STUDIES IN THE BIOLOGY OF CANCER

A THESIS

SUBMITTED TO THE

UNIVERSITY OF EDINBURGH

FOR THE DEGREE

OF

DOCTOR OF SCIENCE

I. The biological characters of spontaneous tumours of the mouse, with special reference to rate of growth.

II. The influence of carcinogenic compounds and related substances on the rate of growth of spontaneous tumours of the mouse.

III. The influence of carcinogenic substances on sarcomata induced by the same and other compounds.

IV. On secondary colony development in bacteria and an analogy with tumour production in higher forms.

F O R E W O R D

The work embodied in this thesis was carried out partly in the Department of Bacteriology of the University of Edinburgh and partly in the Research Institute of the Royal Cancer Hospital (Free), and with the aid of grants from the British Empire Cancer Campaign. I wish to acknowledge my indebtedness to Professor J.W. Cook, F.R.S. for many of the compounds employed (Sections II and III), to Professor E.L. Kennaway, F.R.S., for a specimen of styryl 430 (obtained from Professor C.H. Browning F.R.S.), and to Dr E. Boyland for another sample of the same compound. Thanks must also be expressed to Mr H. Burrows, F.R.C.S., Dr P.A. Gorer, Dr R.D. Mackenzie and Dr L.D. Parsons for the gift of mice bearing spontaneous tumours, and to Mr D.L. Stevens of the Galton Laboratory for much help in connexion with the statistical examination of data. Figs. 25 and 26 were obtained through the kindness of Professor J. Woodburn Morison.

15th April, 1938.

THE BIOLOGICAL CHARACTERS OF SPONTANEOUS TUMOURS OF THE MOUSE, WITH SPECIAL REFERENCE TO RATE OF GROWTH.

The biological characters of spontaneous tumours of the mouse, with special reference to rate of growth.

The biological characters of spontaneous tumours of the mouse, with special reference to rate of growth.

I. Introduction.

In the course of the succeeding study --- the influence of carcinogenic substances on spontaneous tumours in the mouse --- material accumulated which illustrates the behaviour of these neoplasms under normal conditions. Apart from the general interest of the data they are of considerable practical importance for control purposes in these and other experiments, and are treated here with special reference to rate of growth.

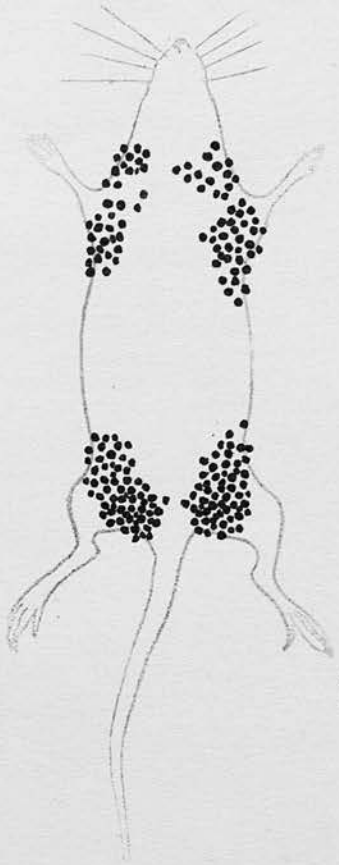
II. Incidence, type and anatomical distribution of spontaneous tumours.

336 tumours occurred in 269 mice, the majority of these animals being obtained from stock sources and some from the laboratory colony of the Little dilute brown pure line (Little dba; see Murray 1934). Histological examination of tissues obtained at autopsy or biopsy was carried out in every case. With one exception --- a large liver-celled growth in a mouse with breast cancer --- all tumours in

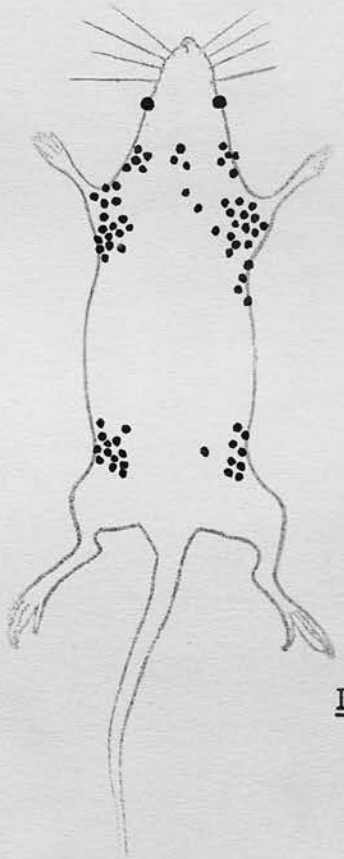
females proved to be derived from the epithelium of the mammary gland, and Fig. 1 shows the anatomical distribution of some two hundred such carcinomata. Four male animals provided three examples of spindle-celled sarcoma arising subcutaneously, (for a study of primary spontaneous sarcoma in mice see Slye, Holmes and Wells 1917), and one case of an osteo-:genic sarcoma.

III. Frequency of multiple primary carcinomata.

An important and well recognised feature of mammary cancer in mice is the not infrequent occurrence of multiple primary neoplasms. These are often apparently quite distinct in such features as histogenesis and characteristic growth-rate, and other evidence for their independence was put forward by Fischer (1919) on anatomical grounds. It is of considerable theoretical interest to determine the statistical nature of the distribution of such tumours, and some attention may be given to this subject before proceeding further. In the present material the incidence of single and multiple primary mammary tumours was as follows:



Ventral aspect



Dorsal aspect

Fig. 1. Anatomical distribution of spontaneous mammary carcinomata.

TABLE I

Number of mice bearing 1 tumour	221
" " " " 2 tumours	34
" " " " 3 "	11
" " " " 4 "	2
" " " " 5 "	0
" " " " 6 "	1
	269

Inspection of these data may suggest a chance distribution, and they are strikingly similar to those of Haaland's study (1911), in which the numbers of mice with one, two, three and four tumours were 239, 41, 6 and 2 respectively. In the present case however Mr D.L. Stevens kindly examined the fit between the Poisson distribution and these frequencies and concluded....."The observed frequencies differ from the theoretical expectations by an excess of number of mice with one or (3 or more) tumours, and a deficit of those with two tumours. A χ^2-test shows the discrepancy between observation and expectation to be significant, and hence we may not assume that the tumours are distributed at random." (The expected numbers of mice with one, two and three or more tumours were 211.6, 49.1 and 8.4 respectively). It here appears that any verdict

must be a non-committal one, although Wells (1924) suggested that the co-existence of primary mammary tumours in mice of high cancer susceptibility may be more frequent than can be accounted for by chance. Bugher (1934), by computation from the human cancer mortality data in the United States from 1920 to 1928 inclusive, determined the expected occurrence of coincidental multiple primary malignant neoplasms. The actual incidence exceeded that expected from chance alone, although the form of the frequency curves and the magnitude of incidence agreed with those expected. In this case Bugher concluded..... "The apparent contradiction between theory and experience is understandable if the risk of acquiring cancer is not spread uniformly over the entire population but over some large portion of it: cancer morbidity and mortality rates based upon this reduced population would give a frequency of coincidental cancer multiplicity in agreement with that found to occur in fact. This is equivalent to saying that there is an inherent susceptibility to cancer not possessed by all people, a conclusion abundantly corroborated by many other lines of evidence."

IV. Measurement of rate of growth.

Bashford (1911, pp. 198-9; see also Bashford, Murray and Bowen 1906, and Russ and Chambers 1913) was perhaps the first to recognise clearly that transplantable mouse tumours have characteristic growth rates, and as a suitable measure he employed the quantity "1000 x the reciprocal of the number of days required by the tumour to produce one gram of tissue upon the inoculation of a standard dose." Mayneord (1932) found that the Jensen rat sarcoma increases in linear dimensions linearly with time, and Schrek (1935) showed that the growth of the Walker and Flexner-Jobling tumours can be represented graphically by a straight line, the mean diameter being taken as the cube root of the product of the three dimensions of the tumour. (For a discussion of the mathematical analysis of tumour growth see Ottensooser 1930). Schrek also found (1936a) that the growth rates of rat tumours in vivo and in vitro are of the same order of magnitude as the growth rate of rat embryonic tissue, which may also be represented linearly ($\sqrt[3]{\text{weight}}$; see also Bashford 1911 p. 199 and also Sugiura and Benedict (1921) who found close agreement between the rate of growth of the rat foetus and of the Flexner-Jobling rat carcinoma). Schrek further concluded (1936b) that the average growth rate is a

physiological constant of any given tumour and is as characteristic as histological structure. Another study of the mensuration of transplantable animal tumours was made by Okada (1930).

Few comparable data are however available for the rate of growth of spontaneous tumours: yet the information they provide must be more valuable than that derived from transplantable tumours alone, in the sense that variations may safely be regarded as due to causes other than genetic differences between the tissues of tumour and host.

In the present investigation the animals were housed separately in metal cages, and provided with a diet of brown bread, oats, millet, canary seed and drinking water, with additions of cod liver oil and marmite on occasion. Tumours were inspected at frequent and often at daily intervals, and the length of two suitable diameters determined by the use of calipers. At the same time a record was kept of the condition of the animal and tumour, with any other relevant facts.

It soon became apparent that a high proportion of these spontaneous mammary carcinomata behaved similarly to the transplantable tumours studied by Mayneord and by Schrek, since the sum of tumour length and breadth proceeded linearly with time.

This fact, together with the large number of observations available and the relative accuracy of the method, permitted a reasonably good estimate of growth-rate to be arrived at without the calculation of regression lines. It was shown by Mayneord that the law of growth for the Jensen sarcoma can be explained by the circumstance that cell-division in that tumour is largely confined to the periphery. From histological examination of the tumours in the present work it may be said that this factor is of rather less importance here, and that the same result is produced partly at least by other means. As might be expected, however, a number of tumours increased their length exponentially, but as these are relatively few in number they are omitted from the following account. Fig. 2 indicates the rate of growth of 190 tumours in 183 mice, and Fig. 3 shows the frequency of occurrence of various rates.

V. The inter-relation of various characters including rate of growth.

It is thus common to find these carcinomata maintain a steady rate of increase over considerable periods of observation. The relationship between this property and other tumour characters may now be examined.

1. Growth-rate and the formation of metastases.

Of 226 tumour-bearing mice examined at autopsy, 92 (40.5 per cent.) presented gross secondary deposits in the lungs. This figure may be compared with the occurrence of pulmonary metastasis in 39.6 per cent. of 68 tumour-bearing mice (Murray 1908); 38.0 per cent. in 273 mice (Haaland 1911); and 39.1 per cent. in 314 mice (Marsh 1929). Ashburn (1937) observed gross lung deposits in 37.0 per cent. of 480 tumour-bearing mice, and the proportion increased to 45.2 per cent. when microscopic findings were included. In the present series adequate data were obtained in 169 cases, and the following figures indicate the tumour growth-rate (in degrees on an arbitrary scale) in animals with and without pulmonary metastases at autopsy.

T A B L E II

Growth-rate of tumours in mice.....

A. With metastases at autopsy.

2.0	3.5	5.0	5.5	6.0	6.0
6.5	7.0	7.5	7.5	8.0	9.0
9.0	10.0	10.0	10.0	10.0	10.0
11.5	12.0	12.0	12.0	12.5	12.5
12.5	13.0	13.5	14.0	14.0	14.0
15.0	15.0	16.0	16.0	16.5	17.0
17.0	19.0	20.0	21.0	22.0	22.0
22.5	22.5	23.5	23.5	23.5	24.5
25.0	26.0	26.0	26.0	26.0	27.0
27.0	27.5	28.5	29.0	29.0	29.5
30.0	30.0	32.5	33.0	33.0	34.0
34.0	35.0	37.0	38.0	38.0	39.0

Mean 19.20

TABLE II continued.

Growth-rate of tumours in mice.....

B. Without metastases.

0.5	3.0	4.0	4.0	4.5	5.0
5.0	5.5	7.0	7.0	7.0	7.0
7.5	8.0	8.0	8.5	8.5	8.5
9.0	9.0	9.0	9.0	10.0	10.0
10.0	10.0	10.0	11.0	11.0	11.5
12.0	12.0	12.5	12.5	13.0	13.0
13.5	13.5	13.5	15.0	15.0	15.5
16.0	16.0	16.0	16.5	17.0	17.0
17.0	17.0	17.0	17.5	18.0	18.0
18.5	19.0	19.0	19.5	20.0	20.0
20.0	20.0	21.0	21.0	21.0	21.0
21.5	22.0	22.0	23.0	23.0	24.0
24.5	25.0	25.0	25.5	26.0	26.0
26.5	26.5	27.0	27.0	28.0	28.0
29.0	30.0	30.0	32.0	32.0	32.0
32.5	33.0	34.5	36.5	38.0	39.0
47.5					

Mean 17.82

Statistical examination of the data in Table II gives a value for P of 0.4-0.3: hence there is no correlation in this series between rate of growth and production of metastases. Of 45 mice with tumours of rates 10 and less, 17 (37.7 per cent.) presented metastases at death, while the correspond-:ing proportion in the case of 22 mice with tumours of rates 30 and over was 50 per cent. In this case by the contingency table method P c. 0.30. Metastases were present in 2 of 10 mice with tumours of rates 5 and less and in 5 of 9 with tumours of rates 35 and over (P 0.20-0.10).

Although these data are insufficient to attain statistical significance, it must be mentioned that Ashburn (1937) found 41 (49.4 per cent.) cases of metastasis in 83 mice with growth rates above a median rate established for 185 mice, and 28 (30.4 per cent.) in 92 mice with tumours less than this rate (P 0.02-0.01). On the other hand it is improbable that rate of growth per se has any fundamental relation to the production of metastases, and it is well recognised that induced sarcomata in the mouse and rat produce metastases only rarely, although these tumours not infrequently possess extremely high growth-rates. Again, similarly in-:duced sarcomata in the fowl are most often associated with voluminous and widespread deposits in the internal organs.

2. Metastasis production and the sizes attained by tumours.

Table III shows the sizes attained by tumours in the mice presenting gross pulmonary metastases at death and in those not so affected. The difference between the means is significant (P c. 0.02). That increasing size of tumour is associated with increase in metastasis formation is also indicated by Table IV ($P < 0.01$).

Table V gives the sizes attained by tumours of growth rates 5 and less and of growth rates 35 and over. The difference between the means is highly significant ($P < 0.01$). Thus, although in any given case other factors may kill the animal and so prevent a rapidly growing tumour from reaching the size it would otherwise attain, examination of a sufficient number shows the expected relation to hold.

T A B L E III

Maximum size (sum of two diameters in cm.) attained by tumours in animals.....

A. With metastases at autopsy.

1.75	2.00	2.10	2.10	2.25	2.30
2.50	2.70	2.70	2.90	2.90	2.90
2.95	3.10	3.15	3.20	3.30	3.30
3.40	3.50	3.55	3.70	3.75	3.90
4.00	4.00	4.05	4.05	4.10	4.20
4.20	4.25	4.35	4.35	4.35	4.35
4.40	4.40	4.45	4.50	4.50	4.50
4.55	4.70	4.75	4.80	4.80	4.85
4.85	4.95	4.95	5.00	5.00	5.10
5.10	5.10	5.15	5.20	5.30	5.40
5.40	5.45	6.15	6.20	6.20	6.25
6.35	6.40	6.85	6.85	7.10	

Mean 4.30

T A B L E III continued.

Maximum size (sum of two diameters in cm.) attained by tumours in animals.....

B. Without metastases.

1.50	1.60	1.75	2.05	2.10	2.15
2.20	2.25	2.25	2.30	2.45	2.55
2.55	2.60	2.65	2.70	2.70	2.75
2.75	2.80	2.95	3.00	3.00	3.00
3.05	3.05	3.05	3.10	3.10	3.10
3.20	3.25	3.30	3.30	3.30	3.35
3.40	3.40	3.50	3.50	3.50	3.55
3.55	3.60	3.60	3.60	3.60	3.65
3.65	3.65	3.70	3.70	3.75	3.75
3.75	3.80	3.85	3.85	3.90	3.90
3.90	3.95	4.00	4.00	4.00	4.05
4.05	4.10	4.10	4.10	4.15	4.15
4.15	4.20	4.25	4.30	4.30	4.30
4.30	4.30	4.35	4.40	4.40	4.45
4.50	4.50	4.55	4.65	4.70	4.70
4.75	4.75	4.80	4.90	4.95	5.10
5.10	5.45	5.50	5.50	5.55	6.35
6.45	6.50	6.55	6.90	6.95	7.60

Mean 3.85

T A B L E IV

(after Ashburn 1937)

Relation of size of primary tumour to incidence of lung metastases.

Size of largest tumour $(\sqrt{l \times b})$ mm.	No. of mice	Metastases	
		Number	Percent
10 or less	47	3	6.4
10.1-15	80	26	32.5
15.1-20	97	40	41.2
20.1-25	109	54	49.5
25.1-30	67	43	64.2
30.1-35	58	40	69.0
35.1 and over	22	11	50.0

T A B L E V

Maximum size (sum of two diameters in cm.) attained by tumours.....

A. Of growth-rates 5 and less.

1.40	1.60	2.10	2.50
2.80	3.10	3.25	3.40
3.50	4.30	4.55	

Mean 2.95

B. Of growth-rates 35 and over.

3.50	4.55	4.75	4.90
5.10	5.20	6.20	6.25
6.85			

Mean 5.25

3. Duration of primary tumour and the incidence of metastasis.

However, Ashburn (1937) believed that tumour size in itself had only a slight influence on metastasis formation, and a general study of all the results available suggests that the incidence of metastasis may be influenced less by rate of growth and size attained than by the time of duration of the tumour. Ashburn found that while in mice having had their tumours less than 50 days, metastasis occurred in 36.6 per cent. of 41 with tumours above the mean growth-rate for 185 mice, and in 18 per cent. of 50 mice with tumours below the mean (P 0.05-0.02), in animals having had their tumours for 50 days or more, metastasis occurred in 61.9 per cent. of 42 with tumours above the mean rate and in 45.2 per cent. of 42 with tumours below the mean (P 0.2-0.1). Table VI makes clear the relation between approximate (calculated) duration of primary tumour and incidence of lung metastasis in Ashburn's series ($P < 0.01$).

For the present series Table VII gives the interval between date of receipt of 173 mice bearing single tumours and their death, the animals being divided into two groups according to whether they presented pulmonary deposits at autopsy (69 cases) or did not (104 cases). Although the time during

T A B L E VI

(after Ashburn 1937)

Relation of approximate (calculated) duration of primary tumour to incidence of lung metastases.

Duration of oldest tumour, in days	Total no. of mice	Metastases	
		Number	Percentage
10	2	0	0
11-20	29	6	20.7
21-30	69	20	29.0
31-40	68	26	38.2
41-50	81	28	34.6
51-60	78	40	51.3
61-70	56	34	60.7
71-80	37	22	59.5
81-90	30	19	63.3
91-100	13	9	69.2
100	17	13	76.5

T A B L E VII

Time of observation (in days) of single tumours.....

A. In mice presenting metastases at autopsy.

2	4	5	8	9	9
9	11	12	13	14	15
16	18	23	23	23	23
24	25	25	26	27	28
30	30	31	31	33	33
34	35	35	35	36	40
42	43	46	47	48	48
49	50	50	51	53	56
57	57	59	61	61	63
65	75	76	76	77	78
82	91	94	98	113	128
134	143				

Mean 45.65

T A B L E VII continued

Time of observation (in days) of single tumours....

B. In mice without metastases.

1	1	1	2	4	6
7	9	9	10	10	10
10	10	10	10	10	11
11	12	12	12	13	14
15	15	15	16	16	16
17	17	18	19	19	19
20	20	20	21	21	21
21	21	21	22	23	23
24	25	25	25	27	27
27	28	29	31	31	31
32	33	35	36	39	39
40	40	41	41	41	43
44	45	47	52	53	53
53	53	58	60	61	62
62	63	65	65	67	69
71	75	76	78	78	80
81	81	88	88	90	91
91	99	106			

Mean 35.91

which individual tumours were available for study must bear a highly variable relation to the total period of duration of the tumour, it seems probable from experience that accidental factors (determin-:ing at what stage a tumour was submitted) must cancel out for these numbers to a sufficient extent to justify comparison of the groups. The mean period of observation was 45.65 days for mice with metastases and 35.91 days for those without metastases; this difference is significant (P 0.05-0.02). It therefore seems probable that time of duration of the primary tumour is an important single factor in determining the occurrence of metastasis. This suggestion is confirmed by the following observation recently made by Mr Burrows (personal communication) and the writer independently. While the Walker rat carcinoma 256 possesses an exceedingly high rate of growth and may attain a weight of c. 70 g. in three weeks, metastasis is not frequent in animals dying under 40 days from the date of implantation. On the other hand, rats which continue to live and bear their tumours for longer periods (and especially those which are treated by x-radiation and are so enabled to survive for periods up to say 80 days) show a high proportion of pulmonary metastasis.

4. Multiplicity of tumours and the production of metastases.

Pulmonary metastases were detected at death in 69 of 185 animals bearing single tumours, in 14 of 32 with two tumours and in 6 of 10 with three tumours. Suggestive as these data may appear, a χ^2-test of association between production of metastases and number of tumours per mouse indicates that the proportions cannot be accepted as significant (P c. 0.3). It is however of interest to compare the figures with those provided by Ashburn (1937) and derived from 480 tumour-bearing mice of the strain 3 described by Marsh (1929) (see Table VIII). In this case there is a significant association (P 0.02) between number of tumours and production of metastases.

T A B L E VIII

Relation of multiple primary tumours to incidence of lung metastasis.

(after Ashburn 1937)

	Total number of mice	Metastases	
		Number	Percent
Mice with 1 tumour	185	69	37.3
" " 2 tumours	153	71	46.4
" " 3 "	88	43	48.9
" " 4 "	34	20	58.8
" " 5-8 "	20	14	70.0

5. Rate of growth and location of tumour.

136 tumours were divided into two categories according to their cephalic or caudal situation, and Table IX gives the individual growth rates in these groups. The difference between the means is significant ($P < 0.01$), although no further facts can be offered to explain the higher average growth-rate of tumours caudal in position. It is possible that the observation is a chance one, but apart from this it is probably unlikely that the difference is due to factors inherent in the malignant cells of different sites. An anatomical basis may be indicated, and it is of interest that Voegtlin (1937), in discussing differences in growth-rate of multiple primary mammary carcinomata in mice, suggested that these differences might be due to variations in vascularization.

T A B L E IX

Growth-rate of tumours.....

A. Cephalic in position.

3.5	4.0	5.0	5.0	5.0	5.5
6.0	6.5	7.0	7.0	7.5	7.5
8.0	8.0	8.5	8.5	9.0	9.0
9.0	9.0	10.0	10.0	10.0	10.0
10.0	10.0	11.0	11.5	12.0	12.5
12.5	13.5	14.0	14.0	15.0	15.0
16.0	16.0	16.0	17.0	20.0	20.0
21.0	21.0	21.0	22.0	22.0	22.5
24.5	26.0	26.0	27.0	27.0	28.0
30.0	30.0	32.0	33.0	34.0	34.5
37.0	39.0				

Mean 16.00

T A B L E IX continued.

Growth-rate of tumours.....

