Sarcoma tumours, and which had induced the acute inflammatory changes, the stress of which had mainly fallen on the interstitial tissue of the kidney and produced Acute Interstitial Nephritis.

The essential nature of the lesions which were present in the three cases which have been described, has been seen to be the extreme degree of interstitial change associated with a very limited amount of involvement of the parenchymatous tissue; this similarity being especially noticeable in the first and third cases quoted. In these the lesions were seen to be virtually identical in nature. They have been shown to have occurred in animals, both of which succumbed to the effects of a virus to which they had been exposed for a relatively similar period, and in one case to have developed after the introduction of a portion of the tumour; in the other to have followed the introduction of a fluid obtained by filtration for the same.

The lesion of the kidney which they displayed in common, is identical with that described by Councilman[41] as Acute Interstitial Nephritis. This form of Nephritis was, according to him, first described by Wagner under the name of lymphomatous nephritis, and is a variety of kidney inflammation which is frequently the result of acute infective fevers being found more especially in association-ship with diphtheria and scarlet fever. It has been generally looked upon as arising from the operation of a soluble toxin carried to that organ in/

the blood stream, which leads at the same time to a degeneration of the parenchyma and proliferation of the interstitial tissue, whereof the essential lesions are in the interstitial tissue and are so prominent and accompanied by so little change in the parenchyma that they cannot be regarded as secondary to parenchymatous lesions. These may be followed by a complete restoration of the integrity of the kidney, or by a gradual organisation of the inflammatory tissue present in the stroma and the development of chronic interstitial nephritis. This would appear to have been the case in the second animal.

In the cases we have examined the reaction present in the parenchymatous tissue has been even less severe than is usually met in those, described by Councilman as Acute Interstitial Nephritis with this corresponding advantage, that the changes which occur in the interstitial tissue can be more readily examined.

In order to study the early inflammatory changes of the kidney by means of experimental investigation a number of different poisons have been used, such as Corrosive sublimate, Chromic acid, and other metallic salts. The action of various organisms/

organisms and their toxins has also been examined. Of recent years attention has been especially directed to the study of the interstitial changes, and in order to examine these, various soluble toxins have been experimented with. Thus Morse[42] succeeded in producing in the kidneys of three rabbits interstitial changes after the repeated injections over several months, of a toxin prepared from a virulent culture of Staphylococcus pyogenes aureus. The constancy with which these changes can be induced has been so irregular, as to render them almost useless for the purpose of investigating this special feature. Thus, for example, when Lyon[43] repeated Morse's experiments he was only able to obtain a slight evidence of interstitial change in the kidney of one rabbit out of six treated with the toxin. Lyon[43] also found in experimenting with the action of a number of other substances, such as - Diphtheria Toxin, Cantharidin, and Corrosine Sublimate the production of what we may call a pure interstitial nephritis occurred in none of them, and only in a few did any definite interstitial change take place - I have, therefore, as I have had occasion to mention already, considered it right to specially investigate the kidney changes in Infective Sarcoma, not so much/

much with the view of, at present, endeavouring to contribute to the knowledge which we possess of the pathogenesis of certain of the more insidious forms of Bright's Disease, as to establish the relative value of this, as a means for a future investigation, when it may be conducted, not as a side issue, but, one in which the animals may be killed without regard to any other factor.

The number of animals in which the kidneys were examined, was, as already said, twenty two.

The filtrate obtained from the tumour was injected into five of these with the following result.

One animal died after 36 days with acute interstitial nephritis.

Two were killed after 41 days and showed definite fatty degeneration, mainly on the ascending limbs of the looped tubules of Henle. In one, slight evidence of interstitial change was noticed.

One was killed after 51 days, and showed no obvious lesion in its kidneys.

One was killed after 119 days, and showed advanced chronic interstitial nephritis.

A single injection was given in each case.

As/

As no method of standardising the dose can be used, it is difficult to explain the amount injected. I may, however, be allowed to say, that in the last case the quantity was approximately similar to that used in the first case, and was obtained from the same stock of tumours. The other three animals all received about half that amount, from the second stock of tumour.

The animals which lived, showed virtually no alteration in their body weight, except in the case of the last, which lost weight steadily for three and a half weeks and then gradually regained it again, and was, when killed, looked upon as it appeared so healthy, as a negative result, until it was found to show the condition referred to.