B. Caudal in position.

0.5	4.0	4.5	5.5	6.0	7.0
7.0	7.0	8.0	10.0	11.0	11.5
12.0	12.0	12.5	12.5	13.0	13.0
14.0	15.0	15.0	15.5	16.0	16.5
16.5	17.0	17.0	17.0	17.5	18.0
18.0	19.0	19.0	19.0	19.5	20.0
20.0	20.0	21.0	21.5	22.0	22.0
22.5	23.0	23.0	23.5	23.5	24.0
24.5	25.0	26.0	26.0	26.5	26.5
27.0	27.5	28.0	28.5	29.0	29.0
29.5	30.0	30.0	32.0	32.0	32.5
33.0	33.0	35.0	36.5	38.0	38.0
39.0					

Mean 20.63

6. Location of tumour and production of metastases.

Of 76 tumours cephalic in position, 32 produced gross pulmonary deposits, while of 95 tumours caudal in position 35 metastasised in the same way. A test of this association shows that there is no significance in the difference between these proportions (P lies between 0.7 and 0.5). In a study of mammary tumours in ninety animals of the Strong albino A stock, Williams, Silcox and Halpert (1935) were unable to detect any correlation between site of tumour and production of metastases. Similarly Ashburn (1937) observed metastases in 35 (40.2 per cent.) of 87 mice with single tumours of the cephalic half of the body and in 35 (35.7 per cent.) of 98 mice with tumours in the caudal half.

7. Growth-rate of tumour and histological type.

Some two hundred tumours, the growth-rates of which had been determined, were studied histologically and classified as far as possible. It is commonly agreed that the histological grouping of mammary cancer in mice is far from satisfactory, on account of the frequency with which individual tumours exhibit widely varied types of cell and arrangement. According to Ashburn (1937), ".....the most undifferentiated areas can be traced by easy gradations from the hyperplastic breast lobule. Small, well-differentiated acini, larger and atypical acini or tubules, epithelial cysts of variable size, with or without true papillary proliferation, and solid cellular areas inter-grading or well circumscribed are not infrequently seen in a single tumour. It is infrequent to find a tumour entirely glandular or solidly cellular....." Over the whole series no very clear impression could be obtained of any association between rate of growth and structural type. However, detailed examination of eleven tumours of growth-rates 5 and less, and of nine tumours with rates of 35 and over, did appear to justify such a correlation. The majority of tumours in the former group showed a comparatively uniform adenomatous or acinar structure, differentiated in type (adenoma simplex of Apolant 1906; epithélioma

glandulaire simple of Nicod 1936). On the other hand, five of the most rapidly-growing tumours were obviously of lower differentiation, being characterised by solid and sometimes even symplastic areas consisting of masses or cords of malignant cells (epithelioma trabeculaire, Nicod). The most dedifferentiated cells appeared also to be significantly larger in size, and to stain more deeply, than the cells from slowly growing tumours. It must be stressed however that although the inherent or potential growth-rate of a tumour may be related to cell type, it is not infrequently affected by adverse environmental factors. Hence the observed growth-rate may be low on account of bacterial infection, a feature which was much more prominent in tumours of the first group than in those of the second.

Figs. 4-13 illustrate some of the types of histological structure encountered.

Ashburn found an additional association between histological structure and the incidence of metastases, since deposits were found in 51.6 per cent. of 31, 41.2 per cent. of 102, 24.2 per cent. of 33, and 10 per cent. of 10 mice with cystadenocarcinoma, adenocarcinoma, papillary cystadenocarcinoma and adenoma malignum respectively ($P = 0.05$). "In 126 mice with tumours

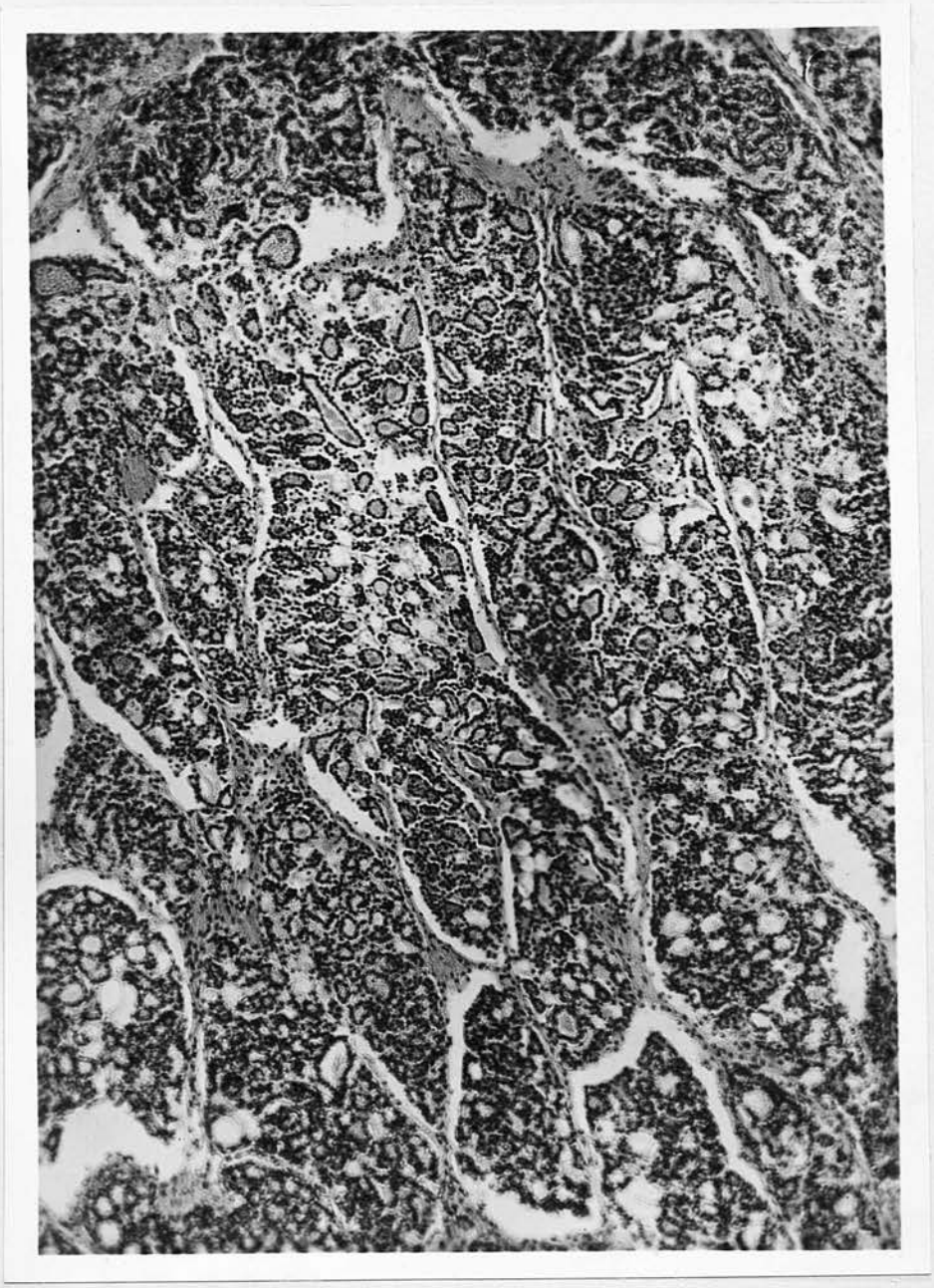


Fig. 4. x 120.

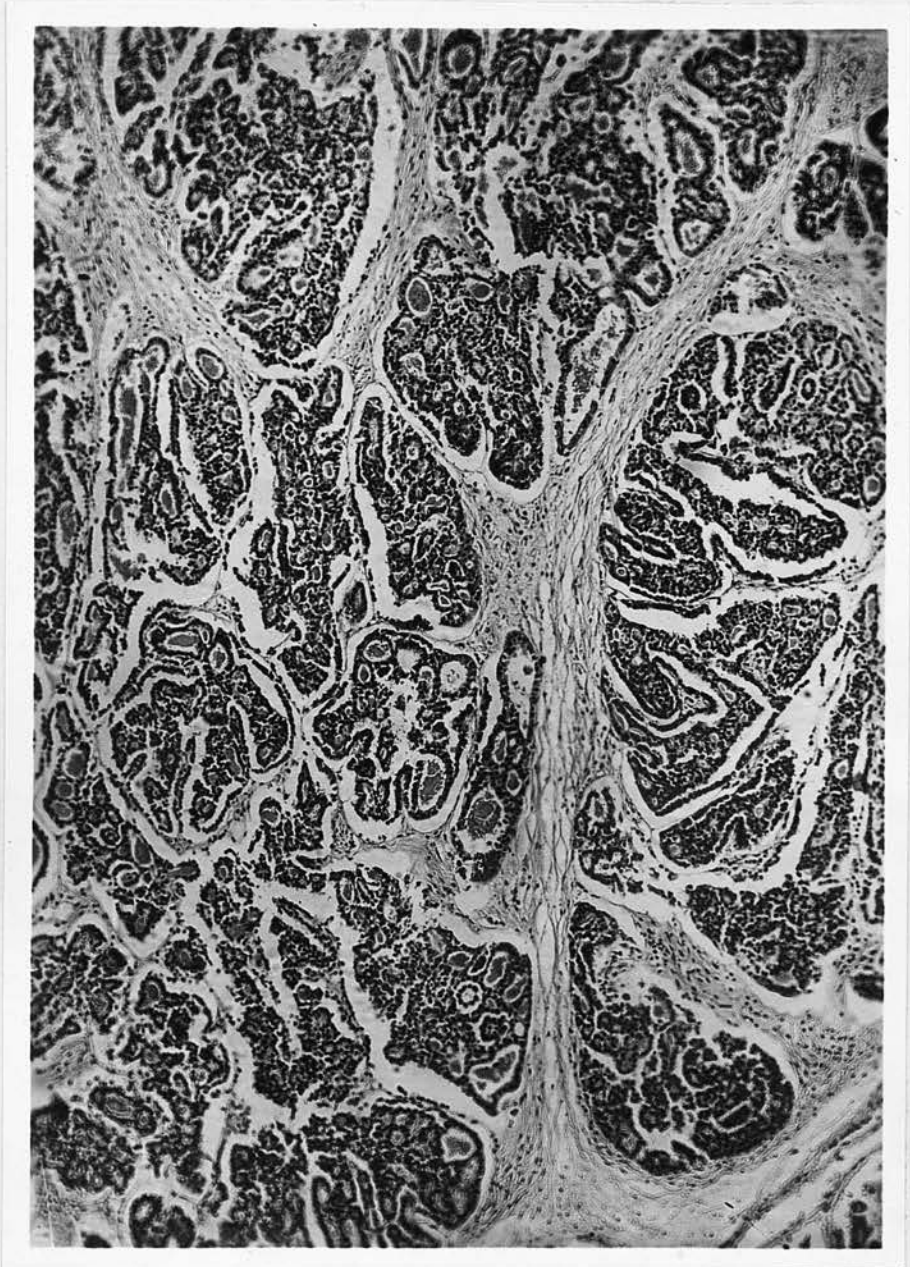


Fig. 5. x 120.

Fig. 6. x 120.

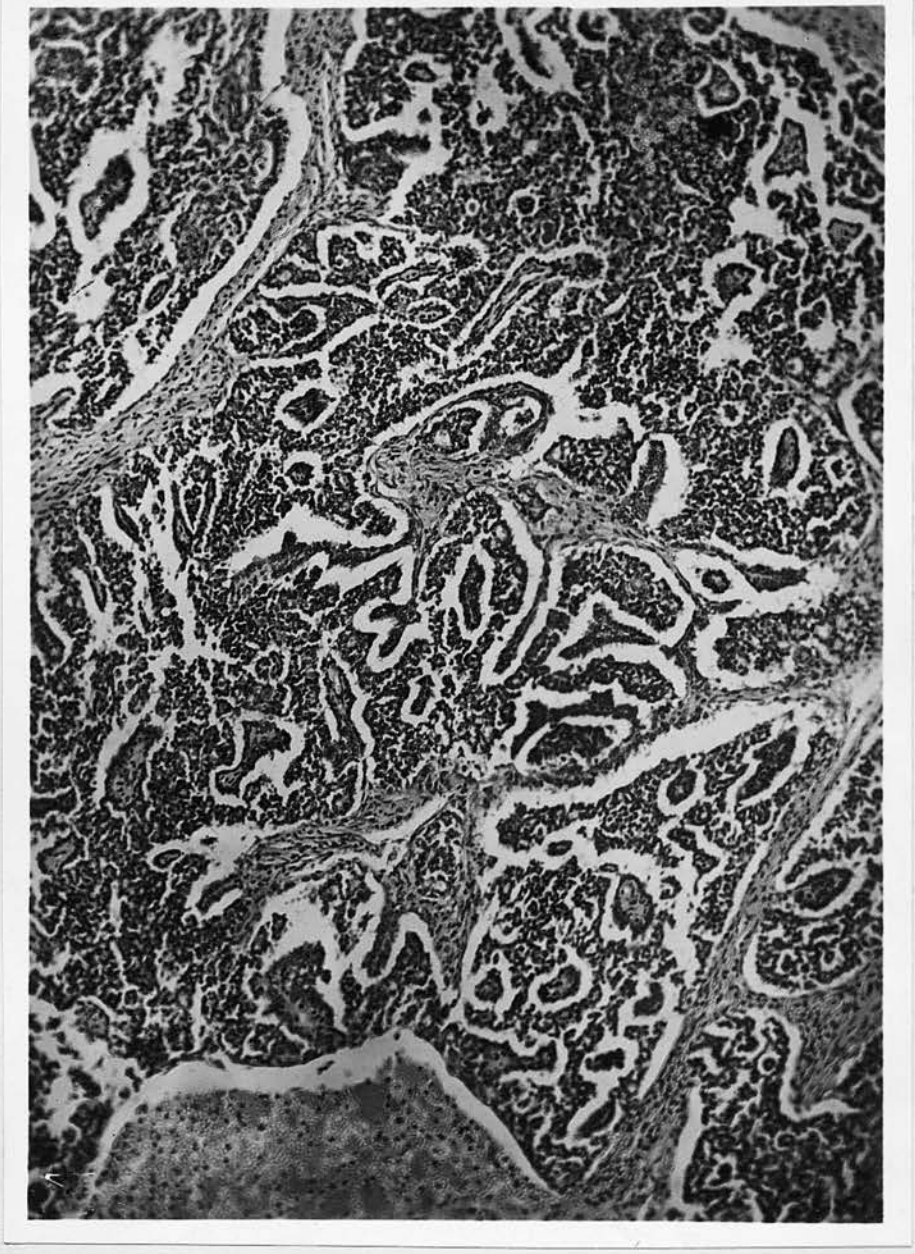


Fig. 7. x 120.

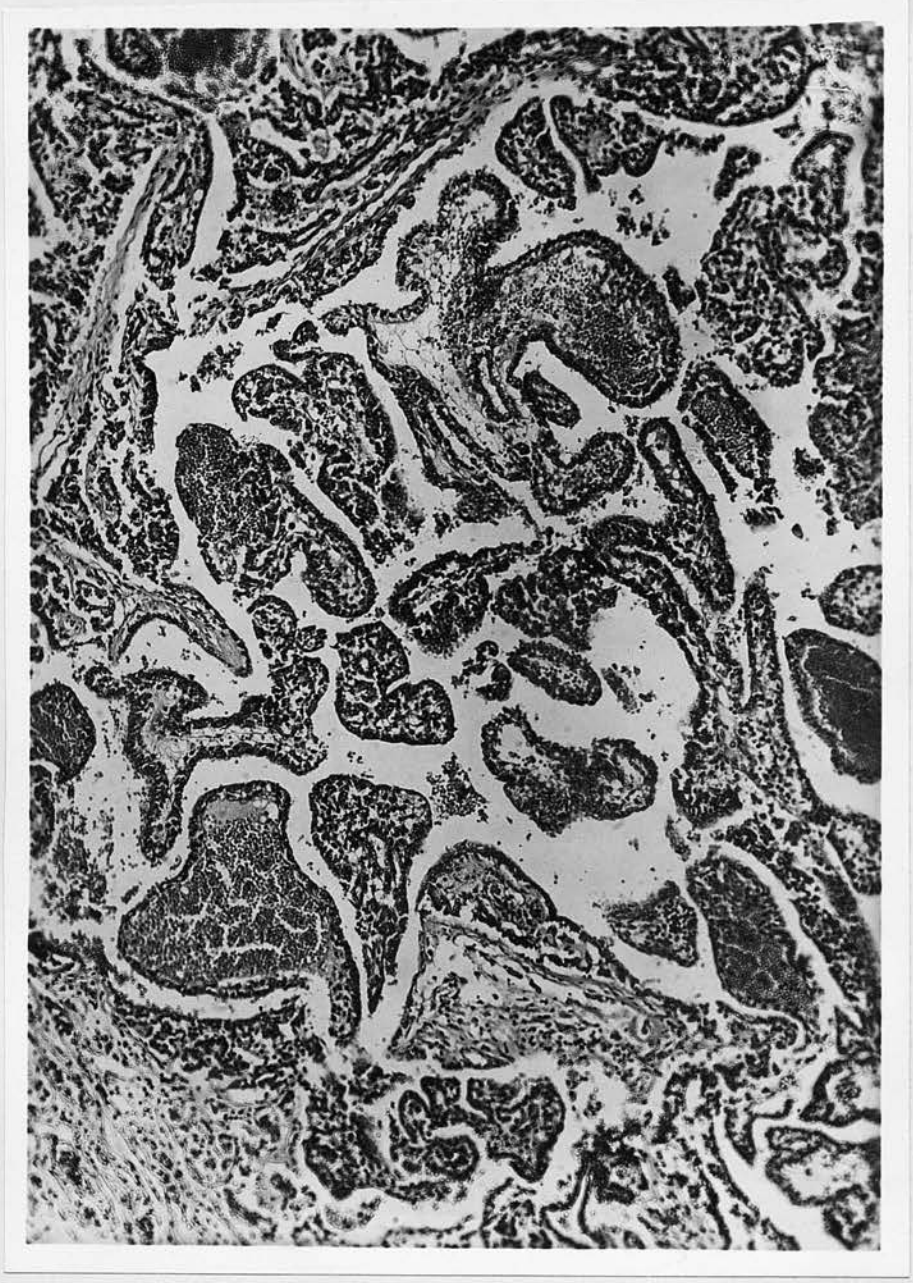


Fig. 8. x 120.

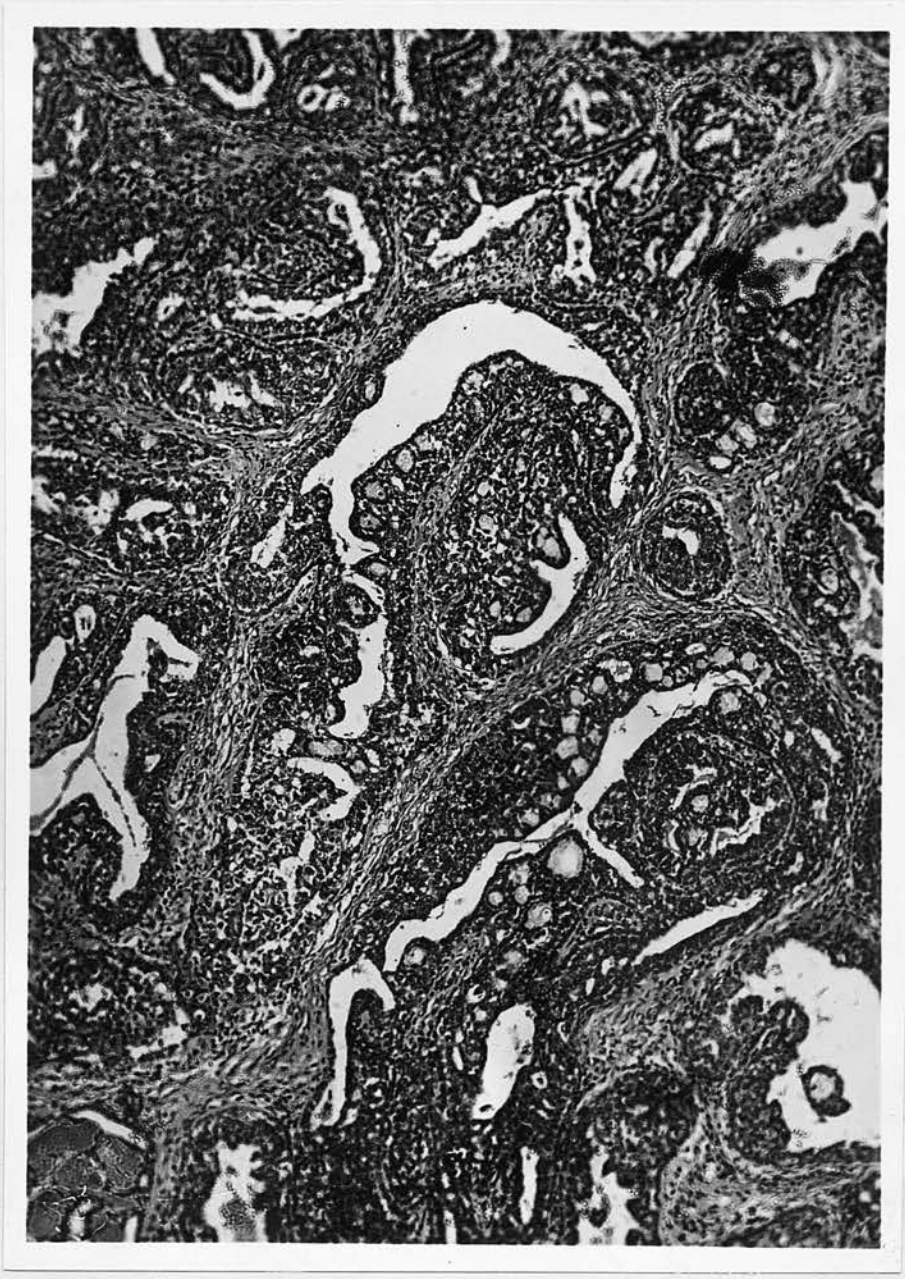


Fig. 9. x 120.

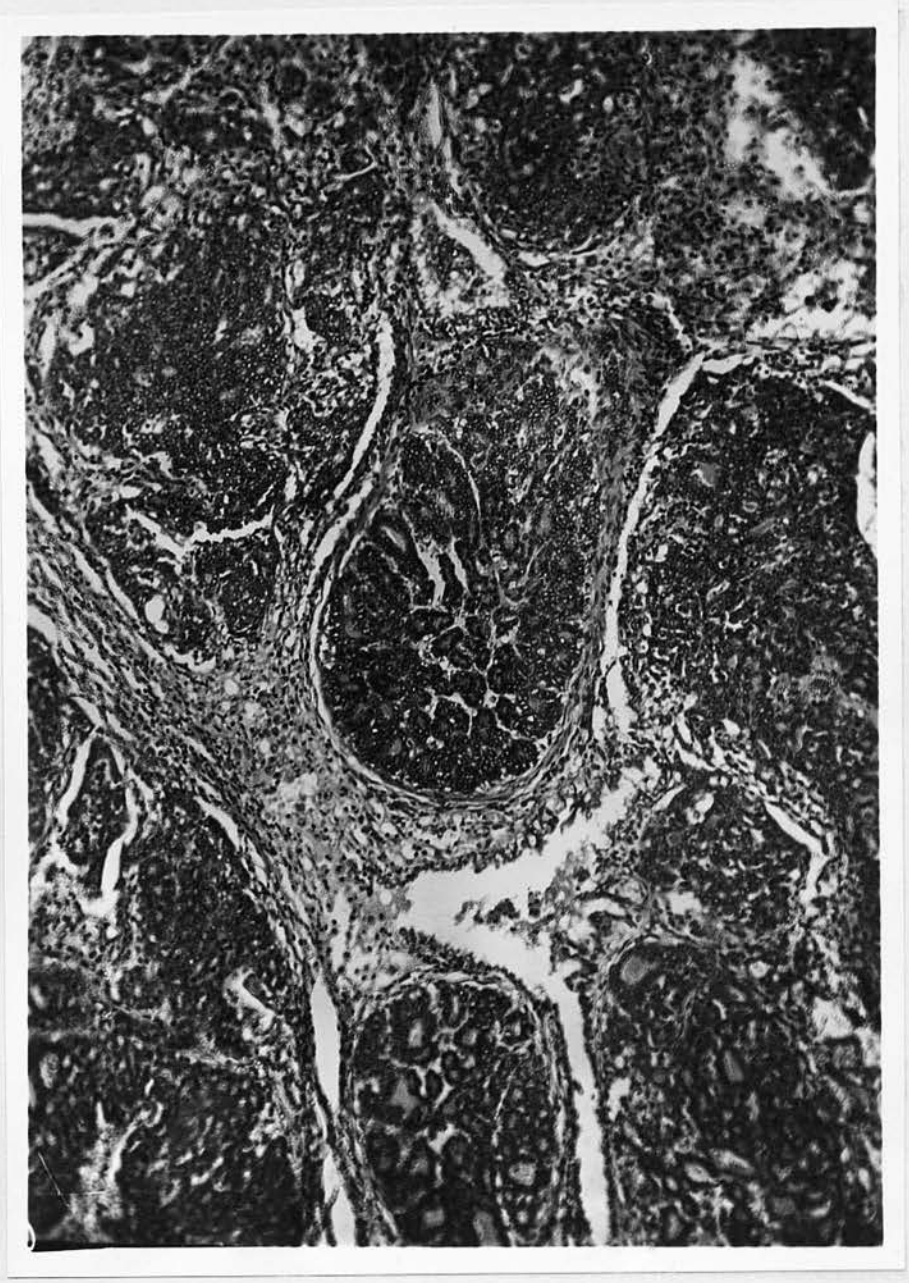


Fig. 10. x 120.

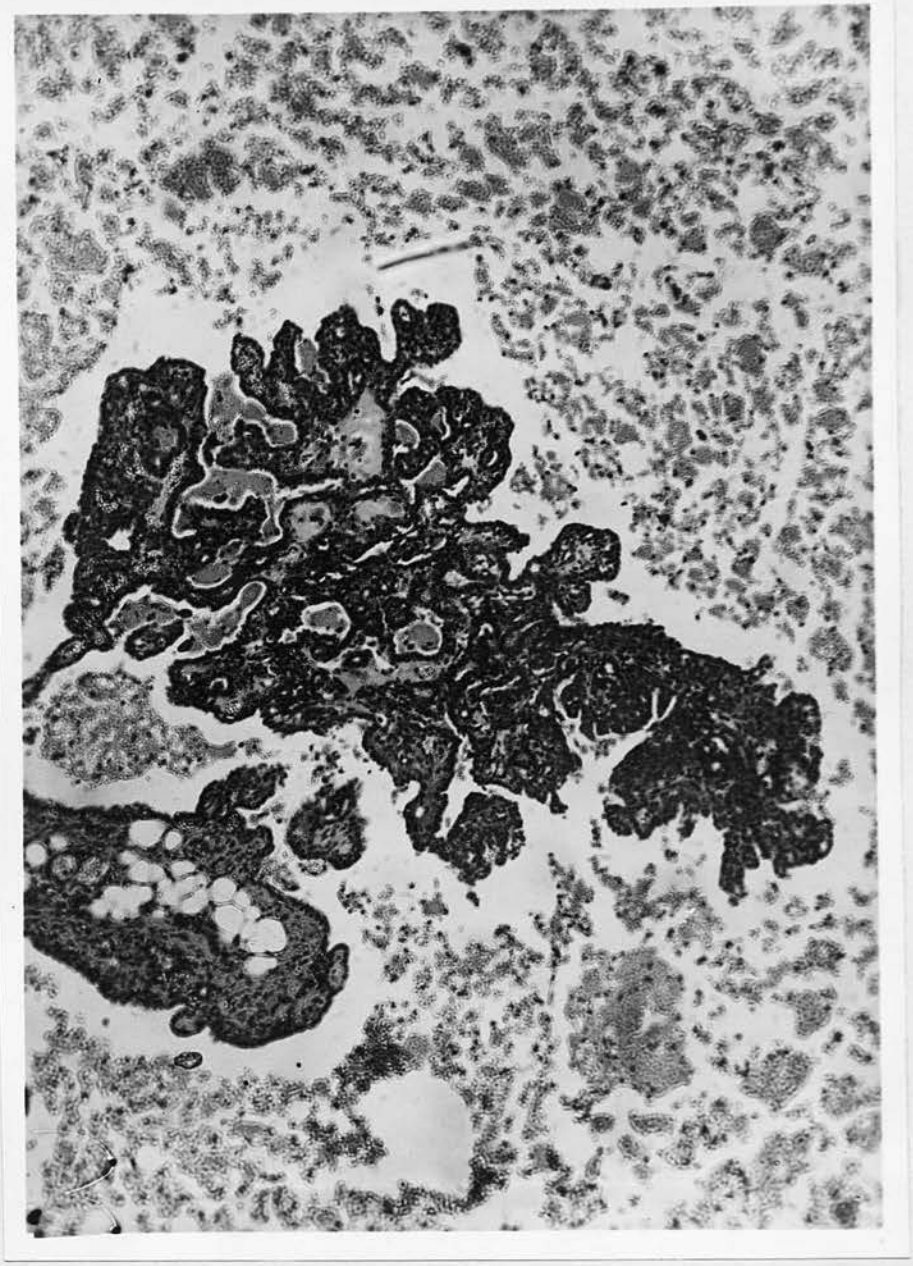


Fig. 11. x 120.

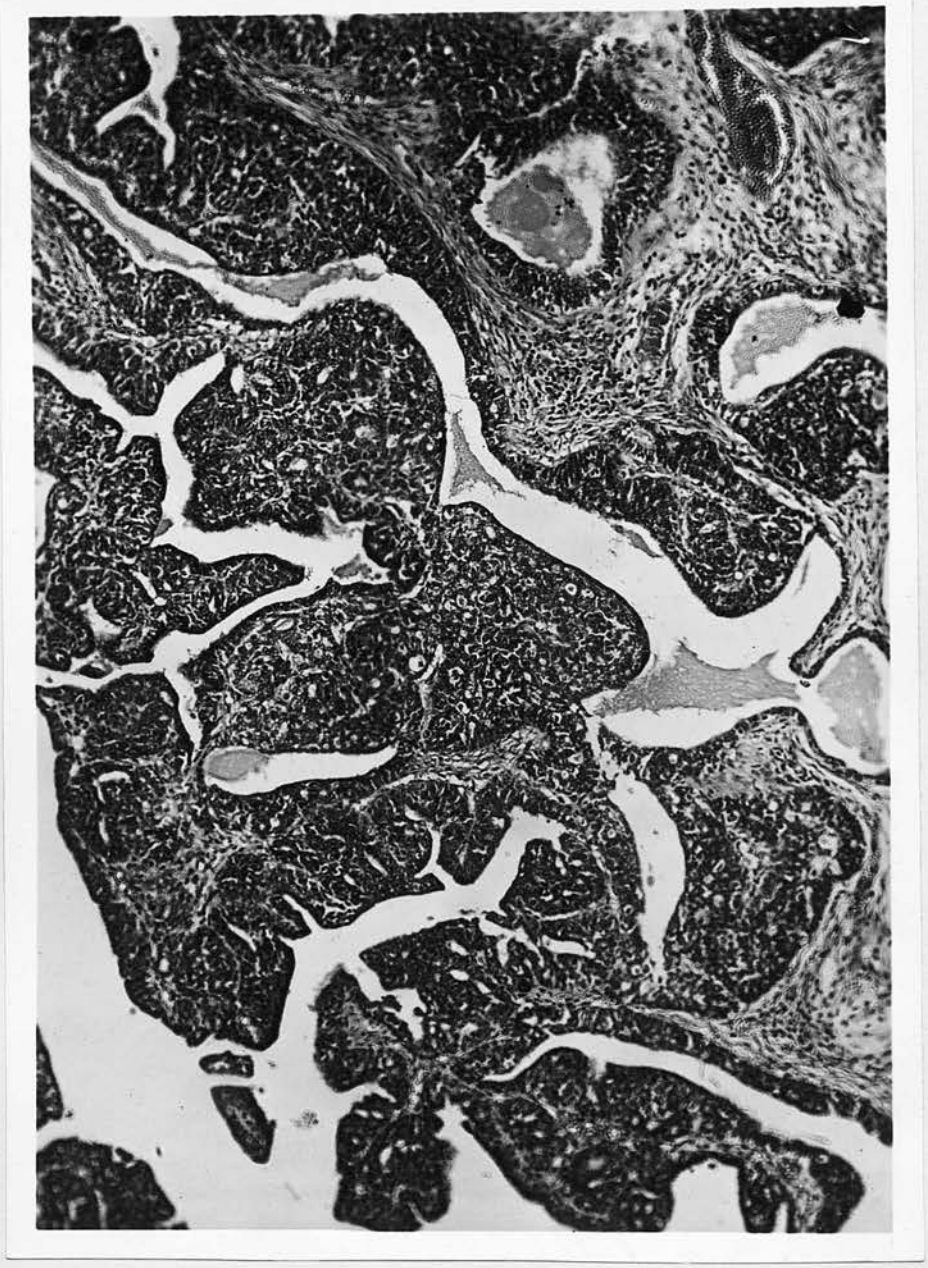


Fig. 12. x 120.

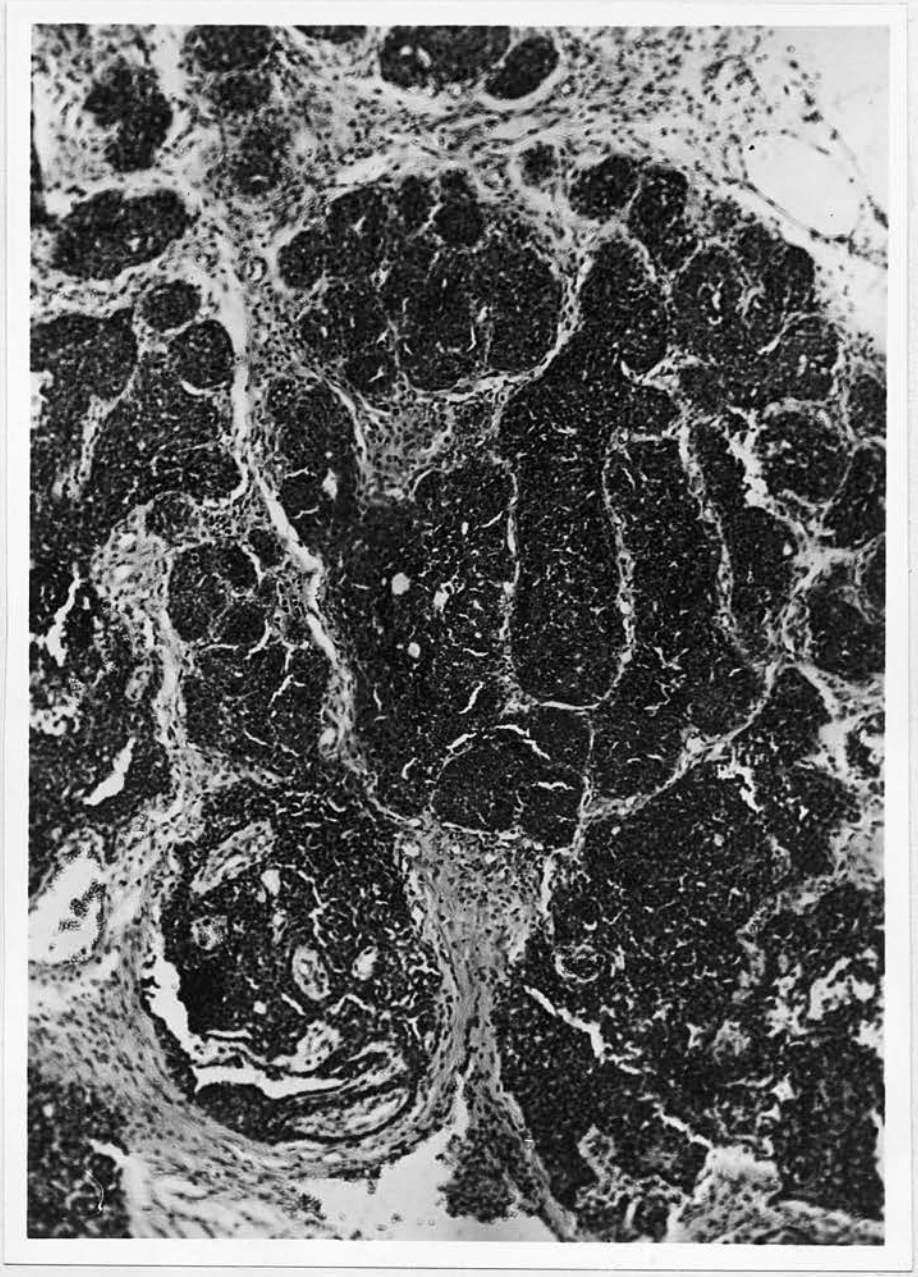


Fig. 13. x 120.

of relatively pure glandular structure, metastases occurred in 29, or 23 per cent., whereas in 59 mice whose tumours showed solidly cellular areas of varying extent, metastases occurred in 24 or 40.7 per cent." (P 0.02-0.01).

VI. Influence of reproduction on tumour growth-rate.

In the present study no evidence was found to suggest that gestation itself has any influence on the rate of growth of mammary cancer in the mouse, but in approximately half of the available cases it was observed that parturition was followed by a temporary decline in growth-rate. The duration of this retardation varied from several days to many weeks, and Figs. 14-17 illustrate four examples of this undoubted phenomenon. Although no adequate explanation can be offered from evidence, it appears likely that the effect is dependent on lactation and the marked increase in functional activity of the mammary tissue. In this connexion it is of interest that Cuenot and Mercier (1909) noticed that Borrel's mouse tumour 'B', which rarely underwent spontaneous absorption if inoculated before fecundation, developed during gestation and receded during lactation. The tumour however did not regress if one mouse only was born, nor did absorption occur even after the birth of several young if the tumour was so situated that its vascular supply was independent of that of the mammary gland (see also Apolant 1911 p. 320 and Kross 1921). Slye (1920) also recorded that the growth of spontaneous cancer in mice was often strikingly less in reproducing

females. Dobrovolskaia-Zavadskaia (1930) further drew attention to the slow rate of growth, sometimes even interrupted by periods of partial regression, of similar tumours during lactation in the host. Lastly, Emge and Wulff (1934), in a study of the influence of pregnancy on the growth of fibroma and adenofibroma in the rat, observed "depression of the growth curves of small tumours during lactation."

VII. Influence of other factors on tumour growth-rate.

Figs. 18 and 19 show a number of examples of fluctuations in the rate of growth of spontaneous mouse cancer under control conditions. In many of these cases (e.g., A 142) slow growth or retardation was almost certainly due to bacterial infection of the tumour substance, and A 19 is of interest as illustrating the prompt recovery in growth-rate which occurs when such an infection is overcome. The writer has on no occasion encountered any instance of complete regression of spontaneous mammary cancer in the mouse, and this is apparently the experience of the majority of workers (see however Woglom 1922). In his study of partial or complete regression in human neoplasms Rohdenburg (1918) attributed the phenomenon to nutritional causes, acute general infection, local fibrosis or calcification.

But quite apart from incidental impeding factors of this kind it was found in the present investigation that those tumours which were detected in their earliest stages, and which were extremely small and probably quite free from infection, not infrequently grew with extreme slowness for a variable period. The same observation was made in another investigation in many cases of induced sarc-:omata, where the tumour may be studied from its

inception, and it appears to illustrate the important etiological principle that the local conditions which lead to tumour emergence are not such as primarily favour growth, but are rather the reverse (see also Orr 1937). In any case it would seem that the malignant cell rarely attains its full capacity for proliferation before it reaches the healthy tissues and so escapes from the restricted and pathological environment in which it arose.

VIII. Note on some tumours of special interest.

Among a total of 336 tumours there occurred a small number presenting various exceptional features. In three cases of mammary carcinoma the histological examination showed numerous foci of keratinization, an appearance described in detail by Murray (1908) and Haaland (1911), encountered also by Williams, Silcox and Halpert (1935), and referred to by Nicod (1936) as "epithelioma à évolution malpighienne: adeno-cancroïde."

Tumour K 4 was of special interest since the primary growth in the bulk of its substance seemed to be sarcomatous in nature, with occasional giant cells (Figs. 20, 21). But careful study of certain areas showed an apparent morphological transition from undoubted epithelium in glandular formation to spindle cells in the arrangement shown (epithelioma à cellules fusiformes: sarcomatoïde, Nicod 1936). Further, the lungs contained large nodules, undoubtedly secondary, and presenting a highly typical papilliferous structure (Fig. 22). Bonser, Stickland and Connal (1937) recently described spindle-celled areas in a mammary carcinoma (of the mouse) induced by oestrone.

Apart from this type of case two tumours were found in which the appearances suggested sarcomatous

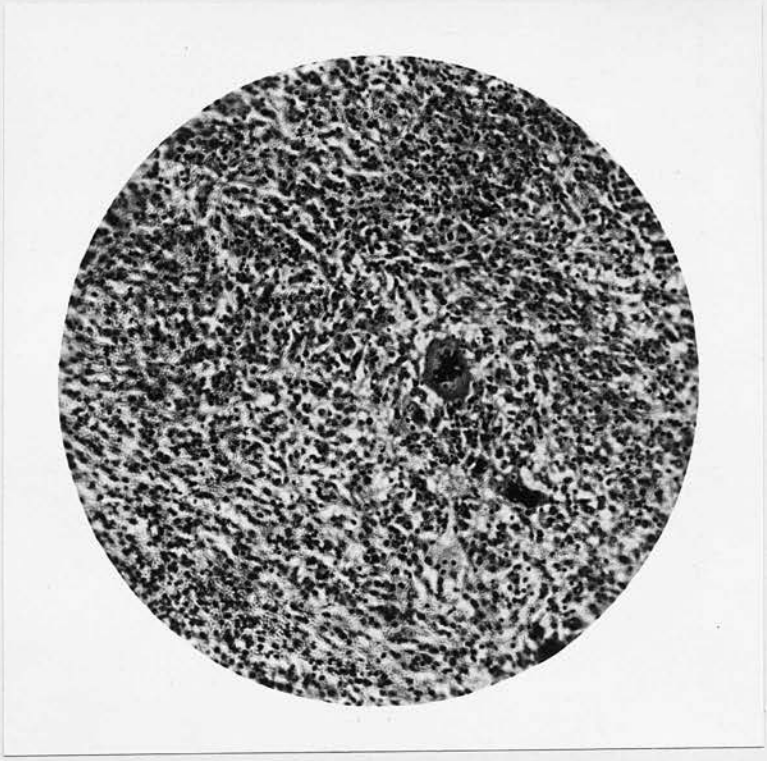


Fig. 20. K 4 primary tumour
x 120.

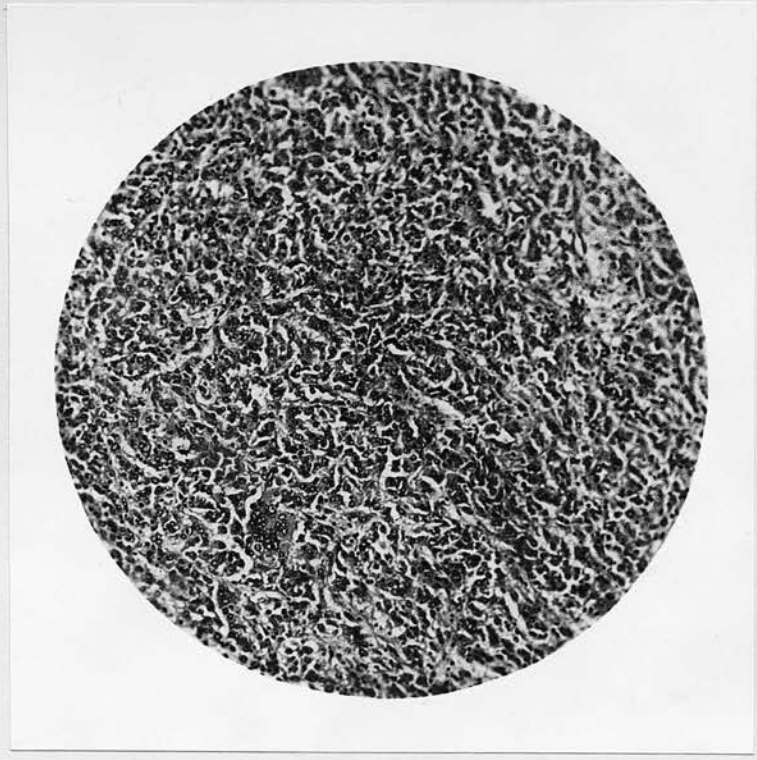


Fig. 21. K 4 primary tumour
x 120.

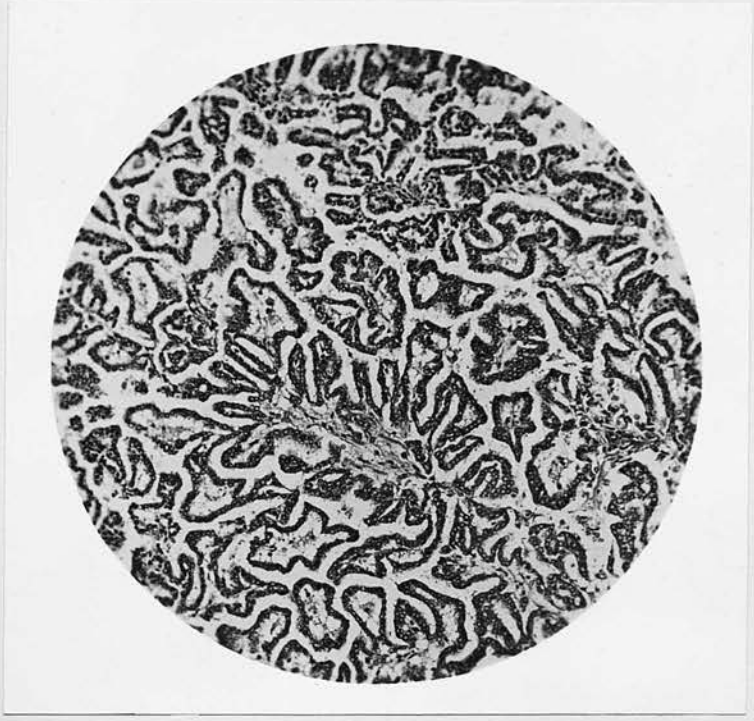


Fig. 22. K 4 pulmonary deposit
x 120.

alteration in the stroma, a phenomenon the classical description of which was given by Haaland (1908). Woglom (1917, 1918) accepted the possibility of such a change, and described the presence of cartilage in the sarcomatous portion of a carcinosarcoma in the mouse as strongly suggestive that this part was indeed of connective tissue and not of epithelial origin.

In case A 139 the animal bore two primary mammary carcinomata, and the most striking features at autopsy were an enormous hypertrophy of the normal mammary tissue and of the uterus, each horn of the latter measuring about 3.2 cm. x 1.0 cm. (see Fig. 23). The vagina was also greatly enlarged and showed pronounced keratinization. Both ovaries appeared to be normal, but the pituitary was moderately enlarged. The general appearances suggested considerable over-secretion of oestrin, and the case may be compared with one described by Gardner, Strong and Smith (1936) in which the animal presented a pituitary adenoma, bilateral ovarian granulosa-cell tumours and multiple mammary adenocarcinomata. These authors observed no definite physiological activity which might be attributed to the pituitary adenoma, but they found a uterine endometrial hyperplasia and a general cystic and hyperplastic condition of the mammary

Fig. 23. A 139 uterus x 120

glands which they ascribed to a hyperovarian state associated with the ovarian tumours.