Fifteen animals had been inoculated with the tumour of these:-

One died as described with acute interstitial nephritis.

Four were killed after the tumours had disappeared. Three of these had distinct chronic interstitial nephritis. One showed no evidence of interstitial nephritis, the opportunity of examining in this case for fatty degeneration was unfortunately lost.

One/

One was killed at eighteen hours after inoculation. It showed congestion of the vessels, and slight cloudy swelling of the epithelium of certain of the secreting tubules.

One was killed at 24 hours after inoculation. It showed distinct cloudy swelling, no fatty degeneration.

One was killed 3 days after inoculation. It showed distinct fatty degeneration mainly in the ascending limbs of Henle's tubules, and infiltration of the cortex and the medulla at certain parts with numerous lymphocyte like cells, and other evidence of early interstitial change. (Plate XXXVIII)

Seven were killed when the growth was mainly at its summit. Of these - three showed definite interstitial nephritis which could be described as subacute. Two showed fatty degeneration. One showed no interstitial change, and was not examined for fatty degeneration.

The Kidneys from the animal which had recovered from second sporadic tumour, also showed fatty degeneration, and early interstitial nephritis

The constancy with which these changes occurred justifies the assumption, in my mind that in/

in many cases especially after inoculation with portions of the tumour, infective sarcoma causes mild Interstitial Nephritis.

THE/

THE GENERAL OUTLINE OF THE CHANGES PRODUCED.

The toxin appears to be one which shows its effects earliest upon the epithelium of the secreting tubules. At first it causes cloudy swelling, and later fatty degeneration of the epithelium, more especially that lining the ascending looped tubules of Henle. This is definitely evident by three days. By this date, also, there appears evidence of reaction on the part of the interstitial tissue. This may go on to the production of definite acute interstitial nephritis or may rapidly pass away. The evidence of necrobiosis in the parenchyma persists, however, considerably longer, but it may also pass off and leave no trace of its presence.

A noticeable feature in the morbid anatomy of the kidneys was the frequency with which no positive evidence of inflammatory, or degenerative changes, could be detected to the naked eye.

The changes induced in the Glomerular Structure were very slight. The glomerular vessels, at/

at no time, could be seen undergoing hyaline degeneration, and never were thrombosed in the earlier cases. A very occasional haemorrhage could, however, be made out to have occurred from them.

The Capsular space, in many, contained an albuminous exudate with an occasional Red Blood Cell in it.

In Bowman's Capsule the most noticeable feature was the early evident reaction of the endothelial cells lining it. They readily became swollen and frequently multiplied. Apart from this, the reaction was often slight, but in the capsule itself, hyaline swelling could be detected in the earlier cases, and the membrane was noticed in some, swollen up and fibrillated, having a laminated appearance, with distinct nuclei between the fibres. Fatty change would appear to occur in some of these fibrils.

The Glomerular Epithelium showed no constant reaction.

In addition to no thrombosis, no obstructive vascular changes could be made out to account in any way for such reaction as was present.

THE TUBULAR STRUCTURE. The relative degree of vulnerability of the different tubules is found to correspond/

correspond to that seen in acute toxic poisoning. The secreting tubules show early evidence of necrobiotic change, no actual necrosis of them occurs; but a fatty metamorphosis, which is preceded by a granular swelling of the protoplasm of the cell, is early and very constantly seen, in the Ascending Looped tubules and Convoluted tubules. In certain cases this would appear to be due to direct pressure from without, but in others it can only be due to the direct action of a toxin. The Loops of Henle are most vulnerable, and there is evidence that obstruction of the blood supply to the glomerular unit, plays little or no part in producing this change.

In some of the acute cases, tube casts of an albuminous nature are noticed.

INTERSTITIAL TISSUE, shows in the early cases, evidence of acute Interstitial Nephritis characterised by the accumulation of lymphocyte-like cells, especially in the boundary zone around the interlobular* vessels, and extending for a varying distance into the cortex. These cells are in the earlier stages almost entirely Polyblasts and Plasma cells; later, however, they have interspersed throughout them larger cells of endothelial and connective tissue origin/

origin. They surround many of the Glomeruli and are interspersed through the tubules.