In addition to a mammary carcinoma, mouse A 193 possessed a large pedunculated adenoma of the liver. Similar tumours were described by Murray (1908), Slye, Holmes and Wells (1916) and Itami (1918).

Of the non-mammary tumours occurring in male mice may be mentioned the osteogenic sarcoma A 86 (Fig. 24). This tumour arose in relation to the right tibia, and Figs. 25 and 26 show the radiological appearances at an interval of 40 days. There is clear evidence of elevation of the periosteum, and spicules of bone can be seen radiating in characteristic fashion into the tumour substance. The neoplasm grew slowly and was observed over a period of some ninety days. Two cartilaginous nodules, similar in structure to the primary tumour, were discovered in the lungs at autopsy.

Finally, numerous examples were encountered of the varied but related conditions which have been described under the terms lymphoid hyperplasia (Hill 1930), lymphomata (Haaland 1911), lymphomatosis (Furth, Siebold and Rathbone 1933), lymphadenoma (Mercier and Gosselin 1931, Dobrovolskaia-Zavadskaia 1932) and leukemoid and leukemic states (Hueper 1934).

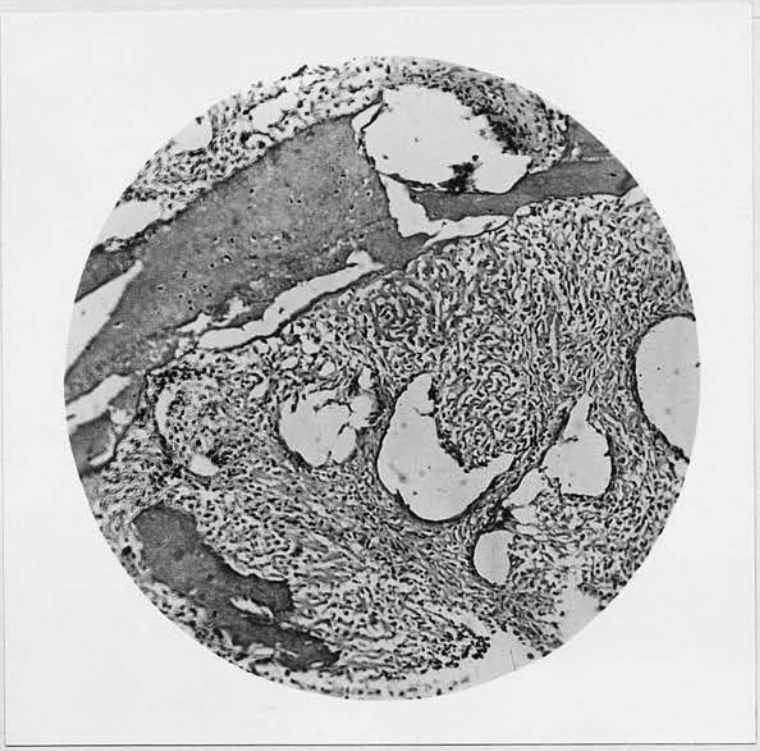


Fig. 24. A 86 primary tumour
x 120.

Fig. 25. A 86.

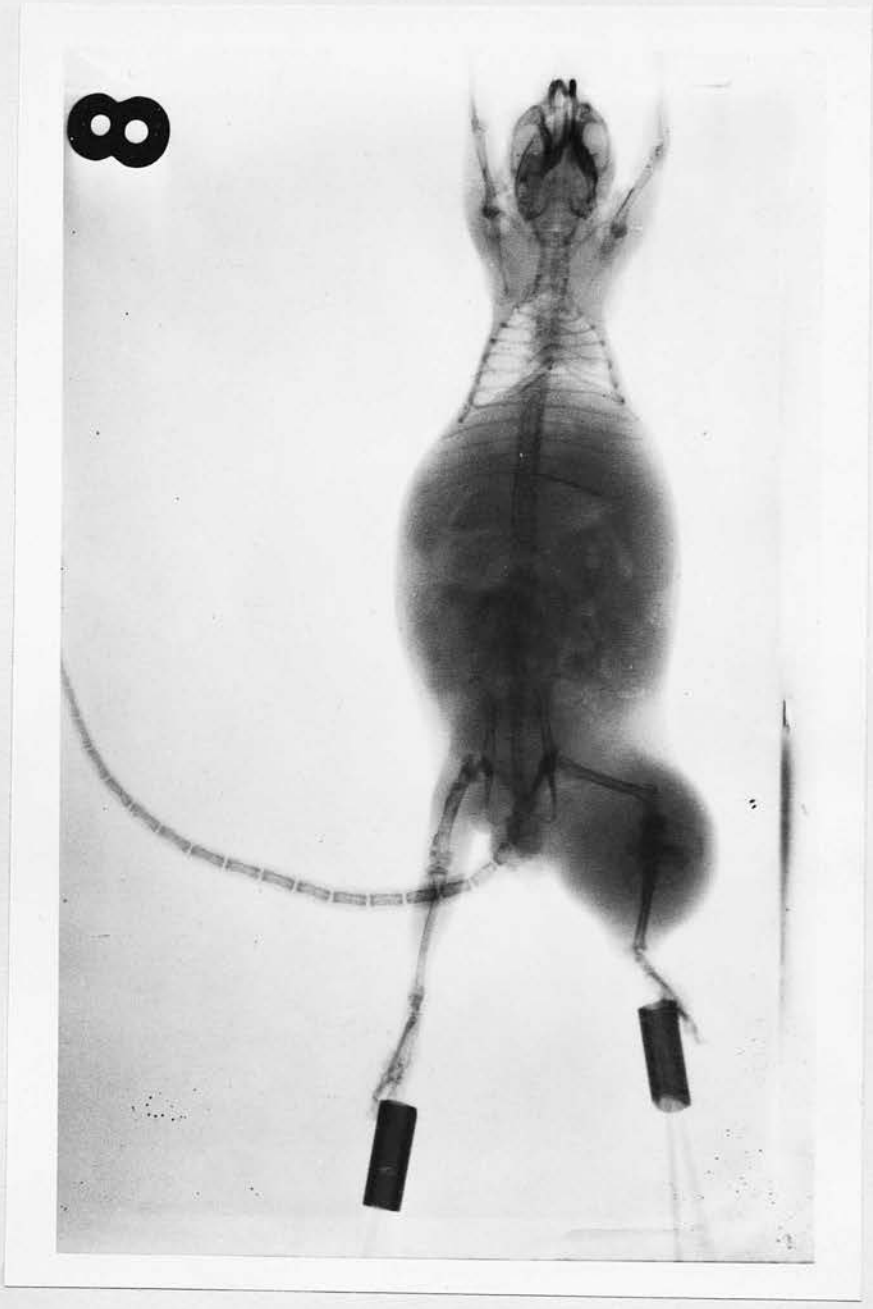


Fig. 26. A 86; see text.

IX. Summary.

In a study based on 336 spontaneous tumours in the mouse.....

1. The occurrence of multiple primary neoplasms approached a chance distribution, although the approximation was statistically inadequate to justify the assumption of actual random incidence.

2. In the great majority of cases the linear measurement of tumour size increased linearly with time, although a small proportion showed exponential increase.

3. No significant association could be established between rate of growth and the incidence of metastasis, although such an association was present in a series studied by Ashburn (1937).

4. A significant correlation was found between tumour-size attained and the incidence of metastasis.

5. Time of duration of the primary tumour was found to be a highly important single factor in determining the occurrence of metastasis.

6. No significance could be attributed to a somewhat increased proportion of metastasis in mice with multiple primary tumours.

7. Tumours situated in the caudal half of the body were observed to possess a mean growth-rate significantly higher than the mean for similar tumours cephalic in position.

8. No relation was found to exist between location of tumour and production of metastases.

9. When tumours of extreme types were studied, it appeared justifiable to conclude that those of differentiated adenomatous structure more often possessed low rates of growth as compared with the higher rates frequently manifested by dedifferentiated and anaplastic histological types.

10. In the case of pregnant animals bearing spontaneous mammary carcinomata no evidence was found to suggest that gestation influenced the rate of tumour growth, but parturition and the onset of lactation were not uncommonly followed by a temporary retardation.

11. Slow growth was frequently observed in the earliest stages of tumour development, such examples only attaining their maximal rate after a variable and often considerable interval. Mention is made of the possible etiological significance of this phenomenon.

12. Apart from the effect described as consequent on parturition and lactation, retardation during the later stages of growth was mainly due to incidental factors such as bacterial infection of the tumour substance.

X. References.

Apolant, H. 1906 Die epithelialen Geschwulste der Maus.

In Arbeiten a. d. Koniglichen Institut fur exper. Ther. zu Frankfurt A.M. Heft 1.

Jena 1906

--------- 1911 The question of athrepsia.

Jour. Exp. Med., 14, 316-321.

Ashburn, L.L. 1937 Spontaneous mammary tumors in mice; factors influencing the incidence of metastases.

Publ. Health Repts., 52, 915-929.

Bashford, E.F. 1911 The behaviour of tumour cells during propagation.

4th Sci. Rept. Imperial Cancer Research Fund, 131-214.

Bashford, E.F., Murray, J.A. and Bowen, W.H. 1906 The experimental analysis of the growth of cancer.

Proc. Roy. Soc., B, 78, 195-222.

Bonser, G.M., Stickland, L.H. and Connal, K.I.	1937	The carcinogenic action of oestrone: induction of mammary carcinoma in female mice of a strain refractory to the spontaneous development of mammary tumours. Jour. Path. Bact., 45, 709-714.
Bugher, J.C.	1934	The probability of the chance occurrence of multiple malignant neoplasms. Amer. Jour. Cancer, 21, 809-824.
Cuenot, L. and Mercier, L.	1909	Etudes sur le cancer des Souris. Relations entre la greffe de tumeur, la gestation et la lactation. C. rend. Acad. sci., 149, 1012-1013.
Dobrovolskaia-Zavadskaia, N.	1930	Sur une tumeur de souris a evolution lente et discontinue et son comportement hereditaire. C. rend. soc. biol., 103, 994-996.
----------	1932	Distribution de cas de lymphadenome dans une lignee de souris. Rapprochement de differentes lignees cancereuses de souris au point de vue de la distribution des cas de lymphadenome.

C. rend. soc. biol., 109, 263-266; 339-342.

Emge, L.A. and Wulff, L.M.R. 1934 Influence of pregnancy on experimental tumor growth in the white rat: volumetric studies on adenofibroma and fibroma. West. Jour. Surg., Obst. Gyn., 42, 45-54.

Fischer, A. 1919 Multiple tumors of the mouse mamma: are they independent or metastatic? Jour. Cancer Res., 4, 325-347.

Furth, J., Siebold, H.R. and Rathbone, R.R. 1933 Experimental studies on lymphomatosis of mice. Amer. Jour. Cancer, 19, 521-604.

Gardner, W.U., Strong, L.C. and Smith, G.M. 1936 An observation of primary tumors of the pituitary, ovaries and mammary glands in a mouse. Amer. Jour. Cancer, 26, 541-546.

Haaland, M. 1908 Contributions to the study of the development of sarcoma under experimental conditions.

3rd. Sci. Rept. Imperial Cancer Research Fund, 175-261.

-------- 1911 Spontaneous tumours in mice.

4th Sci. Rept. Imperial Cancer Research Fund, 1-113.

Hill, F. McC. 1930 Lymphoid hyperplasia in mice.

Jour. Cancer Research, 14, 325-358.

Hueper, W.C. 1934 Leukemoid and leukemic conditions in white mice with spontaneous mammary carcinoma.

Folia haematologica, 52, 167-178.

Itami, S. 1918 Adenocarcinoma or adenoma of the liver in a mouse.

Jour. Cancer Research, 3, 275-277.

Kross, I. 1921 Pregnancy and tumor growth.

Jour. Cancer Research, 6, 245-250.

Marsh, M.C. 1929 Spontaneous mammary cancer in mice.

Jour. Cancer Research, 13, 313-339.

Mayneord, W.V. 1932 On a law of growth of Jensen's rat sarcoma.

Amer. Jour. Cancer, 16, 841-846.

Mercier, L. and Gosselin, L. 1931 Sur la possibilite d'obtenir chez la souris les manifestations ganglionnaires caracteristiques du lymphome malin de Borrel-Haaland.

C. rend. soc. biol., 106, 1216-1218.

Murray, J.A. 1908 Spontaneous cancer in the mouse; histology, metastases, transplantability, and the relation of malignant new growths to spontaneously affected animals.

3rd. Sci. Rept. Imperial Cancer Research Fund, 69-114.

Murray, W.S. 1934 The breeding behavior of the dilute brown stock of mice (Little dba).

Amer. Jour. Cancer, 20, 573-593.

Nicod, J.L. 1936 Essai de classification des cancers spontanes de la glande mammaire chez la souris blanche.

Bull. de l'Assoc. franc. pour l'etude du cancer, 25, 743-758.

Okada, O. 1930 On the mensuration of the transplantable malignant tumours.

Trans. Jap. Path. Soc., 20, 753-756.

Orr, J.W. 1937 The results of vital staining with phenol red during the progress of carcinogenesis in mice treated with tar, dibenzanthracene and benzpyrene.

Jour. Path. Bact., 44, 19-27.

Ottensooser, F. 1930 Uber die Schwankungen der Virulenz von Mauseimpf-:tumoren.

Zeits. f. Krebsforsch., 30, 553-572.

Rohdenburg, G.L. 1918 Fluctuations in the growth energy of malignant tumors in man, with especial reference to spontaneous recession. Jour. Cancer Research, 3, 193-225.

Russ, S. and Chambers, H. 1913 On the action of radium rays upon the cells of Jensen's rat sarcoma. Arch. Middlesex Hospital, 30, 120-135.

Schrek, R. 1935 A quantitative study of the growth of the Walker rat tumor and the Flexner-Jobling rat carcinoma. Amer. Jour. Cancer, 24, 807-822.

------- 1936a Comparison of growth curves of malignant and normal (embryonic and post-embryonic) tissues of the rat. Amer. Jour. Path., 12, 525-530.

------- 1936b Further quantitative methods for the study of transplantable tumors; growth of R39 sarcoma and Brown-Pearce carcinoma. Amer. Jour. Cancer, 28, 345-363.

Slye, M.	1920	The relation of pregnancy and reproduction to tumor growth. Jour. Cancer Research, 5, 25-52.
Slye, M., Holmes, H.F. and Wells, H.G.	1916	Primary spontaneous tumors of the liver in mice. Jour. Cancer Research, 1, 107.
---------	1917	Primary spontaneous sarcoma in mice. Jour. Cancer Research, 2, 1-37.
Sugiura, K. and Benedict, S.R.	1921	Jour. Cancer Research, 6, 100.
Voegtlin, C.	1937	Biochemistry of malignant tissues. Physiol. Rev., 17, 109-110.
Wells, H.G.	1924	Jour. Cancer Research, 8, 147.
Williams, A.C., Silcox, L.E. and Halpert, B.	1935	Carcinoma of the mammary gland in an inbred stock of albino mice. Amer. Jour. Cancer, 24, 823-830.

Woglom, W.H. 1917 Loss of the power to produce sarcomatous transformation in the stroma.

Jour. Cancer Research, 2, 471-491.

---------- 1918 Significance of the cartilage in a carcinochondrosarcoma of the mouse.

Jour. Cancer Research, 3, 47-61.

---------- 1922 The regression of spontaneous mammary carcinoma in the mouse.

Jour. Cancer Research, 7, 379-394.

THE INFLUENCE OF CARCINOGENIC COMPOUNDS AND RELATED SUBSTANCES ON THE RATE OF GROWTH OF SPONTANEOUS TUMOURS OF THE MOUSE

The influence of carcinogenic compounds and related substances on the rate of growth of spontaneous tumours of the mouse.

The influence of carcinogenic compounds and related substances on the rate of growth of spontaneous tumours of the mouse.

I. Introduction.

Previous papers (Haddow 1935, Haddow and Robinson 1937, Haddow, Scott and Scott 1937) described the production by certain of the carcino-:genic hydrocarbons of a characteristic retardation in the body growth of the rat and in the rate of growth of transplantable animal tumours. The effect was absent in the majority of a number of related but non-carcinogenic compounds, and the implications of this association have been further discussed (Haddow 1937 and 1938 in press) in relation to the mode of action of carcinogenic substances in general. The present section describes similar experiments carried out on a considerable number of spontaneous tumours (mostly adenocarcinomata of the mammary gland) in the mouse. The origin and general biology of this material have already been described in the foregoing paper. As compared with transplantable tumours it is obvious that the spontaneous tumour in many respects is a more valuable test-object for the study of any effect such as the one under discussion. Although temporary retardation of growth may of course occur from non-experimental

causes, as already described, this difficulty may very easily be overcome by employing adequate numbers of selected animals bearing healthy and actively growing tumours. A more serious disadvantage is that many tumours when received are so advanced or septic that death of the animal occurs within a comparatively short period. Thus in a total of some three hundred tumour-bearing mice in the present series no fewer than seventy-six died after a mean period of only 16.4 days from receipt, the chief causes being the advanced state of the tumour, ulceration, infection of its substance, haemorrhage and the presence of pulmonary metastases.

In order that the influence of the substance administered might be reliably assessed in individual cases, the tumour growth-rate was estimated by caliper measurements over a preliminary control period of usually 10-20 days, and only those tumours were employed which showed a satisfactory and reasonably uniform increase during this time. The substance to be tested was then administered (by the intra-peritoneal injection of a 0.5 per cent. solution in sesame oil, unless otherwise stated) and daily measurements continued to the death of the animal. Histological examinations were carried out in every case. Experiments of this kind are described in detail in the following account.

II. Carcinogenic compounds.

1. 1:2:5:6-dibenzanthracene.

A 11. When its tumour had shown steady growth for 14 days this animal was given 12 mg. 1:2:5:6-dibenzanthracene intraperitoneally in oil. Fig. 27 shows the resulting degree of inhibition, which was considerable and persisted to the time of death some 60 days after the first observation. This result may be regarded as typical of a considerable number of cases. The animal maintained its weight but shortly before death the tumour surface broke down leading to discharge and haemorrhage. Peritoneal effusion was discovered at autopsy. Microscopic section showed papillary cystadenocarcinoma of the mamma, with a few nodules of similar structure in the lungs.

A 11.

T A B L E X

Day	Tumour diameters and sum (cm.)	Weight	Remarks
1	1.90, 1.40, 3.30		
2	2.00, 1.65, 3.65		
3	2.00, 1.70, 3.70		
4	2.05, 1.60, 3.65		
5	2.05, 1.65, 3.70	38.0 g.	
7	2.10, 1.65, 3.75		
8	2.15, 1.80, 3.95		
9	2.20, 1.65, 3.85		
10	2.25, 1.70, 3.95		
11	2.25, 1.70, 3.95		
12	2.40, 1.80, 4.20	39.5 g.	
14	2.35, 2.00, 4.35		
15	2.40, 1.85, 4.25		
---	12 mg. 1:2:5:6-dibenzanthracene ---		
16	2.30, 1.95, 4.25		
17	2.40, 1.85, 4.25		
18	2.60, 1.80, 4.40		
19	2.40, 1.95, 4.35	42.0 g.	
21	2.55, 2.10, 4.65		
22	2.50, 2.00, 4.50		
23	2.50, 1.85, 4.35		
24	2.50, 2.10, 4.60		
25	2.40, 2.10, 4.50		
26	2.50, 1.90, 4.40		
28	2.55, 2.15, 4.70		
29	2.60, 2.00, 4.60	41.5 g.	
30	2.55, 2.05, 4.60		
31	2.55, 2.00, 4.55		
32	2.45, 1.95, 4.40	42.5 g.	
35	2.45, 1.90, 4.35		
36	2.50, 2.00, 4.50		
37	2.60, 2.00, 4.60		
38	2.65, 1.95, 4.60		
39	2.65, 2.05, 4.70		
40	2.70, 2.00, 4.70		
42	2.55, 2.00, 4.55		
43	2.65, 2.00, 4.65		
44	2.60, 1.95, 4.55		
45	2.65, 1.95, 4.60		
46	2.60, 1.95, 4.55		
47	2.65, 1.95, 4.60	43.0 g.	
49	2.60, 1.95, 4.55		
50	2.65, 2.00, 4.65		
51	2.65, 1.95, 4.60		
52	2.65, 2.00, 4.65		
53	2.60, 1.95, 4.55		
54	2.65, 2.00, 4.65		

A 11 continued.

Day	Tumour diameters and sum (cm.)	Weight	Remarks
56	2.60, 1.95, 4.55		
57	2.60, 2.10, 4.70		
58	2.55, 1.95, 4.50	45.0 g.	
59	2.40, 2.15, 4.55		
60	2.50, 2.00, 4.50		Tumour discharging
61	2.60, 2.15, 4.75		
63	2.60, 1.95, 4.55		Haemorrhage.
64	2.45, 1.95, 4.40		
65	2.30, 1.95, 4.25	43.0 g.	"
66	Died.		

A 1. This example shows a similar effect following the injection of 10 mg. 1:2:5:6-dibenzanthracene (Fig. 28). Haemorrhage from the tumour was a troublesome feature, and the animal showed loss of weight before death. At autopsy the primary tumour was found to be extremely degenerate, and metastases were present in the lungs. The microscopic appearances are shown in Figs. 29 and 30.

A 20. Fig. 31 shows the alteration in rate of tumour growth following administration of 12 mg. 1:2:5:6-dibenzanthracene. The effect was followed to the 90th day, when the animal was still in good condition and was used for another purpose. It survived to a total of 133 days from the start of the experiment, maintaining weight to the end. The lungs contained numerous secondary deposits at autopsy. Section of the primary tumour showed a highly cellular mammary carcinoma with areas of secretion and haemorrhage.

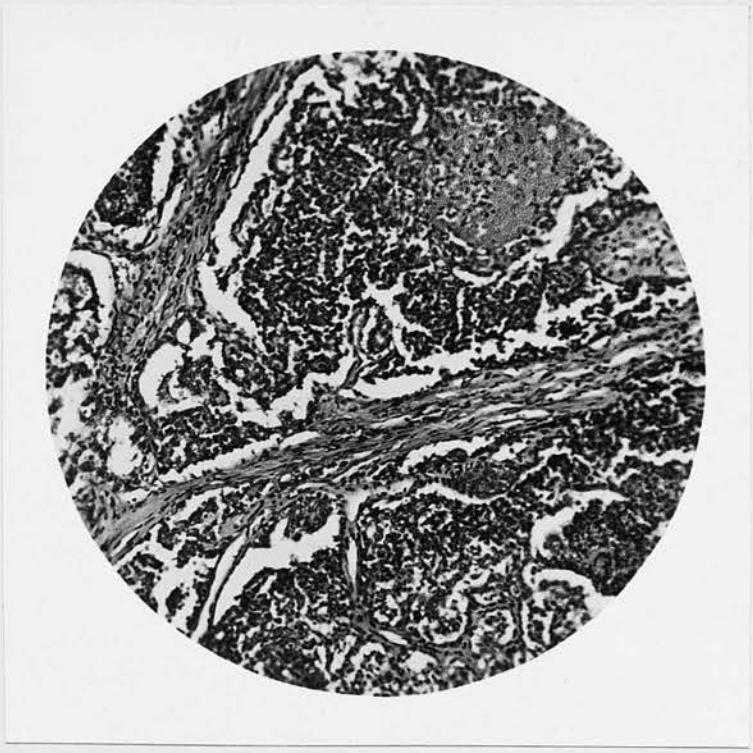


Fig. 29. A 1 primary tumour
x 120.

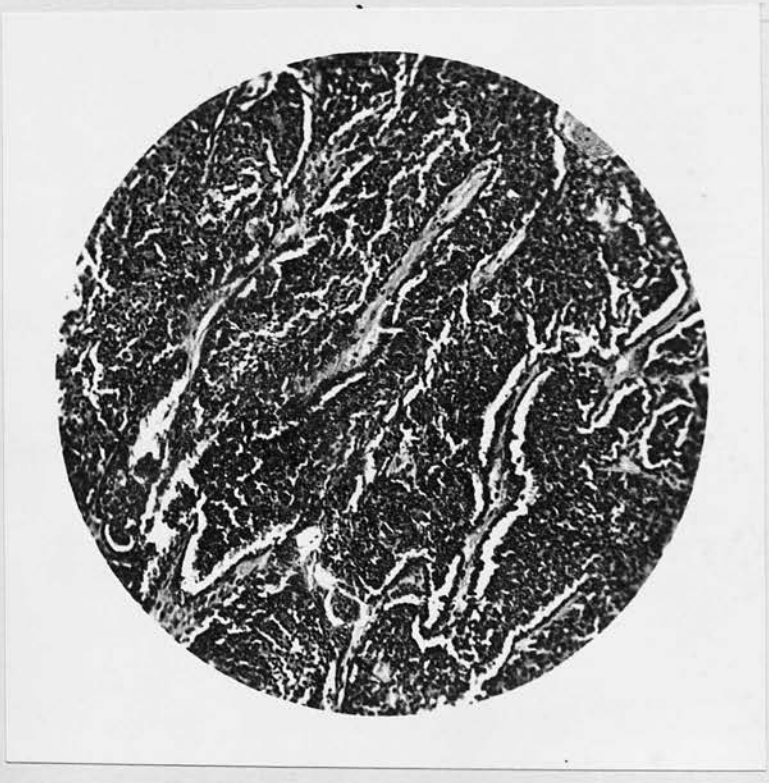


Fig. 30. A 1 pulmonary deposit
x 120.

TABLE XI

A 20.

Day	Tumour diameters and sum (cm.)	Weight	Remarks
1	1.35, 1.20, 2.55		
2	1.60, 1.30, 2.90		
3	1.85, 1.40, 3.25		
4	1.80, 1.35, 3.15		
5	1.80, 1.40, 3.20	42.0 g.	
7	1.70, 1.40, 3.10		
8	1.80, 1.40, 3.20		
9	1.85, 1.30, 3.15		
10	1.80, 1.55, 3.35		
11	1.80, 1.50, 3.30		
12	1.80, 1.50, 3.30		
14	2.15, 1.65, 3.80		
15	2.10, 1.90, 4.00	40.5 g.	
---	12 mg. 1:2:5:6-dibenzanthracene	---	
16	1.90, 1.55, 3.45		
17	1.85, 1.75, 3.60		
18	2.00, 1.70, 3.70		
19	2.00, 1.70, 3.70	45.0 g.	
21	2.05, 1.90, 3.95		
22	1.90, 1.75, 3.65		
23	1.85, 1.70, 3.65		
24	1.95, 1.90, 3.85		
25	1.80, 1.65, 3.45		
26	1.85, 1.70, 3.55		
28	1.95, 1.75, 3.70		
29	1.95, 1.70, 3.65		
30	1.85, 1.70, 3.55		
31	1.95, 1.85, 3.80		
32	1.80, 1.70, 3.50		
33	1.85, 1.75, 3.60	42.0 g.	
35	1.95, 1.90, 3.85		
36	2.00, 1.80, 3.80		
37	1.95, 1.80, 3.75		
38	2.00, 1.80, 3.80		
39	1.90, 1.85, 3.75		
40	1.95, 1.85, 3.80		
42	2.00, 1.80, 3.80		
43	1.90, 1.80, 3.70		
44	2.00, 1.80, 3.80	43.5 g.	
45	1.95, 1.85, 3.80		
46	1.95, 1.85, 3.80		
47	2.05, 1.80, 3.85		
49	2.00, 1.90, 3.90		
50	2.00, 1.85, 3.85		
51	2.00, 1.95, 3.95	42.5 g.	
52	2.10, 2.00, 4.10		
53	1.95, 1.85, 3.80		

A 20 continued.

Day	Tumour diameters and sum (cm.)	Weight	Remarks
54	1.95, 1.80, 3.75		
56	2.00, 1.85, 3.85		
57	1.95, 1.80, 3.75		
58	2.05, 1.95, 4.00		
59	2.05, 1.95, 4.00	47.0 g.	
64	2.15, 2.05, 4.20		
65	2.20, 2.05, 4.25		
67	2.15, 2.10, 4.25	45.0 g.	
68	2.35, 2.10, 4.45		
70	2.35, 2.15, 4.50		
71	2.35, 2.25, 4.60		
72	2.30, 2.20, 4.50		
73	2.35, 2.05, 4.40		
74	2.45, 2.10, 4.55	50.0 g.	
75	2.30, 2.10, 4.40		
77	2.50, 2.40, 4.90		
78	2.50, 2.40, 4.90		
79	2.40, 2.35, 4.75		
80	2.55, 2.25, 4.80		
81	2.50, 2.35, 4.85	48.0 g.	
82	2.50, 2.35, 4.85		
84	2.60, 2.35, 4.95		
85	2.65, 2.35, 5.00		
86	2.55, 2.40, 4.95		
87	2.50, 2.25, 4.75		
88	2.60, 2.40, 5.00	51.0 g.	
89	2.60, 2.50, 5.10		
91	2.60, 2.45, 5.05		

A 8. This animal presented an ulcerating mammary tumour which however showed fair growth during the control period. 12 mg. 1:2:5:6-dibenzanthracene produced marked inhibition with a slight tendency to regression (Fig. 32). The ulcerated tumour surface discharged blood and pus at intervals, and the animal suffered a gradual loss in weight. Autopsy revealed pronounced toxic changes in the myocardium and a large pleural effusion. The liver contained two cysticercus cysts (larval Taenia crassicollis), one of which was grossly infected. The tumour (Fig. 33) consisted for the most part of large-celled acini.

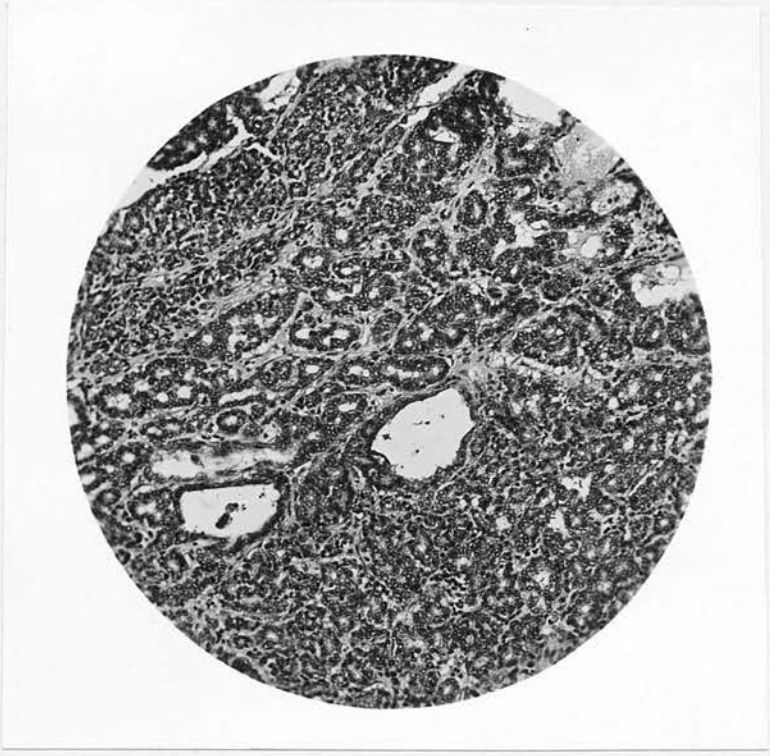


Fig. 33. A 8 primary tumour
x 120.

A 16. While results of the above type are frequent, other tumours may show a less effect, and A 16 is of some interest as possibly representing a case of complete resistance to the action of 1:2:5:6-dibenzanthracene. The tumour increased with remarkable uniformity over a control period of three weeks, and no alteration in the rate of growth was detected in the ten days following intraperitoneal injection of 12 mg. 1:2:5:6-dibenzanthracene. Subsequent administration of 5 mg. 1:2:5:6-dibenzacridine however led to a sharp change in rate which persisted for 25 days to the time of death (Fig. 34). The general condition remained good throughout, and the weight record reflects the increase in tumour size. Autopsy indicated pneumonia as the probable cause of death, and one lung contained a small secondary nodule. A single parasitic cyst was present in the liver. The primary carcinoma was exceptionally bulky and haemorrhagic, with enormous sinuses, and its microscopic structure proved comparatively uniform, consisting for the most part of masses of undifferentiated cells with only occasional indications of acinar formation.

TABLE XII

A 16.

Day	Tumour diameters and sum (cm.)	Weight	Remarks
1	1.75, 1.25, 3.00		
2	2.00, 1.40, 3.40		
3	2.10, 1.35, 3.45		
4	2.10, 1.50, 3.60		
5	2.10, 1.40, 3.50		
6	2.10, 1.50, 3.60	41.5 g.	
8	2.25, 1.55, 3.80		
9	2.20, 1.60, 3.80		
10	2.30, 1.55, 3.85		
11	2.40, 1.65, 4.05		
12	2.40, 1.55, 3.95		
13	2.45, 1.60, 4.05	39.0 g.	
15	2.55, 1.70, 4.25		
16	2.55, 1.80, 4.35		
17	2.70, 1.80, 4.50		
18	2.60, 1.90, 4.50		
19	2.80, 1.80, 4.60		
20	2.85, 1.80, 4.65		
22	3.00, 2.05, 5.05		
23	3.05, 2.05, 5.10	47.0 g.	
---	12 mg. 1:2:5:6-dibenzanthracene	---	
24	3.10, 1.95, 5.05		
25	3.15, 2.05, 5.20		
26	3.25, 2.10, 5.35		
27	3.45, 2.05, 5.50	49.5 g.	
29	3.50, 2.25, 5.75		
30	3.55, 2.20, 5.75		
31	3.65, 2.25, 5.90		
32	3.70, 2.35, 6.05		
33	3.85, 2.30, 6.15		
34	3.80, 2.35, 6.15		
---	5 mg. 1:2:5:6-dibenzacridine	---	
36	3.80, 2.25, 6.05		
37	3.85, 2.25, 6.10		
38	3.85, 2.40, 6.25		
39	3.80, 2.45, 6.25		
40	3.85, 2.45, 6.30		
41	3.75, 2.30, 6.05	57.0 g.	
43	3.85, 2.30, 6.15		
44	3.95, 2.35, 6.30		
45	3.95, 2.55, 6.50		
46	3.90, 2.50, 6.40		
47	4.05, 2.45, 6.50		
48	4,00, 2.50, 6.50		
50	3.90, 2.60, 6.50		
51	4.15, 2.65, 6.80		
52	4.05, 2.65, 6.70	66.0 g.	
53	3.75, 2.50, 6.25		
54	4.15, 2.70, 6.85		
55	4.10, 2.60, 6.70		
57	4.15, 2.60, 6.75		
58	Died		

D.B. 127/128. This tumour occurred in an animal of the laboratory colony of the Little dba pure line, the characters of which were described by Murray (1934). The mouse was 8-9 months of age and had littered on three occasions, the tumour being first observed some ten weeks after the birth of the last litter. Initially growth proved slow, possibly on account of a persistent discharging sinus, but later was satisfactory. The case is of interest since administration of 10 mg. 1:2:5:6-dibenzanthracene produced a rapid alteration in the size and naked-eye appearance of the tumour. Over a period of ten days it became smaller and drier. The surface then disintegrated and the bulk appeared to slough, when a remaining fragment was taken for biopsy. During the following week the resulting wound healed considerably and the scar retracted almost completely. Final healing did not however occur, and after remaining stationary for some days the remaining edges became distinctly thickened and vesicular. This change progressed and recurrence was definite about one month after the administration of hydrocarbon. Thereafter growth continued at a rate little short of that during the control period (Fig. 35). Death occurred when the mouse had borne its tumour for 134 days, and was attributed to pneumonia and a grossly infected primary tumour.

Fig. 36 shows the microscopic appearance of the biopsy specimen, and Fig. 37 the histological structure of the primary tumour.

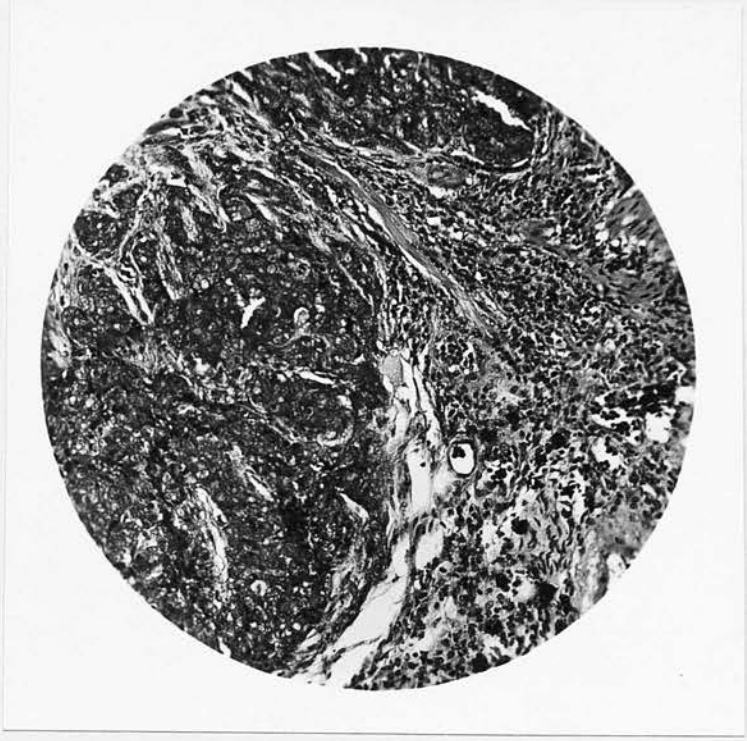


Fig. 36. D.B.127/128 biopsy specimen x 120.

Fig. 37. D.B. 127/128 primary tumour x 120.

TABLE XIII

D.B. 127/128.

Day	Tumour diameters and sum (cm.)	Weight	Remarks
1	1.25, 0.90, 2.15		
2	1.10, 0.90, 2.00		
3	1.00, 0.80, 1.80		Sinus
5	1.00, 0.90, 1.90		discharging
6	1.20, 0.90, 2.10		blood and
7	1.00, 1.00, 2.00		pus.
8	1.10, 0.90, 2.00		"
10	1.10, 0.90, 2.00		
12	1.10, 0.80, 1.90		"
13	1.20, 0.90, 2.10		
14	1.10, 0.95, 2.05		"
15	1.10, 0.80, 1.90		
16	1.00, 0.80, 1.80		
17	1.10, 1.10, 2.20	29.0 g.	
19	1.10, 1.00, 2.10		
20	1.20, 1.00, 2.20		
21	1.20, 1.00, 2.20		
22	1.15, 0.90, 2.05		
23	1.20, 1.00, 2.20		
24	1.30, 1.10, 2.40	30.0 g.	
26	1.30, 1.05, 2.35		
27	1.50, 1.10, 2.60		
28	1.50, 1.20, 2.70		
29	1.45, 1.25, 2.70		
30	1.50, 1.25, 2.75		
31	1.60, 1.30, 2.90	30.3 g.	
33	1.70, 1.45, 3.15		
---	10 mg. 1:2:5:6-dibenzanthracene ---		
34	1.70, 1.10, 2.80		
35	1.45, 1.20, 2.65		
36	1.40, 1.20, 2.60		
37	1.30, 1.10, 2.40		
38	1.10, 1.00, 2.10	27.5 g.	
40	1.30, 1.25, 2.55		
41	1.40, 1.30, 2.70		
42	1.35, 1.20, 2.55		
43	1.25, 1.20, 2.45	29.0 g.	
44			Surface of tumour disintegrating and sloughing.
45			Biopsy.
47			Drying.
48			Apparently healing.
49			"
51			Scar retracting.

D.B. 127/128 continued.

Day	Tumour diameters and sum (cm.)	Weight	Remarks
54			Rapid healing.
55		30.5 g.	
57			Slower healing.
59		30.5 g.	Edges thickened and vesicular.
61			"
62			? recurrence.
64	0.90, 0.70, 1.60		recurrence definite.
65	0.95, 0.70, 1.65		
66	1.15, 0.75, 1.90	30.5 g.	
68	1.20, 0.85, 2.05		
69	1.25, 0.95, 2.20		
70	1.30, 0.95, 2.25		
71	1.25, 0.95, 2.20		
72	1.20, 0.90, 2.10		
73	1.40, 1.05, 2.45	32.0 g.	
75	1.40, 1.10, 2.50		
76	1.40, 1.05, 2.45		
77	1.60, 1.10, 2.70		
78	1.55, 1.20, 2.75		
79	1.45, 1.20, 2.65		
80	1.55, 1.10, 2.65		
82	1.45, 1.20, 2.65		
83	1.75, 1.25, 3.00		
84	1.75, 1.30, 3.05	32.0 g.	
85	1.65, 1.35, 3.00		
86	1.70, 1.45, 3.15		
87	1.80, 1.40, 3.20		
89	1.95, 1.50, 3.45		
91	Died.		

A 3. Although the most frequent type of effect is a retardation, this is not as a rule of such a degree as to bring about complete inhibition and check all further growth. On the other hand, in a small proportion of cases the tumour undergoes active regression following similar doses of dibenzanthracene, and Fig. 38 shows an example of this exceptional response. A small portion of the receding tumour was taken for biopsy 17 days after the administration of hydrocarbon. Death occurred one week later. Autopsy showed pneumonic changes and effusion into both pleura and peritoneum. The biopsy appearances are indicated in Fig. 39.

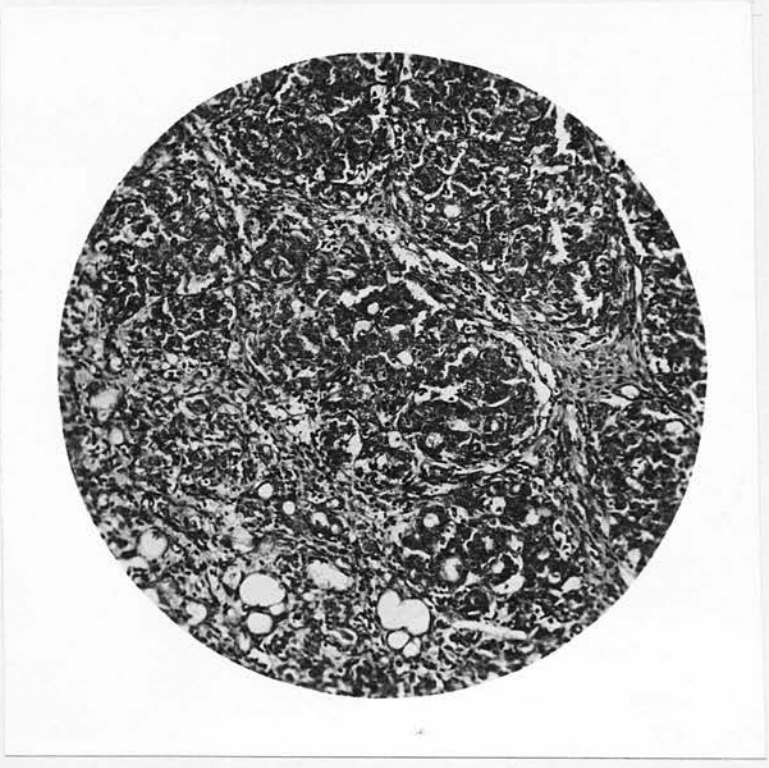


Fig. 39. A 3 biopsy specimen x 120.

TABLE XIV

A 3.

Day	Tumour diameters and sum (cm.)	Weight	Remarks
1	2.10, 1.10, 3.20		
2	1.90, 1.05, 2.95		
3	2.00, 1.10, 3.10		
4	1.95, 1.30, 3.25		
6	2.10, 1.40, 3.50		
7	2.10, 1.50, 3.60		
8	2.05, 1.50, 3.55		
9	2.30, 1.40, 3.70		
10	2.35, 1.60, 3.95		
11	2.20, 1.60, 3.80	47.5 g.	
13	2.35, 1.70, 4.05		
14	2.35, 1.70, 4.05		
15	2.20, 1.80, 4.00		
16	2.20, 1.85, 4.05		
17	2.35, 1.85, 4.20		
18	2.20, 1.90, 4.10	48.5 g.	
20	2.35, 1.95, 4.30		
21	2.50, 2.10, 4.60		
---	12 mg. 1:2:5:6-dibenzanthracene ---		
22	2.40, 2.05, 4.45		
23	2.40, 2.00, 4.40		
24	2.30, 1.80, 4.10		
25	2.30, 1.85, 4.15	47.5 g.	
27	2.10, 1.85, 3.95		
28	2.10, 1.90, 4.00		
29	2.10, 1.80, 3.90		
30	2.10, 1.65, 3.75		
31	1.90, 1.65, 3.55		
32	2.00, 1.55, 3.55		
34	1.95, 1.55, 3.50		
35	1.75, 1.50, 3.25	47.0 g.	
36	1.75, 1.40, 3.15		
37	1.85, 1.45, 3.30		
38	1.80, 1.50, 3.30	47.5 g.	Biopsy.
41	1.95, 1.40, 3.35		
42	1.85, 1.40, 3.25		
43	1.70, 1.30, 3.00		
44	1.80, 1.25, 3.05		
45	1.60, 1.10, 2.70		
46	Died.		

A 26. An even more marked instance of partial regression is given in Fig. 40. The mouse remained in fair condition until shortly before death. Pneumonic changes were present and the liver contained three cysticercus cysts. The tissue of the primary tumour was comparatively free from necrosis or infection, and section revealed an adenocarcinoma markedly papilliform in structure but with abundant stroma formation (Fig. 41).

A 5. Fig. 42 shows similar regression. The animal survived for 65 days, dying from pneumonia 25 days after the administration of 12 mg. 1:2:5:6-dibenzanthracene. Microscopic section showed the tumour to be a haemorrhagic cystadenocarcinoma, with no appearance which could be correlated with the fact of partial regression (Fig. 43).

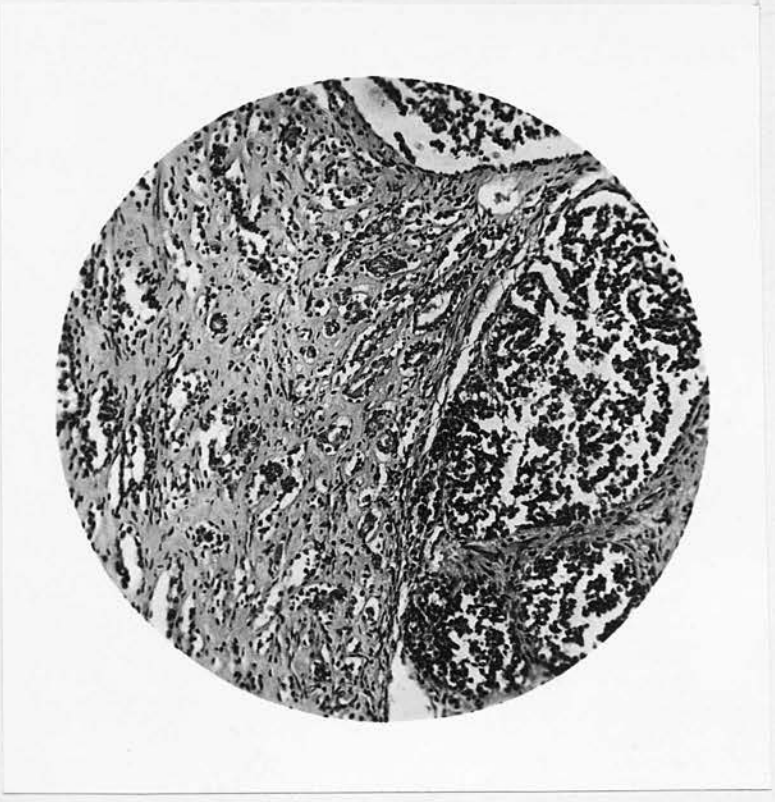


Fig. 41. A 26 primary tumour
x 120.