The formation of fibrous tissue takes place, following the lines of the interlobular vessels towards the surface. The creation of this is brought about by the organisation of the inflammatory exudate, of which the small cells formed part, in the customary manner. It compresses the adjacent tubules and glomeruli. The characteristic feature is, however, well noticed in the manner in which for a long time the process is entirely periglomerular, and only in its later stages does invasion occur, and fibroid glomeruli become produced, which may now be associated with tubular degeneration from an avascular cause; this, however, is not at all evident.

These are the main features of the changes as they are found in the Kidney. I do not propose to discuss them in reference to the bearing they have upon the work that has been done on this subject. A few points may, however, be alluded to.

The site of the primary interstitial lesion in this disease, as pointed out by Greenfield, (44) is in contradistinction to that in the Atrophic Kidney/

Kidney, found in the deeper part of the cortex, adjacent to the boundary zone. He, also, was the first to emphasise in 1879, the further attention which the study of this disease then required.[44] This investigation has also borne out another point referred to - the early reaction of the endothelium lining of Bowman's Capsule, and the similarity of its behaviour to that of the endothelium adjacent to an inflammatory focus in the process of repair.

The lymphoid nodes which have been so constantly described in this disease, and, more especially by Councilman,[41] in its acuter form of Scarlatinal Nephritis, are here also distinctive. The evidence would strongly bear out that, as that observer and others maintain, they consist to a large extent, of Plasma Cells, under which category is included, by most observers, cells which Maximow would differentiate into Polyblasts. These would appear to have emigrated from the blood stream and undergone a further metamorphosis. I have not, however, seen evidence of these locally increasing in numbers by cell division. There has not been seen in this investigation, any evidence of these cells acting, to any appreciable extent, as phagocytes/

phagocytes, their function here would, however, appear to be very similar to that which they fulfil in the former situations in which we found them.

THE/

THE RELATIONSHIP OF INFECTIVE SARCOMA TO CANCER.

As one would naturally expect to find from what has been seen of the nature of this disease, certain diversity of opinion exists, in regard to the relationship it possesses to a true malignant neoplasm, such as sarcoma, as it occurs in man.

We have already seen how it possesses a histological structure which, in my opinion is identical with a true alveolar sarcoma, and how this opinion is in conformity with that of virtually all those who have, more especially in the last few years investigated this disease, with this reservation, that some would designate it a round-celled sarcoma and others a lymphosarcoma.

Beebe and Ewing, in speaking of it describe it as an Infective Lymphosarcoma, but come to the conclusion that the general histological characters suggest to them, more the diagnosis of alveolar sarcoma or endothelioma, although they, in common with other observers, have failed to determine the particular tissue cell giving rise to the tumour. The main source of origin of the tumour cells, formed in the/

the tissues of the host after artificial inoculation, we have seen to be from connective tissue cells, although some undoubtedly arise from endothelial cells, and thus, their latter conclusion has some evidence in support of it. My objections, however, to the use of the term "endothelioma" to describe the tumour, from its histological appearance are, that this is not to any great extent the main source of origin of its cells, and the term "endothelioma" has, to my mind become of late the Cave of Adullam, for the terminological designation of so many new growths.

Those of us, who have worked at this disease are fortunate in one respect, in that Stricker[12] has been able to obtain specimens of the tumours, studied by Wehr, Geissler, Smith and Washbourne, Sanfelice and Bashford, and on submitting them with his own to a large number of German Pathologists, found that they all were of the opinion that the lesions were identical, and of the nature of a round-celled sarcoma.

This state of affairs would help to emphasise a point, that is sometimes overlooked by some clinicians, that the diagnosis of a neoplasm is not always/

always possible, in some cases from the histological examination of it alone, and would also be evidence, in favour of the importance of avoiding the divorce of pathology from clinical work.