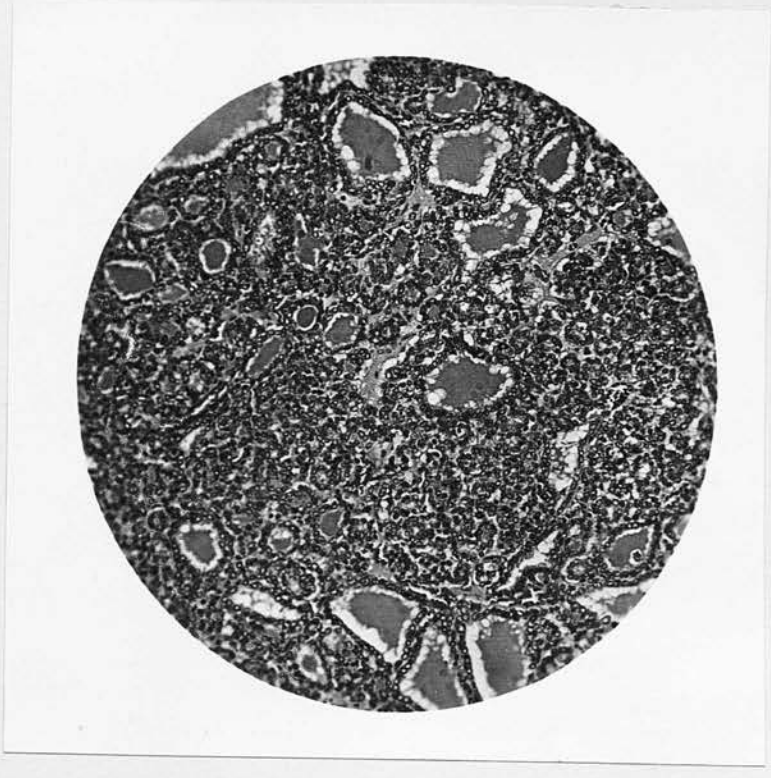


Fig. 43. A 5 primary tumour
x 120.

TABLE XV

A 26.

Day	Tumour diameters and sum (cm.)	Weight	Remarks
1	1.85, 1.80, 3.65		
2	1.80, 1.75, 3.55		
3	1.85, 1.70, 3.55	31.5 g.	
5	2.20, 1.90, 4.10		
6	2.00, 1.85, 3.85		
7	2.20, 2.00, 4.20		
8	2.20, 2.05, 4.25		
9	2.25, 1.95, 4.20		
10	2.05, 2.00, 4.05		
12	2.30, 2.15, 4.45		
13	2.20, 2.05, 4.25	32.5 g.	
14	2.30, 2.10, 4.40		
15	2.40, 2.10, 4.50		
16	2.50, 2.05, 4.55		
17	2.55, 2.25, 4.80	32.0 g.	
19	2.65, 2.30, 4.95		
---	12 mg. 1:2:5:6-dibenzanthracene	---	
20	2.55, 2.30, 4.85		
21	2.55, 2.15, 4.70		
22	2.55, 2.25, 4.80		
23	2.55, 2.25, 4.80		
24	2.50, 2.25, 4.75		
26	2.40, 2.15, 4.55		
27	2.30, 1.80, 4.10		
28	2.25, 1.95, 4.20		
29	2.35, 1.95, 4.30		
30	2.10, 1.90, 4.00		
31	2.10, 1.85, 3.95	32.0 g.	
33	2.20, 1.75, 3.95		
34	2.05, 1.70, 3.75		
35	1.95, 1.75, 3.70		
36	2.10, 1.70, 3.80		
37	1.95, 1.75, 3.70		
38	1.90, 1.75, 3.65		
40	1.95, 1.75, 3.70		
41	1.75, 1.75, 3.50		
42	1.85, 1.60, 3.45		Haemorrhage
43	1.75, 1.65, 3.40	29.0 g.	
44	1.70, 1.65, 3.35		
45	1.85, 1.60, 3.45		

--- continued over

A 26 continued.

Day	Tumour diameters and sum (cm.)	Weight	Remarks
47	1.65, 1.60, 3.25		
48	1.65, 1.55, 3.20		
49	1.65, 1.55, 3.20	27.0 g.	
50	1.65, 1.50, 3.15		
51	1.55, 1.50, 3.05		
52	1.60, 1.55, 3.15		
54	1.55, 1.50, 3.05		
55	1.65, 1.40, 3.05		Haemorrhage
56	1.60, 1.55, 3.15		
57	1.65, 1.50, 3.15		
58	Died.		

TABLE XVI

A 5.

Day	Tumour diameters and sum (cm.)	Weight	Remarks
1	1.50, 1.40, 2.90		
2	1.40, 1.35, 2.75		
3	1.30, 1.30, 2.60		
4	1.40, 1.30, 2.70		
6	1.40, 1.30, 2.70		
7	1.30, 1.20, 2.50		
8	1.30, 1.25, 2.55		
9	1.30, 1.20, 2.50		
10	1.40, 1.35, 2.75		
11	1.55, 1.25, 2.80		
13	1.60, 1.40, 3.00	42.5 g.	
14	1.70, 1.40, 3.10		
15	1.35, 1.30, 2.65		
16	1.70, 1.30, 3.00		
17	1.65, 1.50, 3.15		
18	1.80, 1.45, 3.25	40.5 g.	
20	1.50, 1.45, 2.95		
21	1.70, 1.30, 3.00		
22	1.80, 1.40, 3.20		
23	1.85, 1.40, 3.25		
24	1.85, 1.40, 3.25		
25	1.70, 1.50, 3.20	39.5 g.	
27	1.60, 1.40, 3.00		Tumour firmer.
28	1.65, 1.45, 3.10		
29	1.75, 1.40, 3.15		
30	1.90, 1.50, 3.40		
31	1.95, 1.55, 3.50		
32	2.05, 1.70, 3.75		
34	2.15, 1.65, 3.80		
35	2.20, 1.60, 3.80	39.0 g.	
36	2.20, 1.70, 3.90		
37	2.25, 1.75, 4.00		
38	2.30, 1.85, 4.15		
39	2.25, 1.85, 4.10	40.0 g.	
41	2.00, 2.00, 4.00		
---	12 mg. 1:2:5:6-dibenzanthracene	---	
42	2.00, 1.80, 3.80		
43	2.00, 1.85, 3.85		
44	2.00, 1.70, 3.70		
45	1.95, 1.75, 3.70		

--- continued over

A 5 continued.

Day	Tumour diameters and sum (cm.)	Weight	Remarks
46	1.95, 1.85, 3.80		
48	1.80, 1.75, 3.55		
49	1.95, 1.75, 3.70		
50	1.90, 1.85, 3.75		
51	1.85, 1.75, 3.60		
52	2.00, 1.60, 3.60		
53	1.85, 1.50, 3.35	40.0 g.	
55	1.80, 1.55, 3.35		
56	1.80, 1.70, 3.50		
57	1.75, 1.60, 3.35		
58	1.85, 1.55, 3.40		
59	1.75, 1.65, 3.40		
60	1.70, 1.50, 3.20		
62	1.85, 1.60, 3.45		
63	1.75, 1.50, 3.25		
64	1.70, 1.50, 3.20	37.0 g.	
65	1.55, 1.50, 3.05		
66	Died.		

2. 1:2:5:6-dibenzacridine.

Although the carcinogenic potency of 1:2:5:6-dibenzacridine is considerably less than that of 1:2:5:6-dibenzanthracene (Barry et al. 1935), it had previously been found (Haddow, Scott and Scott 1937) to possess a primary growth-inhibiting action at least as marked as that of the latter compound. Its effect on spontaneous tumours however showed considerable variation, and the following cases may be taken as examples.

A 50. Intraperitoneal administration of 5 mg. 1:2:5:6-dibenzacridine produced a slowly developing retardation followed by gradual recovery to the original rate of growth. A second injection, 35 days after the first, produced little or no effect (Fig. 44). The animal died after 76 days and showed at death an infected primary tumour (Fig. 45) and voluminous lung metastases.

Fig. 45. A 50 primary tumour.
x 120.

A 90. After a period of slow increase (not charted) this tumour accelerated and grew well over eighteen days. The animal then received three intravenous injections, at intervals of one week, of an aqueous colloidal preparation of 1:2:5:6-dibenzacridine, each dose representing 0.6 mg. hydrocarbon. There resulted a significant alteration in the rate of growth, indicated in Fig. 46. The tumour unfortunately broke down and discharged pus, and the mouse was killed after it had been observed for a total of 75 days. At autopsy the tumour was found to be severely infected, and the lungs contained a single metastatic nodule. The histological structure of the primary tumour is shown in Fig. 47.

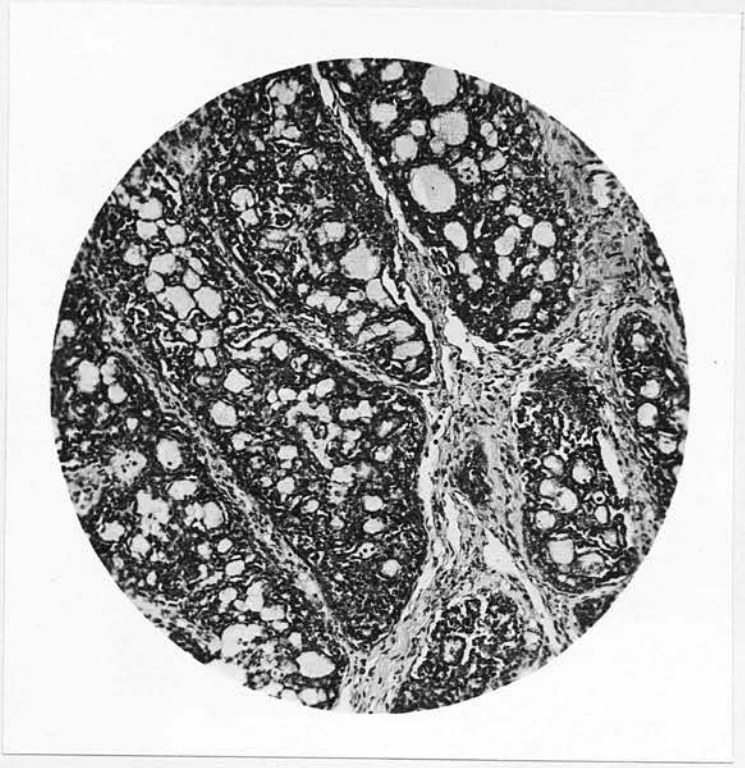


Fig. 47. A 90 primary tumour
x 120.

T2-L. This mouse was pregnant when received, and although its tumour grew steadily and rapidly during gestation, marked inhibition occurred shortly after parturition and continued for about three weeks (see previous paper for an account of this phenomenon with other examples). Recovery to the original growth-rate then took place, and the animal was given a single intraperitoneal injection of 5 mg. 1:2:5:6-dibenzacridine (Fig. 48). Rapid regressive changes followed, and fragments of the tumour were removed for biopsy one week later, when considerable local infection was evident. The tumour bed commenced to granulate, but the resultant ulcer showed no signs of progressive healing. The edges remained rolled and thickened, and this appearance, together with redundant granulations, suggested the possibility of recurrence. In the next few weeks however the condition remained stationary, there being no evidence either of recurrence or of further healing, and the animal continued in fair condition. Death occurred 81 days after the administration of the hydrocarbon, and 128 days from the time of first observation. Autopsy showed the primary area still unhealed and infected, and the local tissue was removed for section. A pleural effusion was present, and also, of considerable diagnostic importance, a solitary nodule in the right lung.

TABLE XVII

T2-L.

Day	Tumour diameters and sum (cm.)	Weight	Remarks
1	1.10, 0.80, 1.90		Pregnant
2	1.15, 0.85, 2.00		
3	1.30, 0.90, 2.20	43.0 g.	
5	1.30, 1.00, 2.30		
6	1.30, 1.05, 2.35		
7	1.40, 1.10, 2.50		
8	1.40, 1.05, 2.45		
9	1.40, 1.10, 2.50		Littered
10	1.60, 1.35, 2.95	40.0 g.	
12	1.50, 1.25, 2.75		
13	1.60, 1.40, 3.00		
14	1.60, 1.20, 2.80		
15	1.55, 1.25, 2.80		
16	1.40, 1.20, 2.60		
17	1.40, 1.20, 2.60	38.5 g.	
19	1.35, 1.10, 2.45		
20	1.35, 1.10, 2.45		
21	1.40, 1.10, 2.50		
22	1.55, 1.20, 2.75		
23	1.40, 1.05, 2.45		
24	1.50, 1.05, 2.55	37.0 g.	
26	1.50, 1.10, 2.60		
27	1.40, 1.25, 2.65		
28	1.50, 1.25, 2.75		
29	1.60, 1.25, 2.85		
30	1.40, 1.30, 2.70	36.0 g.	
31	1.50, 1.20, 2.70		
33	1.50, 1.20, 2.70		
34	1.55, 1.25, 2.80		
35	1.60, 1.30, 2.90		
36	1.70, 1.40, 3.10		
37	1.65, 1.35, 3.00		
38	1.60, 1.45, 3.05		
40	1.70, 1.55, 3.25		
41	1.75, 1.55, 3.30	37.0 g.	
42	1.85, 1.55, 3.40		
43	1.90, 1.70, 3.60		
44	1.80, 1.75, 3.55		
45	1.85, 1.70, 3.55	36.0 g.	
47	2.05, 2.00, 4.05		
--- 5 mg. 1:2:5:6-dibenzacridine ---			

--- continued over

T2-L continued.

Day	Tumour diameters and sum (cm.)	Weight	Remarks
48	2.05, 1.75, 3.80		
49	1.95, 1.70, 3.65		
50	1.95, 1.65, 3.60		
51	1.95, 1.50, 3.45		
52	1.80, 1.40, 3.20	36.0 g.	
54	1.70, 1.45, 3.15		
55	1.45, 1.35, 2.80		Biopsy.
59		35.0 g.	
62			Tumour bed granulating.
65			? recurrence.
68			"
69			still doubtful.
70		34.0 g.	
72			granulations redundant.
77		34.0 g.	Not healing. Edges rolled and thickened.
84			Doubtful.
85		33.0 g.	
90			Not yet healed but stationary. Probably not recurrence.
93		30.0 g.	Edges softer.
111			Still unhealed. Ulcer infected.
128	-- Died --		

3. Methylcholanthrene.

This markedly carcinogenic compound showed considerable toxicity in various experiments, but clear evidence was obtained of its inhibitory activity.

C7-D.B. The tumour occurred in a mouse of the dba pure line. After being observed for a control period of one month the animal received 12 mg. methylcholanthrene, when considerable retardation followed (Fig. 49). This however was accompanied by marked infection of the tumour and discharge from its substance, so that no healthy tissue was available for section when the animal died 50 days after injection.

T A B L E XVIII

C7-D.B.

Day	Tumour diameters and sum (cm.)	Weight	Remarks
1	1.30, 1.20, 2.50		
2	1.25, 1.05, 2.30		
3	1.40, 1.05, 2.45	29.0 g.	
4	1.40, 1.10, 2.50		
5	1.35, 1.10, 2.45		
6	1.35, 1.10, 2.45		
8	1.45, 1.05, 2.50		
9	1.45, 1.05, 2.50		
10	1.50, 1.20, 2.70	28.5 g.	
11	1.60, 1.25, 2.85		
12	1.65, 1.20, 2.85		
13	1.60, 1.25, 2.85		
15	1.65, 1.25, 2.90		
16	1.75, 1.40, 3.15		
17	1.65, 1.35, 3.00		
18	1.85, 1.40, 3.25	29.0 g.	
23	1.80, 1.40, 3.20		
24	1.80, 1.50, 3.30		
26	2.00, 1.40, 3.40	27.0 g.	
27	1.70, 1.40, 3.10		
29	2.05, 1.55, 3.60		
---	12 mg. methylcholanthrene ---		
30	1.90, 1.45, 3.35		
31	1.85, 1.50, 3.35		
32	1.95, 1.45, 3.40		
33	1.95, 1.40, 3.35	30.0 g.	
34	1.90, 1.45, 3.35		
36	1.95, 1.65, 3.60		
37	1.85, 1.55, 3.40		
38	1.95, 1.55, 3.50		
39	2.00, 1.65, 3.65		
40	2.00, 1.55, 3.55	30.0 g.	
41	2.00, 1.50, 3.50		
43	1.95, 1.60, 3.55		
44	1.85, 1.60, 3.45		
45	2.00, 1.60, 3.60		
46	2.00, 1.50, 3.50		
47	1.95, 1.55, 3.50	28.0 g.	
48	2.00, 1.60, 3.60		

--- continued over

C7-D.B. continued.

Day	Tumour diameters and sum (cm.)	Weight	Remarks
50	2.10, 1.65, 3.75		
51	2.05, 1.70, 3.75		
52	2.00, 1.65, 3.65		
53	1.95, 1.65, 3.60		
54	2.00, 1.75, 3.75	28.0 g.	
55	2.00, 1.70, 3.70		
57	2.00, 1.60, 3.60		
58	2.15, 1.70, 3.85		
59	2.05, 1.65, 3.70		
60	2.00, 1.65, 3.65		
61	2.05, 1.70, 3.75		
62	2.05, 1.60, 3.65		
64	2.05, 1.55, 3.60		
65	2.10, 1.60, 3.70		
67	1.95, 1.60, 3.55		
68	2.05, 1.55, 3.60	26.0 g.	
69	1.90, 1.55, 3.45		
72	2.05, 1.55, 3.60		

4. Styryl 430.

It was considered important to test the trypanocidal substance 2(p-amino styryl)6(p-acetyl-:amino benzoylamino) quinoline methoacetate, (styryl 430), the carcinogenic properties of which were discovered by Browning and his co-workers (Browning, Cohen, Cooper, Ellingworth and Gulbransen 1933, Browning, Gulbransen and Niven 1936), as representing an active tumour-producing compound not chemically related to the carcinogenic hydrocarbons. The response produced was in most cases similar to that already described, and in individual cases varied from little or no effect to a considerable and protracted inhibition, although no regressive changes were ever observed.

M 4. After a period of stasis this tumour grew steadily for 25 days, when the animal was given two subcutaneous injections of 1.0 ml. 0.5 per cent. styryl 430 in water. Fig. 50 shows the resulting inhibition, which was maintained for 70 days, the animal dying 118 days from the first observat-:ion.

T A B L E XIX

M 4.

Day	Tumour diameters and sum (cm.)	Weight	Remarks
1	1.35, 1.30, 2.65	38.0 g.	
3	1.30, 1.20, 2.50		
5	1.25, 1.25, 2.50		
6	1.45, 1.25, 2.70		
7	1.35, 1.25, 2.60		
8	1.25, 1.25, 2.50	40.0 g.	
9	1.30, 1.30, 2.60		
10	1.20, 1.20, 2.40		
15	1.20, 1.15, 2.35		
16	1.35, 1.25, 2.60	37.0 g.	
17	1.35, 1.25, 2.60		
19	1.35, 1.25, 2.60		
20	1.25, 1.20, 2.45		
21	1.40, 1.25, 2.65		
22	1.40, 1.35, 2.75		
23	1.45, 1.40, 2.85	40.0 g.	
24	1.55, 1.45, 3.00		
27	1.70, 1.40, 3.10		
29	1.60, 1.45, 3.05		
30	1.60, 1.35, 2.95	41.0 g.	
31	1.60, 1.40, 3.00		
33	1.90, 1.50, 3.40		
34	1.85, 1.60, 3.45		
40	2.00, 1.65, 3.65		
42	1.90, 1.55, 3.45		
---	1.0 ml. styryl 430 0.5 per cent. ---		
43	2.05, 1.55, 3.60		
44	2.00, 1.55, 3.55		
47	1.95, 1.60, 3.55		
---	1.0 ml. styryl 430 0.5 per cent. ---		
48	1.80, 1.60, 3.40		
49	2.00, 1.55, 3.55		
50	1.90, 1.70, 3.60		
51	2.00, 1.75, 3.75	39.0 g.	
54	1.90, 1.70, 3.60		
55	1.90, 1.65, 3.55		
56	1.75, 1.55, 3.30		

--- continued over

M 4 continued.

Day	Tumour diameters and sum (cm.)	Weight	Remarks
58	1.75, 1.75, 3.50		
61	1.80, 1.70, 3.50		
62	1.90, 1.75, 3.65		
63	1.75, 1.70, 3.45		
64	1.80, 1.75, 3.55	37.0 g.	
65	1.80, 1.65, 3.45		
66	1.85, 1.65, 3.50		
69	1.85, 1.70, 3.55		
70	1.90, 1.75, 3.65		
71	2.00, 1.75, 3.75		
72	1.90, 1.75, 3.65	35.0 g.	
73	1.85, 1.70, 3.55		
75	1.90, 1.65, 3.55		
76	2.00, 1.75, 3.75		
77	1.95, 1.55, 3.50		
78	1.90, 1.45, 3.35		
79	1.95, 1.50, 3.45		
82	1.90, 1.55, 3.45	30.0 g.	
83	1.90, 1.50, 3.40		
84	1.80, 1.55, 3.35		
85	1.90, 1.55, 3.45		
89	1.95, 1.55, 3.50		
91	1.65, 1.35, 3.00	28.0 g.	
93	1.80, 1.60, 3.40		
94	1.90, 1.60, 3.50		
97	1.95, 1.60, 3.55		
98	1.85, 1.75, 3.60		
99	1.95, 1.70, 3.65		
100	2.05, 1.80, 3.85		
101	1.85, 1.70, 3.55		
103	1.95, 1.60, 3.55	33.0 g.	
105	2.10, 1.55, 3.65		
106	2.20, 1.80, 4.00		
107	1.95, 1.85, 3.80		
110	1.95, 1.70, 3.65		
119	Died.		

T 51. This rapidly growing tumour increased in size uniformly for 19 days, when the mouse received 1.0 ml. 0.5 per cent. styryl 430 in water subcutaneously. Retardation was considerable and persisted to the time of death 38 days later (Fig. 51). During this period a second tumour was detected in another mamma. Autopsy showed numerous pulmonary deposits, and Fig. 52 shows the structure of the primary tumour.

A 142. Three separate mammary carcinomata were present in this animal when it was received, and three others developed in the succeeding 84 days. Fig. 53 shows the inhibitory influence of two subcutaneous injections of 5 mg. styryl 430 on the rate of growth of the oldest tumour. Autopsy revealed a small metastatic nodule in the heart muscle and a similar deposit in the lungs. The structure of the primary tumour is shown in Fig. 54.

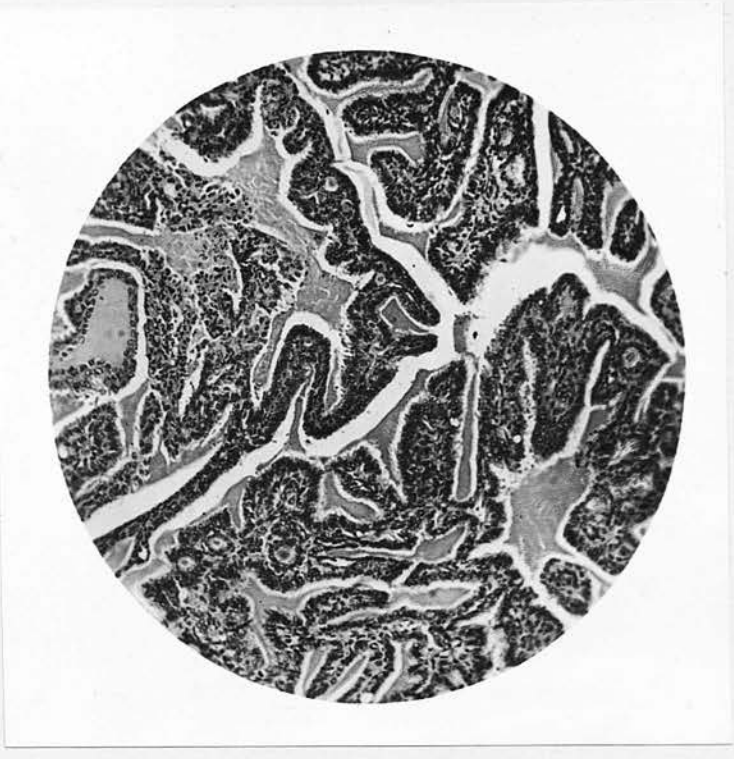


Fig. 52. T 51 primary tumour
x 120.

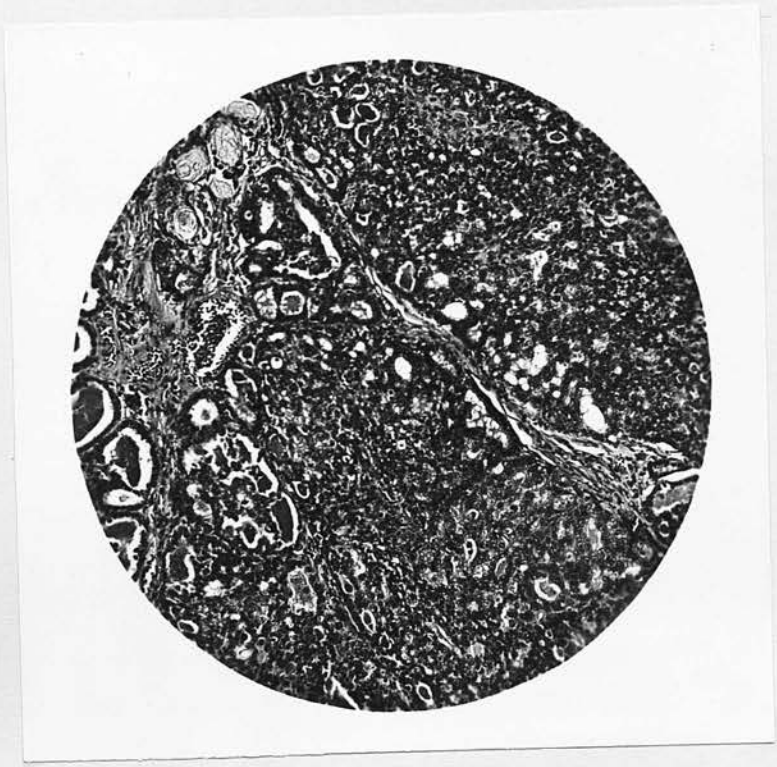


Fig. 54. A 142 primary tumour x 120.

A 140. Fig. 55 represents a similar response following a single intraperitoneal injection of 10 mg. styryl 430. Numerous secondary nodules were present in the lungs at death.

T 40. This animal was given three doses of 5 mg. styryl 430. The tumour continued to increase in size, although at a greatly lessened rate (Fig. 56), and the mouse was killed after a total of 69 days. A small independent tumour was discovered after death, and voluminous metastases were present in the lungs and chest wall. The histological appearance of the primary tumour and a pulmonary nodule are shown in Figs. 57 and 58.

G2. This mouse was $7\frac{1}{2}$ months old at the time of detection of its tumour, which was observed almost from its inception and grew moderately rapidly. Two subcutaneous injections of 5 mg. styryl 430 produced little change in the rate of growth, but some inhibition occurred following a third dose (Fig. 59). The mouse died when it had borne its tumour for some 90 days. It showed considerable loss of weight terminally, and multiple deposits were present in the lungs.

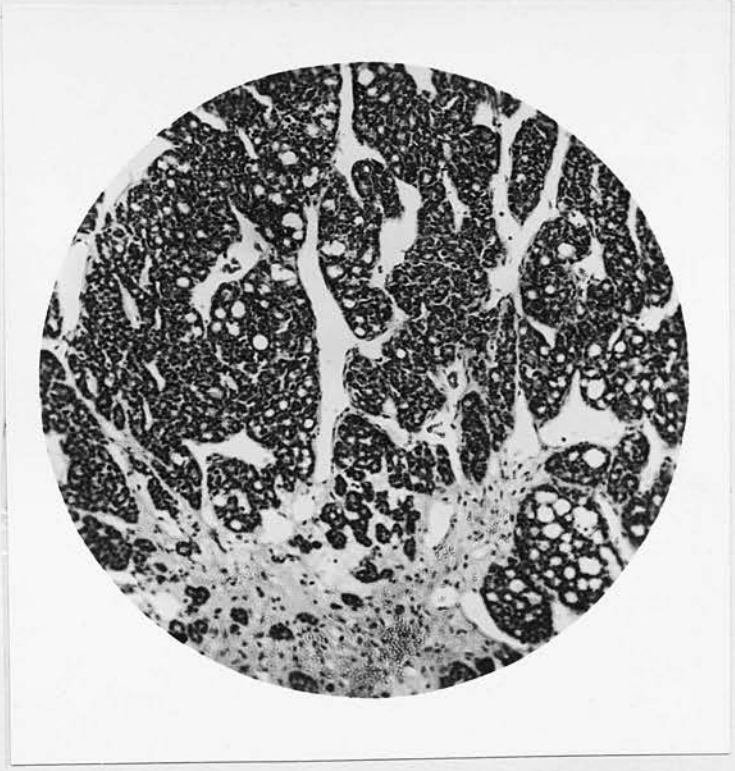


Fig. 57. T 40 primary tumour
x 120.

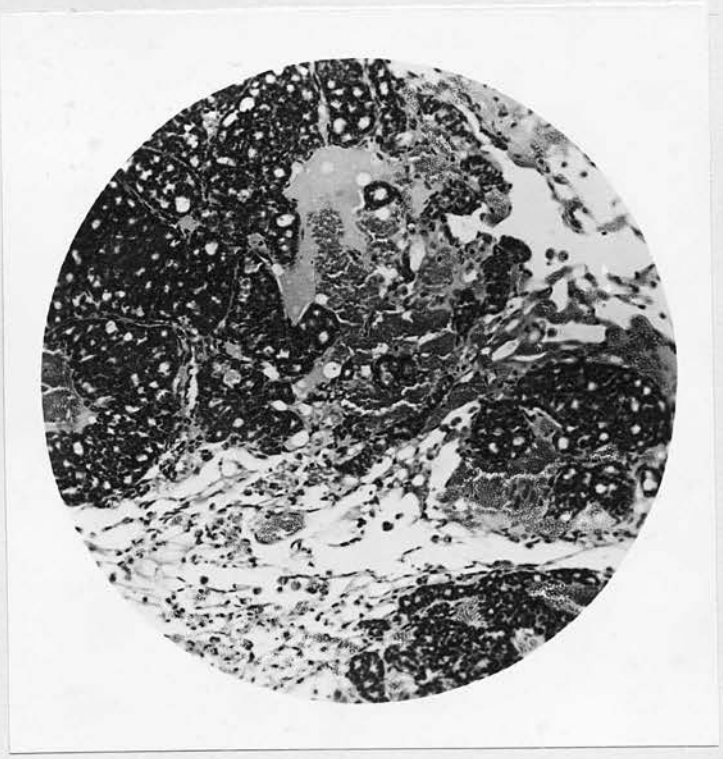


Fig. 58. T 40 pulmonary deposit
x 120.

D.B./172. The tumour was observed three weeks after the birth of the last litter in a dilute brown mouse 12 months old which had littered five times. Fig. 60 shows a moderate alteration in growth-rate produced by a single dose of 5 mg. styryl 430.

T 49; D.B. 7/2; D.B./109.

A number of tumours gave only slight or no response to administration of styryl 430, and Figs. 61-63 show three examples.

III. Non-carcinogenic compounds.

1. Pyrene.

This compound had previously been found to be quite devoid of any effect on body-growth or on the rate of growth of transplantable tumours (Haddow and Robinson 1937, Haddow, Scott and Scott 1937), and experiments with spontaneous tumours have given a similar result.

A 60. Fig. 64 shows that while admininistration of 12 mg. pyrene produced a slight temporary interference, recovery was rapid and the tumour continued to grow at its original rate. This observation was controlled by the later administration of 12 mg. methylcholanthrene, which produced a moderate but undoubtedly significant change. Section of the tumour showed a relatively uniform and simple structure of adenomatous type (Fig. 65).

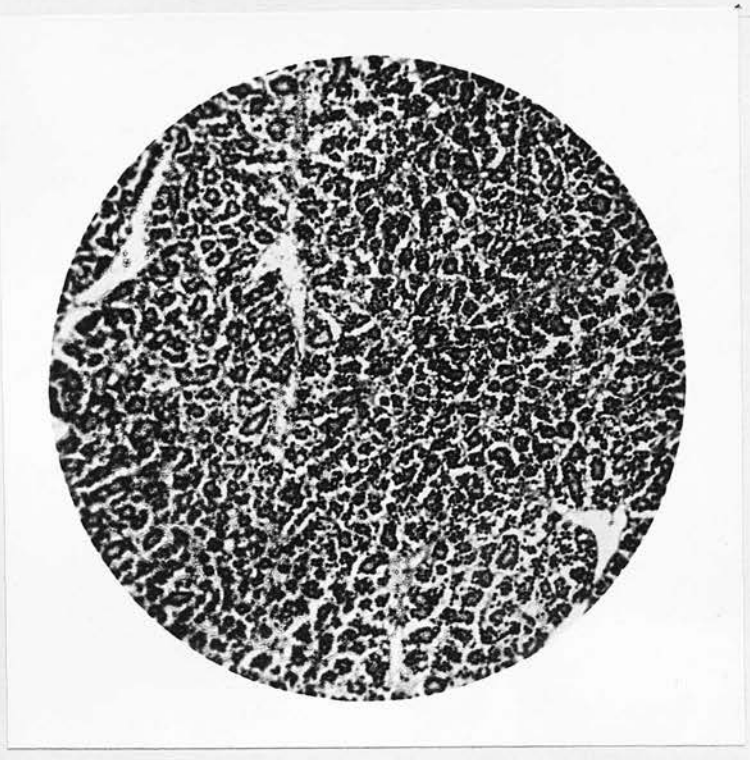


Fig. 65. A 60 primary tumour x 120.

T A B L E XX

A 60.

Day	Tumour diameters and sum (cm.)	Weight	Remarks
1	2.10, 1.60, 3.70		
2	2.10, 1.60, 3.70		
3	2.15, 1.60, 3.75	50.0 g.	
4	2.15, 1.65, 3.80		
5	2.05, 1.80, 3.85		
6	2.15, 1.95, 4.10		
8	2.15, 1.90, 4.05		
9	2.10, 2.00, 4.10		
10	2.10, 2.00, 4.10	49.0 g.	
---	12 mg. pyrene ---		
11	2.15, 1.85, 4.00		
12	1.85, 1.85, 3.70		
13	2.00, 1.90, 3.90		
15	2.25, 1.95, 4.20		
16	2.15, 1.95, 4.10		
17	2.05, 2.05, 4.10		
18	2.15, 2.15, 4.30	49.0 g.	
23	2.15, 2.15, 4.30		
24	2.35, 2.25, 4.60		
26	2.35, 2.25, 4.60	50.0 g.	
27	2.40, 2.20, 4.60		
29	2.55, 2.30, 4.85		
---	12 mg. methylcholanthrene ---		
30	2.65, 2.40, 5.05		
31	2.50, 2.30, 4.80		
32	2.35, 2.15, 4.50		
33	2.30, 2.20, 4.50	55.0 g.	
34	2.50, 2.25, 4.75		
36	2.55, 2.35, 4.90		
37	2.45, 2.30, 4.75		
38	2.30, 2.25, 4.55		
39	2.45, 2.35, 4.80		
40	2.55, 2.50, 5.05	55.0 g.	
41	2.35, 2.30, 4.65		
43	2.70, 2.35, 5.05		
44	2.65, 2.25, 4.90		
45	2.65, 2.35, 5.00		
46	2.60, 2.30, 4.90		
47	2.65, 2.20, 4.85	50.0 g.	
48	2.60, 2.15, 4.75		
50	2.70, 2.15, 4.85		
51	2.75, 2.25, 5.00		
52	2.70, 2.15, 4.85		
53	2.75, 2.25, 5.00		
54	2.85, 2.30, 5.15	49.0 g.	
55	2.90, 2.25, 5.15		
57	2.90, 2.25, 5.15		
58	Died		

A 61. In this case the intraperitoneal injection of 12 mg. pyrene produced no interference of any kind in the rate of growth (Fig. 66). The tumour was observed for 45 days and was then used for another purpose. Its histology was somewhat variable but mainly consisted of regularly disposed and well formed acini. A secondary nodule in the lung possessed a similar structure.

A 55. A similar example (Fig. 67). Section of this tumour revealed a highly malignant adenocarcinoma of large-celled type.

TABLE XXI

A 61.

Day	Tumour diameters and sum (cm.)	Weight	Remarks
1	1.95, 1.85, 3.80		
2	2.00, 2.00, 4.00		
3	2.05, 1.95, 4.00	39.0 g.	
4	2.10, 1.95, 4.05		
5	2.00, 2.00, 4.00		
6	2.05, 1.95, 4.00		
8	2.20, 2.15, 4.35		
9	2.20, 2.10, 4.30		
10	2.20, 2.05, 4.25	38.0 g.	
---	12 mg. pyrene ---		
11	2.30, 2.10, 4.40		
12	2.15, 2.00, 4.15		
13	2.20, 2.10, 4.30		
15	2.15, 2.05, 4.20		
16	2.35, 2.10, 4.45		
17	2.25, 2.10, 4.35		
18	2.25, 2.10, 4.35	39.0 g.	
23	2.35, 2.30, 4.65		
24	2.50, 2.10, 4.60		
26	2.40, 2.20, 4.60	40.0 g.	
27	2.35, 2.20, 4.55		
29	2.50, 2.25, 4.75		
30	2.55, 2.35, 4.80		
31	2.50, 2.30, 4.80		
32	2.60, 2.30, 4.90		
33	2.55, 2.30, 4.85	40.0 g.	
34	2.55, 2.35, 4.90		
36	2.75, 2.40, 5.15		
37	2.75, 2.40, 5.15		
38	2.80, 2.30, 5.10		
39	2.80, 2.55, 5.35		
40	2.80, 2.45, 5.25	43.0 g.	
41	2.75, 2.45, 5.20		
43	2.75, 2.50, 5.25		
44	2.75, 2.60, 5.35		

2. 1:2:3:4-dibenzanthracene.

Cook, Hieger, Kennaway and Mayneord (1932, p. 466) described tumours obtained in a few mice which survived for exceptional periods in painting experiments with 1:2:3:4-dibenzanthracene. But the material used in these experiments was not quite pure, and no tumours arose in a further test in which pure 1:2:3:4-dibenzanthracene was applied to 20 mice (Barry, Cook, Haslewood, Hewett, Hieger and Kennaway 1935, p. 327). In this series the last mouse died on the 487th day, so that it could not be said that no tumours would have been obtained had the mice lived exceptionally long, as in the earlier series with the impure material. It is obvious however that 1:2:3:4-dibenzanthracene can possess no marked carcinogenic activity, and it is possible indeed that it is quite inactive. In the present study experiments were carried out with a specimen of the pure compound.

A 174. When its tumour had shown good growth in the control period over 14 days this mouse was given two doses of 10 and 20 mg. 1:2:3:4-dibenzanthracene at an interval of eleven days. Although slight interference followed, growth was resumed at the initial rate (Fig. 68). The animal died as a result of numerous and massive pulmonary metastases. Section showed a typical mammary adenocarcinoma.

D.B./116. Fig. 69 shows a similar result following the intraperitoneal injection of a single dose of 20 mg. 1:2:3:4-dibenzanthracene. Histological diagnosis: mammary carcinoma with small-celled acini.

A 173. Fig. 70 shows only slight interference after a total dosage of 30 mg., followed by unimpeded growth. Both the primary tumour and metastatic deposits possessed the structure of a large-celled adenocarcinoma, with areas of symplastic arrangement.

M 6. This example again shows a temporary interference, possibly of non-specific toxic origin, following the administration of 20 mg. 1:2:3:4-dibenzanthracene (Fig. 71) and again succeeded by resumed growth at the characteristic rate.

R 1. Fig. 72 is of special interest as showing a particularly marked immediate retardation (after 10 mg. 1:2:3:4-dibenzanthracene) followed by a compensatory recovery at a rate somewhat greater than that in the control period.

3. (i) acenaphthanthracene;
(ii) 1:2:5:6-dibenzphenazine.

It will be recalled (Haddow and Robinson 1937) that while a high degree of correlation was found to exist between growth-retarding power and carcinogenic activity, the parallelism was not absolute, and several non-carcinogenic compounds were shown to produce inhibition. In the present investigation this has been found to apply to acenaphthanthracene and 1:2:5:6-dibenzphenazine, neither of which compounds has produced tumours of the mouse or rat (Cook 1932; Barry et al. 1935).

(i) acenaphthanthracene.

A 84. Fig. 73 demonstrates the retarding effect of 12 mg. acenaphthanthracene on the rate of growth of two independent carcinomata in mouse A 84.

A 86. This tumour occurred in a male mouse and proved to be a slow-growing osteochondrosarcoma of the tibia. After a control period of three weeks the animal was given two injections of 12 mg. acenaphthanthracene at an interval of ten days. The tumour thereafter grew at a greatly decreased rate to the time of death on the 83rd day (Fig. 74). Autopsy showed that death was possibly due to peritoneal adhesions produced by the injections. The lungs contained two nodules of a structure similar to that of the primary tumour. The histological picture, together with the radiographic appearance of the tumour on the 2nd. and 42nd. days of observation, are shown in Figs. 24-26 in the previous section.

(ii). 1:2:5:6-dibenzphenazine.

This compound proved less soluble in oil than 1:2:5:6-dibenzacridine, which in turn is less soluble than 1:2:5:6-dibenzanthracene. Several experiments have indicated that the toxicity of these substances may be in the order dibenzanthracene, dibenzacridine, dibenzphenazine.

T 23. Fig. 75 indicates what is probably a significant degree of inhibition produced by two doses of 6 mg. 1:2:5:6-dibenzphenazine.

D.B./B6. In this tumour a very slight but again probably significant response followed two doses of 6 mg. and 3 mg. 1:2:5:6-dibenzphenazine. (Fig. 76).

A 112. Individual variation in response was again a feature of experiments with 1:2:5:6-dibenzphenazine, and Fig. 77 shows active regression following the administration of 6 mg. of this compound. The mouse however lived for only 17 days following injection. Histological examination of the tumour showed active cellular invasion and disintegration of its structure (Fig. 78), with areas showing considerable increase in stroma (Fig. 79).

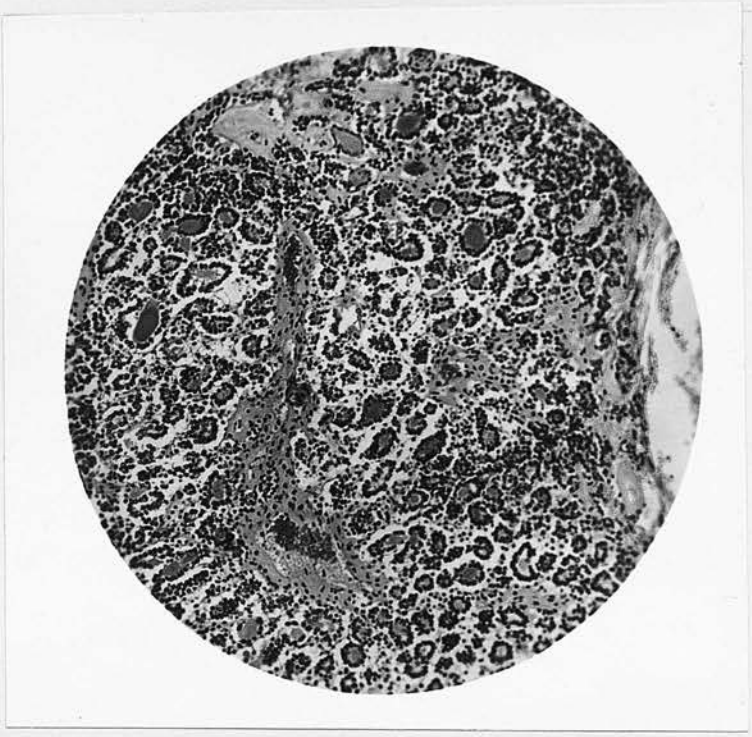


Fig. 78. A 112 primary tumour x 120.

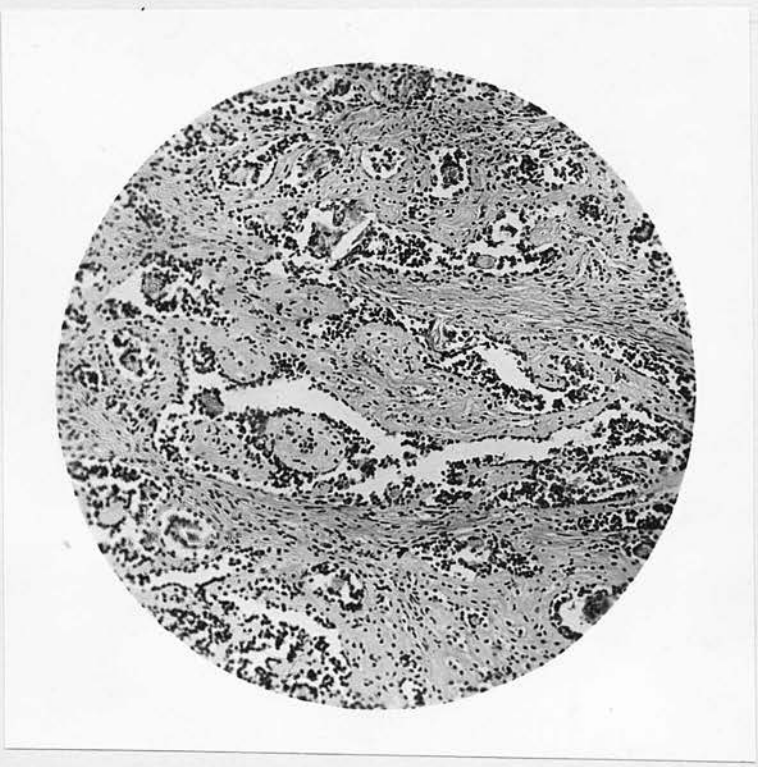


Fig. 79. A 112 primary tumour (stroma increase) x 120.

IV. Oestrone benzoate.

In view of the statement by Zondek (1936a,b) that prolonged treatment with oestrin produces considerable inhibition of body-growth in the rat, a number of experiments were carried out in which large doses of oestrone benzoate in oil were administered to mice bearing spontaneous mammary carcinomata.

P.B./2. Fig. 80 shows a characteristic mild interference in growth-rate on two separate occasions following the injection of 8 mg. oestrone benzoate in oil. Autopsy revealed considerable uterine hypertrophy and dilatation of the bladder, and the lungs contained several metastatic tumour nodules. The histology of the primary tumour is shown in Fig. 81.