Against it being a true malignant neoplasm the workers of the Imperial Cancer Research Laboratory would advance the following arguments.[4]

1. Its Invariable Infective History. This, certainly, is a point worthy of consideration; but in connection with it, although most pathologists will readily concede, that the vast majority of those lesions, classified under the generic term of Cancer or Malignant Neoplasms, do not infect, we have here one, in which it is especially easy to trace the tumours formed to a definite source of contagion, from the nature of the act during which that occurs. This point is well illustrated by the comparison of the venereal and non-venereal warts, which are found growing from various epithelial surfaces of the human body.[45] Cathcart has definitely proved that venereal warts, which are accepted by most pathologists as a variety of tumour, are contagious and independent of local irritation/

irritation, although often associated with the presence of Gonorrhea; whereas on the other hand, it has been extremely difficult to prove that non-venereal cutaneous warts are also, in certain cases at least, contagious. The case described by Payne[46], in which he accidentally inoculated himself from a boy, who had a copious eruption of these, proves conclusively, that this can occur. Other illustrations might be cited to elaborate this point. Before, however, I leave it, I may be allowed to quote the words used by Ohlmacher[47] in speaking of the suspected origin of true lymphoma. "We cannot be too critical regarding this type of neoplasm, for if we are to eliminate those tumours in which an infective cause is suspected, and retain as true tumours only those in which an embryonic or non-infective, post-natal, causal influence can be proved, then it will appear in goodly company."

2. <u>The Transference of the Surrounding Connective tissue cells</u> into tumour cells, even at the surface of fully developed tumours:-

That this occurs in certain cases, is, in my opinion/

opinion correct; but the limited extent to which it is present, may well be realised when such competent observers as Stricker, working under the direction of such a grand master of pathological research as Ehrlich, and Beebe and Ewing, will not concede that it occurs. In answer to this criticism, I would maintain that he, who would claim to be able by microscopical examination of the histological structure of a fully developed sarcoma alone, to be so certain that infection of the surrounding connective tissue cells never occurs, as to base upon this observation the tenet that it was an invariable feature of all malignant neoplasms, is claiming for his powers of observation, a degree of accuracy that few will concede him.

3. The Process Which Occurs In Artificial Transmission is identical with those by which tumour masses are formed, as a result of inoculation with the tubercle or Glandus Bacillus.
This has been discussed fully already.
Points of resemblance do exist, and the deep underlying process is, in many respects, similar.
The analogy is, however, an unfortunate one, and it/

it remains still to be proven, in what respects it differs from that which would occur in the creation of a true sarcoma.

4. The Disease Never Occurs Naturally In Animals Before sexual maturity and is much less frequent in old age.

One would naturally expect this from the source of infection - contagion during copulation.

5. The last point raised by them, is the most important.

The Process Is Definitely Infective In Marked Contrast to the changes which lead to the establishment of new tumours, when a true malignant new growth is successfully transplanted.

We have already seen how, in my opinion, the origin of the new tumour after inoculation is not entirely infective; but, granted, what is probable, that this is the sole explanation of the origin of the sporadic tumours, one has certain criticisms to offer, as it raises a point of great importance in connection with the nature of true malignant neoplasms. It is referred to by Beebe and Ewing, when they say, that/

that all arguments in favour of this being a neoplasm, prove fatally defective, if, on transplantation, the tumour cells grow by infection of other cells. This is, in their opinion, the crucial test of an infective granuloma, as against a neoplasm. Now, on what evidence is the contention based, that all malignant neoplasms grow from a single primary focus, have the power of apparent unlimited growth, and always graft when successfully inoculated, and what are the justifications for Fisch's remark quoted on page 52 of this thesis, that it was finally settled that neoplasms destroyed the surrounding tissues, but never changed the cells of it to tumour tissue?

They are, in my opinion, two:-

1. Histological Investigation of new growths obtained from the operating theatre, and post mortem room, and by such it would appear that for epithelial malignant new growths, the statement is a correct one. I am not prepared to say, that in the case of sarcomata it is possible to be certain of this fact.

2./

2. Experimental Investigation in the course of which, thousands of growths have been inoculated with a negative result, with virtually only one exception - the mouse Adeno Carcinoma of Jensen. Despite the fact of its grafting when all others failed, I refuse to attach that importance to its characteristics that those would make, who endeavour to make it the Mecca, to which all devout pilgrims to the shrine of Cancer Research must journey for the true faith. Especially would I refuse to grant, that it is necessary for all sarcomata, that they must conform to this standard in every respect, before they can be accepted as true malignant neoplasms. When I find, that the only detailed descriptions of cases of successful inoculation of sarcomata I am aware of, are (setting the present disease entirely aside, just now, as being under discussion) the cases of von Eiselberg,[48] Loeb[49] and Velich;[50] the last of whom successfully inoculated a periosteal sarcoma of the rat. Whether it grafted or infected is not mentioned, it is, however, stated that healthy rats kept in the same cage developed tumours. Loeb did a large number/

number of experiments with a sarcoma of the white rat. He carried it through about forty generations. He also worked with other cases of sporadic sarcomata in the same animal, and obtained a similar facility in them, for successful inoculation. The difficulties he encountered, of determining the true nature of the process by which tumours were created, were very much akin to those encountered by me in the dog. A mingling of cells derived from two sources occurred, and tumours were created by a grafting of cells of the portion introduced, along with a process of associated infection. In many other respects his tumours were similar to Infected Sarcoma, as it occurs in the dog, and with it the vexed question also arose, as to whether it was a true sarcoma or infective granuloma. In his opinion it was a true sarcoma. Under these circumstances, I consider we are justified in withholding our opinion, as to whether true sarcomata do or do not infect, and if the power which the cancer cell is claimed to possess of apparent limitless propagation, which is the bed rock of the cytological school of/

of cancer research, is disregarded, the comparison between the behaviour of Jensen's Adeno Carcinoma as will have been already made out, and that of Infective Sarcoma is most striking - in Jensen's tumour a history of likely spontaneous infection, in the sarcoma a constant one.

In both a ready facility of propagation.

In both spontaneous recovery occurs.

In both this is followed by immunity.

If/

If we look at some of the features we have noticed in the tumours we have examined, some further evidence as to the nature of the disease may be obtained. In my opinion the strongest evidence so far brought forward in favour of it being an infective granuloma, is the establishment of the fact that in Infective Sarcomata a toxin is created which leads to the production of interstitial nephritis.

No proof is forthcoming, that in true sarcomata any such virus is created causing similar changes, although these naturally have been noticed to occur in some cases. This is a subject which, I would suggest, is worthy of investigation, as it is quite within the range of possibility that slight manifestations of renal inflammation might be detected, with a regularity which would warrant the assumption, that they were caused by the presence of the neoplasm, and not mere accidental coincidences. The cachexia which accompanies the development of true neoplasms, is undoubtedly due in most cases to a contemporaneous toxic absorption from an associated ulceration or interference with alimentation, there are, however, cases in which it would appear to be due to the absorption of a poison produced by the new growth; but this has never been isolated or definitely/

definitely proven to exist.

The presence of lymphocytes and their derivatives throughout the tumour, which was noticed at certain stages of its growth, and the accumulation of these cells forming extra vascular accumulations beyond its extending border, occurs in true malignant neoplasms. This is found apart from any associated ulceration. The accompanying illustration (Plate XXXII. Fig.2.) shows this taking place in an Alveolar Sarcoma removed by operation from a human being. It also illustrates the similarity of its histological structure, to that which occurs in the dog. No satisfactory explanation of the presence of these cells in human sarcoma is so far forthcoming, and I am unable to find any record of a progressive lymphocytosis being a feature of such cases.

The Spontaneous Recovery which was found so frequently with tumours following subcutaneous inoculation is in accordance with that seen with Jensen's Adeno Carcinoma. The ratio of these has varied materially in the experience of different workers. We have seen how, in the animals inoculated in the course of this investigation, it would probably have been high if they all had been allowed to live.

Stricker/

Stricker had 18 recoveries out of sixty cases. The number of animals in which this occurs after natural infection, is very much lower. It does, however, very occasionally take place.

Spontaneous Recovery after the development of a true malignant neoplasm in man, has been recorded by Orth,[51] and others, more especially with Sarcomata, but it is difficult in these, to exclude all possibility of fallacy.

The Immunity which exists after the disappearance of the growths, is in conformity with that found with the Adeno Carcinoma of the Mouse. The existence of this with true malignant neoplasms is, of course, unable to be demonstrated: but it is in accordance with what one would expect to be present, in the tissues of an organism, which has been able to overcome a similar previous infection.

The production of secondary foci of the disease by metastatic infection by way of the blood stream, has been noticed to happen, in one of the cases. The secondary foci, however, did not show all the characteristics one would expect to see, with the development of them from a single primary focus.

A/

A consideration of the characteristics of this disease, and a comparison of them, with those of true malignant neoplasms will show that it is one, which in certain important features differs from the majority of malignant new growths. In no one prominent trait can it, however, be said to differ from all of those morbid processes, which are at present included under the vague generic term of Cancer, Neoplasm, or New Growth. The differences are mainly in features, which are assumed; but have not been proven to be absent in all true neoplasms. Thus, the ease with which its origin can be traced to a definite process of contagion, is a point in which it differs from the vast majority of the new growths, met with in man. The fact that it is one, where combined with a local lesion a toxin is created and that we have been able to find definite inflammatory changes produced through the body, with other evidences of a general reaction, inclines one to the belief, that we have here a disease, which approximates in its nature to certain of the infective granulomata such as syphilis. Its local lesion, however, resembles true sarcomata in histological structure, and clinical characteristics. I would prefer, therefore, to describe it by the term by which it has been known, Infective Sarcoma, a venereal/

venereal disease, and to consider it a tumour belonging to the borderland between the infective granulomata, and the true neoplasms, one which, probably, in the near future will be added to the group of processes caused by a reaction of the tissues to a ~~living~~ virus, but one in which that virus is intra- and not extra-cellular, and the leading characteristic of which, is the creation by this means of a structure resembling a true neoplastic new growth.

CONCLUSIONS/

C O N C L U S I O N S.

The conclusions concerning the nature of Infective Sarcoma which have been arrived at, as a result of this investigation are:-

1. That Infective Sarcoma is a general disease characterised by the production of a local lesion, possessing the histological structure of an alveolar sarcoma.

2. That it is caused by an intracellular virus, conveyed by contagion from a living cell to a living cell.

3. That the tumours formed after natural infection, probably consist of cells entirely derived from the tissues of the host.

4. That the nature of the virus cannot be detected, and

   (a) cannot be revealed by any method of staining.

(b)/

(b) does not pass through a Berkefeld Filter.

(c) is not an ultramicroscopical micro-organism.

(d) cannot be isolated apart from the tumour.

(e) is not a Spirochete.

5. That the disease can be reproduced readily by subcutaneous inoculation into animals of the same species.

6. That by the same method, it can be reproduced in the fox.

7. That the tumours following subcutaneous inoculation are created from the cells introduced, live, and by a process of direct contagion, lead to the formation of additional tumour cells, from the connective tissue cells of the host.

8. That it grows by multiplication of the cells thus formed, combined with a limited formation of additional tumour cells, from the tissues of the/

the host.

9. That this latter ceases, with the development of the power of increased resistence on the part of the host, and a capsule is thus formed around the tumour.

10. That the tumours disappear by gradual cytolysis associated with the presence of, and probably caused by, lymphocytes and polyblasts.

11. That the growth of the tumour is accompanied by a gradual increase in the number of mononucleated cells in the circulation.

12. That immunity follows spontaneous recovery.

13. That this is brought about by a similar process, occupying a much shorter duration of time - a cytolysis of the tumour cells accompanied by, and probably arising from, the presence of lymphocytes and polyblasts.

14. The growth of the tumour is associated with the development/

development of a toxin, which can be isolated from it by filtration, and produces interstitial nephritis.

15. That the results of this investigation, emphasise the present unsatisfactory classifaction of neoplasms, which include a heterogeneous collection of pathological processes, and indicates the probability that, in the near future, a differentiation will be possible, of processes that are really neoplastic, from those, that are merely reactive attempts by the host against an invading virus.

The experimental part of this research was conducted in the Laboratory of the Royal College of Physicians, to the President and Fellows of which College I desire to express my indebtedness for the opportunity thus afforded. The Histological portion was carried out in the Laboratory of the Royal College of Surgeons. To the President and Fellows of that College, I owe a similar debt of gratitude.

I/

I have also to thank Dr Graham, for the assistance he gave me, in the daily examination of the blood, and Mr Richard Muir of the Pathological Department of the University for the illustrations he has done for me, with his customary skill and accuracy.

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