A 73. This tumour gave a similar type of response (Fig. 82). Autopsy showed congestion of the tumour substance and considerable enlargement of the uterus.

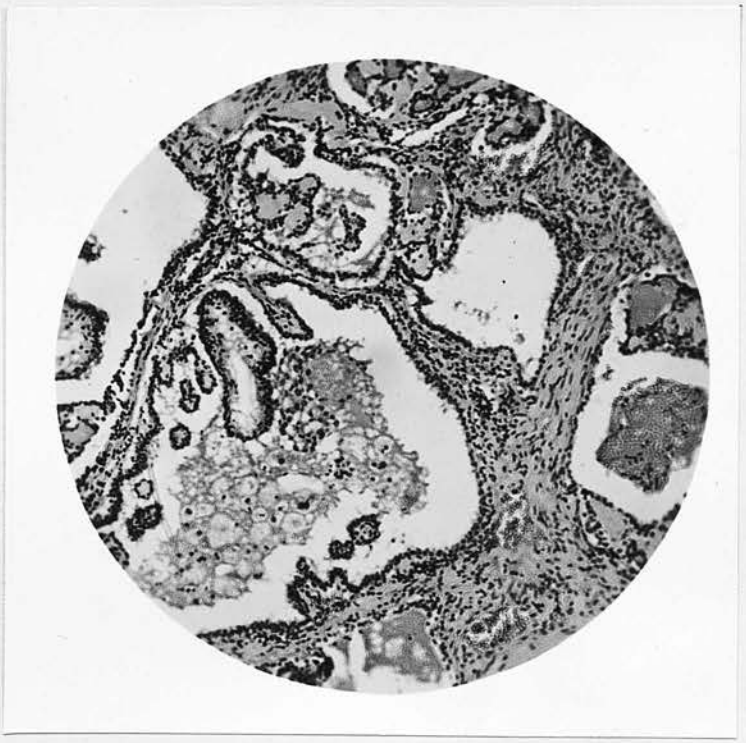


Fig. 81. P.B./2 primary tumour.
x 120.

A 66. In the first place this tumour was employed to test the response to 12 mg. pyrene. It is evident from Fig. 83 that following a temporary disturbance growth proceeded at an unaltered rate for some thirty days (see also above). The mouse then received two doses of 8 mg. oestrone benzoate which, as in the above experiments, produced some slight degree of retardation. Section revealed a histological structure of unusual uniformity, consisting of small-celled acini in regular arrangement.

D.B./58. Fig. 84 illustrates an example of complete absence of response on the part of the tumour to 8 mg. oestrone benzoate. Autopsy however showed the expected uterine hypertrophy together with cystic ovarian changes.

A 63. In this case, as in the last, no evidence of inhibition was obtained (Fig. 85). Section showed a papillary adenocarcinoma with areas of active secretion.

V. Discussion.

When account has been taken of the individual variation in response which is a feature of all such experiments, it will be seen that the above data are in general agreement with those previously obtained in the case of transplantable tumours and body growth (Haddow and Robinson 1937, Haddow, Scott and Scott 1937). Somewhat similar results were obtained by Pybus and Miller (1937), who treated sixty mice bearing spontaneous mammary tumours with intraperitoneal injections of a colloidal solution of 1:2:5:6-dibenzanthracene (to a maximum total dosage of 10 mg.) and obtained temporary inhibition in 11 cases and partial or complete regression in 8, in three of which the tumour did not re-appear before death. On the other hand, the formation of new tumours during treatment was not prevented. In additional experiments by these authors, massive doses of 10 or 15 mg. caused a temporary regression of the tumour in 26 of 61 cases, the effect reaching its maximum about the third and fourth weeks. Such treatment however caused considerable mortality from peritoneal adhesions and ascites.

As to the means by which retardation is produced little at present can be said. Although the carcinogenic hydrocarbons are of undoubted toxicity, it is probably unwise to refer their inhibitory effect to toxic action in any general sense, and the writer believes it to be attributable to toxicity of a special and possibly specific kind.

The possible etiological significance of the inhibitory phenomenon will not be further discussed in the present paper (see Haddow 1938), but attention may be drawn to the positive results obtained with the non-carcinogenic compounds acenaphthanthracene and 1:2:5:6-dibenzphenazine. In earlier work similar activity was shown by 3-methyl-1:2-benzanthracene and 7-methyl-1:2-benzanthracene, (Haddow and Robinson 1937), and although neither of these compounds had produced tumours at that date it is of interest that in recent experiments (personal communication from Professor Kennaway) the former substance has given rise to four sarcomata of the usual type in mice to which it had been administered by injection sub cutem in sesame oil. Two of these tumours have now been transplanted.

In previous papers emphasis was laid on the comparative prolongation of the inhibitory response, and this is also a feature of the present results. Those experiments suggested that the relative persistence of this type of effect might reasonably be regarded as a characteristic of carcinogenic compounds, and equally for spontaneous tumours there is no evidence to show that it can readily be produced by miscellaneous substances, although the available data are in this case fewer. Comparison may be made with experiments by Boyland (see Chemistry and Industry 1938 p. 188) on the inhibition of the growth of spontaneous mammary tumours of the mouse by various aromatic compounds. Substances such as sulphanilic acid, p-aminobenzenesulphonamide and the pp'-dinitrodiphenyl and pp'-diaminodiphenyl sulphides, sulphoxides and sulphones were given daily by mouth, but the inhibitory effect was apparent only so long as the substances were being administered, or passed off within a few days when they were discontinued.

VI. Summary.

1. Parenteral administration of 1:2:5:6-dibenzanthracene in mice bearing spontaneous neoplasms (mainly carcinomata of the mammary gland) resulted in most cases in a prolonged inhibition of the rate of tumour-growth. The degree of individual response varied from slight to marked retardation, and a few cases manifested active regression, either partial or complete.

2. The same result, with a corresponding degree of variation, was produced by the carcinogenic substances 1:2:5:6-dibenzacridine, methyl cholanthrene and styryl 430.

3. The non-carcinogenic compounds pyrene and 1:2:3:4-dibenzanthracene provoked either no response or a transient interference with growth-rate followed by complete recovery.

4. Instances are given in which the non-carcinogenic substances acenaphthanthracene and 1:2:5:6-dibenzphenazine led to a retardation of growth not different from that produced by carcinogenic compounds.

5. Administration of large doses of oestrone benzoate produced in some cases no inhibition and in others a moderate retardation with a tendency to recovery.

6. The inhibitory response brought about by carcinogenic compounds and by certain related non-carcinogenic compounds is attributed to the possession by such substances of toxicity of a special kind.

VII. References.

Barry, G., Cook, J.W., Haslewood, G.A.D., Hewett, C.L., Hieger, I. and Kennaway, E.L. 1935 The production of cancer by pure hydrocarbons. III. Proc. Roy. Soc., B, 117, 318-351.

Browning, C.H., Cohen, J.B., Cooper, K.E., Ellingworth, S. and Gulbransen, R. 1933 Antiseptic and trypanocidal action of some benzoylamino quinoline anil and styryl compounds. Proc. Roy. Soc., B, 113, 300-307.

Browning, C.H., Gulbransen, R. and Niven, J.S.F. 1936 Sarcoma production in mice by a single subcutaneous injection of a benzoylamino quinoline styryl compound. Jour. Path. Bact., 42, 155-159.

Cook, J.W. 1932 The production of cancer by pure hydrocarbons. II. Proc. Roy. Soc., B, 111, 485-496.

Cook, J.W., Hieger, I., Kennaway, E.L. and Mayneord, W.V. 1932 The production of cancer by pure hydrocarbons. I. Proc. Roy. Soc., B, 111, 455-484.

Haddow, A.	1935	Influence of certain polycyclic hydrocarbons on the growth of the Jensen rat sarcoma. Nature, 136, 868-869.
-------	1937	On secondary colony development in bacteria and an analogy with tumour production in higher forms. Acta, Union internationale contre le cancer, 2, 376-401.
-------	1938	Cellular inhibition and the origin of cancer. Acta, Union internationale contre le cancer, 3, (in press).
Haddow, A. and Robinson, A.M.	1937	The influence of various polycyclic hydrocarbons on the growth-rate of transplantable tumours. Proc. Roy. Soc., B, 122, 442-476.
Haddow, A., Scott, C.M. and Scott, J.D.	1937	The influence of carcinogenic and other hydrocarbons on body growth in the rat. Proc. Roy. Soc., B, 122, 477-507.

Murray, W.S. 1934 The breeding behavior of the dilute brown stock of mice (Little dba).

Amer. Jour. Cancer, 20, 573-593.

Pybus, F.C. and Miller, E.W. 1937 On the effect of 1:2:5:6-dibenzanthracene on spontaneous mouse tumours.

Brit. Jour. Exp. Path., 18, 126-137.

Zondek, B. 1936a The inhibitory effect of follicular hormone on the anterior lobe of the pituitary gland.

Lancet, i, 10-12.

-------- 1936b Impairment of anterior pituitary functions by follicular hormone.

Lancet, ii, 842-847.

THE INFLUENCE OF CARCINOGENIC SUBSTANCES ON SARCOMATA INDUCED BY THE SAME AND OTHER COMPOUNDS.

THE INFLUENCE OF CARCINOGENIC SUBSTANCES ON SARCOMATA INDUCED BY THE SAME AND OTHER COMPOUNDS.

The influence of carcinogenic substances on sarcomata induced by the same and other compounds.

I. Introduction.

In a recent review (Haddow 1938a) and in other papers (see Haddow 1936, Haddow and Robinson 1937) the author propounded the theory that the cancer cell represents a discontinuous and irreversible variant of the corresponding somatic cell, differing from the latter in the characters of fission-rate and metabolic behaviour. It was suggested that the problem of the origin of cancer might therefore be regarded as a special case in the origin of cellular variation, and suggestions were made as to the fundamental nature of the environmental changes leading to tumour production. In particular, it was postulated that these changes are frequently inhibitory in nature, and that the increase in growth-rate which marks the emergence of malignancy is due in many cases not to any primary stimulation of growth but represents the emancipation of potentially variable cells from chronic inhibition. Evidence was presented to show that a number of the carcinogenic hydrocarbons exhibit growth-inhibitory properties not possessed by a considerable number of related non-carcinogenic substances, and it was

further found (Haddow, Scott and Scott 1937) that the inhibitory effect thus produced differed from that manifested by diverse poisons in its relative prolongation, even after a single administration, and in its primary independence of toxic action in any general or non-specific sense. The stages in the production of malignancy by the carcinogenic hydrocarbons (and possibly by other tumour-producing agents such as x- and gamma-radiation) were therefore conceived to be (1) the enforcement of a sustained retardation in the rate of growth of the treated normal cells, and (2) an irreversible dedifferentiation of the affected but viable units accompanied by permanent metabolic alterations, increase in growth-rate, and functional release from environmental inhibition.

According to this argument, cancer cells arise and commence their career of proliferation under conditions which impair the life of normal cells. This result they presumably accomplish in virtue of their altered metabolism, which probably possesses distinct survival value and in effect confers a biological advantage upon those cells which manifest it. But it is also important to determine the precise relationship between the malignant variant and the dysgenic factors of the environment in which

it arose. Haddow and Robinson (1937) recorded a few observations on the behaviour of two transplantable rat sarcomata of known causation. The first tumour appeared some five months after the subcutaneous injection of 3:4-benzpyrene and proved to be easily propagable by grafting. A number of experiments were carried out within the first few generations, with results which showed that the tumour was considerably less sensitive to the action of 3:4-benzpyrene than was the Walker carcinoma, although it proved moderately sensitive to inhibition by 1:2:5:6-dibenzanthracene and 3-methyl-1:2-benzanthracene. The second tumour was a sarcoma (LR-10) induced in the Research Institute of the Royal Cancer Hospital by means of 1:2:5:6-dibenzanthracene and subsequently maintained in serial transplantation for over three years: when tested in its 87th generation it was found to be fairly sensitive to inhibition by the compound used for its induction.

In view of the great theoretical interest of the problem, this branch of the investigation was extended, and the present paper records the results of over thirty experiments in which tumours induced by various hydrocarbons were later exposed to the action of the same and other carcinogenic substances.

II. Experimental.

Sarcomata were induced by the following compounds:

1. 1:2:5:6-dibenzanthracene.

Twelve hooded rats (Lister colony) each received two subcutaneous injections, on successive days, of 1.0 ml. of a 0.5 per cent. solution of 1:2:5:6-dibenzanthracene in sesame oil. Three sarcomata appeared 6 months later, two at 7 months, and three at $7\frac{1}{2}$, 8 and $8\frac{1}{2}$ months respectively.

2. Sodium-1:2:5:6-dibenzanthracene-9:10-endo-$\alpha\beta$-succinate.

A number of mice received subcutaneous injections thrice weekly of 0.25 ml. of a 0.4 per cent. aqueous solution of sodium-1:2:5:6-dibenzanthracene-9:10-endo-$\alpha\beta$-succinate. The injections were continued to the appearance of the tumour after an average period of some $5\frac{1}{2}$ months.

3. 3:4-benzpyrene.

Twelve Wistar rats each received three subcutaneous injections, at intervals of one week, of 2 mg. 3:4-benzpyrene dissolved in 1 ml. lard. Five tumours were detected 18 weeks after the last injection and three in the 21st week.

4. cholanthrene.

20 Wistar rats each received four subcutaneous injections, within 3 weeks, of 2 mg. cholanthrene in 1 ml. lard. Eight weeks after the last injection the majority showed nodules or diffuse masses at the site of injection. These remained stationary until the 18th week, when all except four animals developed rapidly growing tumours. Two further sarcomata arose within the following week.

5. methylcholanthrene.

15 Wistar rats each received seven subcutaneous injections of 2 mg. methylcholanthrene in 1 ml. lard, at intervals of 3-4 days. Two tumours arose explosively twelve weeks after the last injection and three at the 13th week, while all the animals showed actively growing tumours at the 15th week.

A number of mice of the Little CBA (agouti)

strain (originally obtained from Professor J.B.S. Haldane) were given a single subcutaneous injection of 2 mg. methylcholanthrene in sesame oil. Of those employed in the present investigation nos. H 9 and H 12 developed tumours at the 4th month and H 14 at the 7th month following injection.

The growth-rate of these tumours was estimated, by means of caliper measurements, from their emergence, and it is of interest that a considerable proportion showed exponential increase of their linear dimensions. After a suitable control period the animals then received intraperitoneal injections (in sesame oil) either of the compound used for induction or of another compound (1:2:5:6-dibenzanthracene, 1:2:5:6-dibenzacridine, 3:4-benzpyrene, methylcholanthrene, 6:7-dimethyl-1:2-benzanthracene, 10-methyl-1:2-benzanthracene, 1:2:5:6-dibenzphenazine or styryl 430). All these substances had previously been shown, in experiments with transplantable or spontaneous tumours, to possess inhibitory activity, and all are carcinogenic with the exception of 1:2:5:6-dibenzphenazine. In addition, a rat sarcoma induced by means of methylcholanthrene was

grafted to three Wistar litter-mates and the growing transplants used for a similar test (Fig. 114).

The results of these experiments are shown graphically in Figs. 86-114, and a few representative data are given in the Appendix (Tables XXVI-XXVIII). Figs. 115 - 140 illustrate the histological appearances of the tumours studied.

In view of the difficulty of arriving at any quantitative assessment of the degree of response, the results were arbitrarily grouped according to whether the observed inhibition was nil, a trace, slight, moderate or marked. On this basis, Tables XXII and XXIII summarise the results of experiments in which sarcomata were exposed to the action (1) of the compound used for induction, or (2) of another compound.

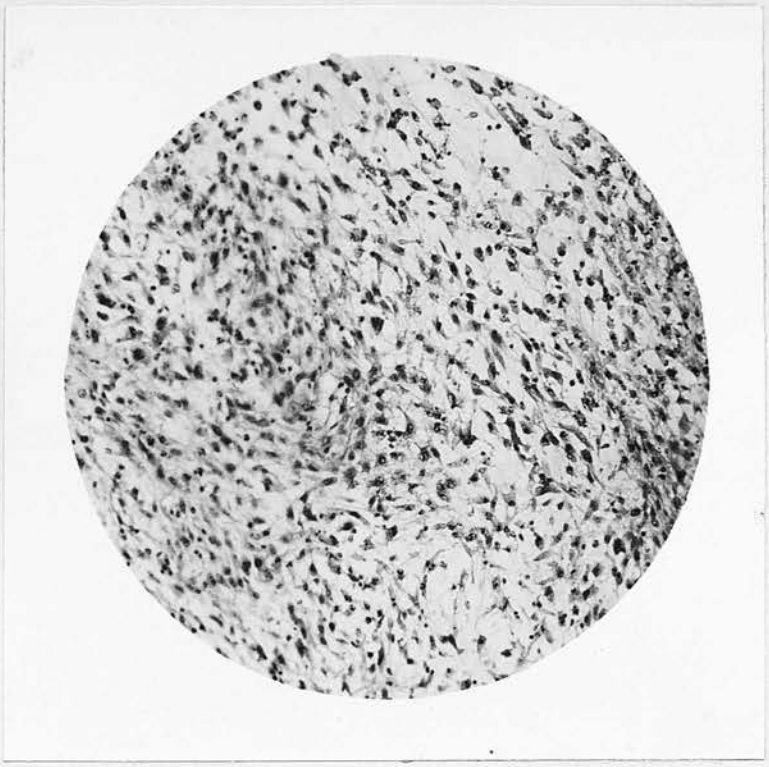


Fig. 115. 1:2:5:6-dibenzanthracene rat sarcoma no. V. x 120.

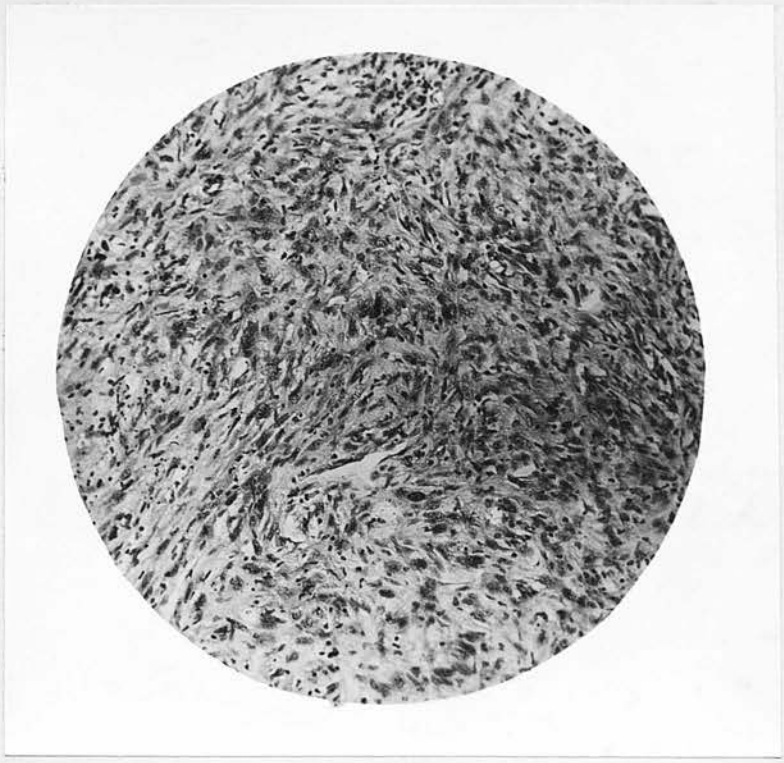


Fig. 116. 1:2:5:6-dibenzanthracene rat sarcoma no. VII. x 120.

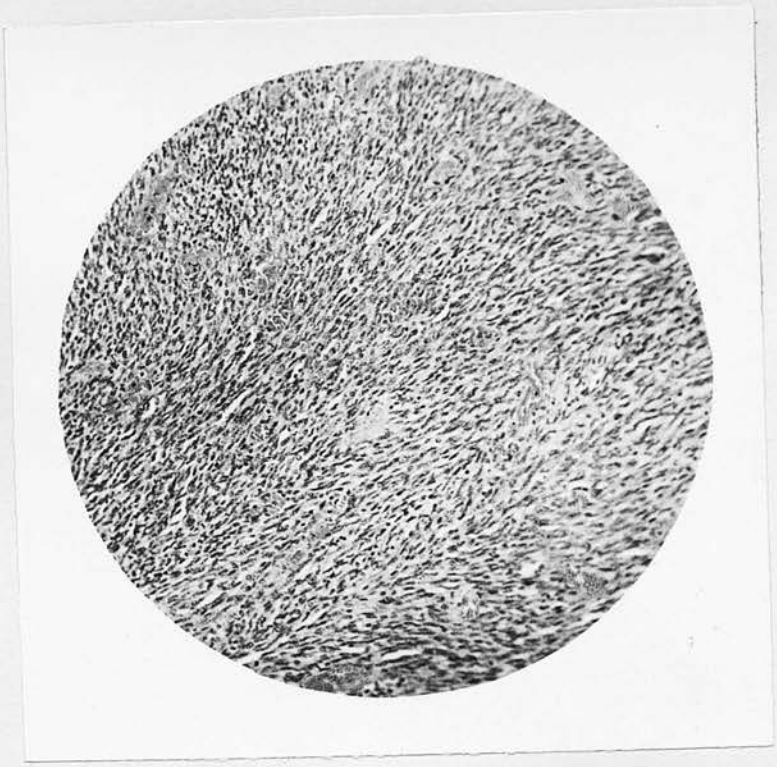


Fig. 117. Sodium-1:2:5:6-dibenzanthracene-9:10-endo-$\alpha\beta$-succinate mouse sarcoma no. L 1. x 120.

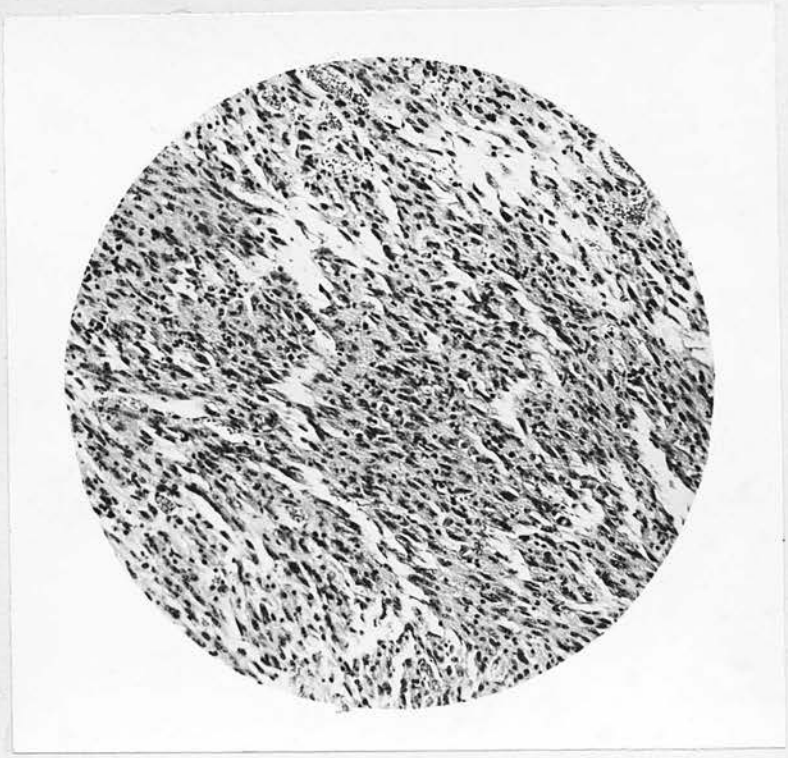


Fig. 118. Sodium-1:2:5:6-dibenzanthracene-9:10-endo-$\alpha\beta$-succinate mouse sarcoma no. 83. x 120.

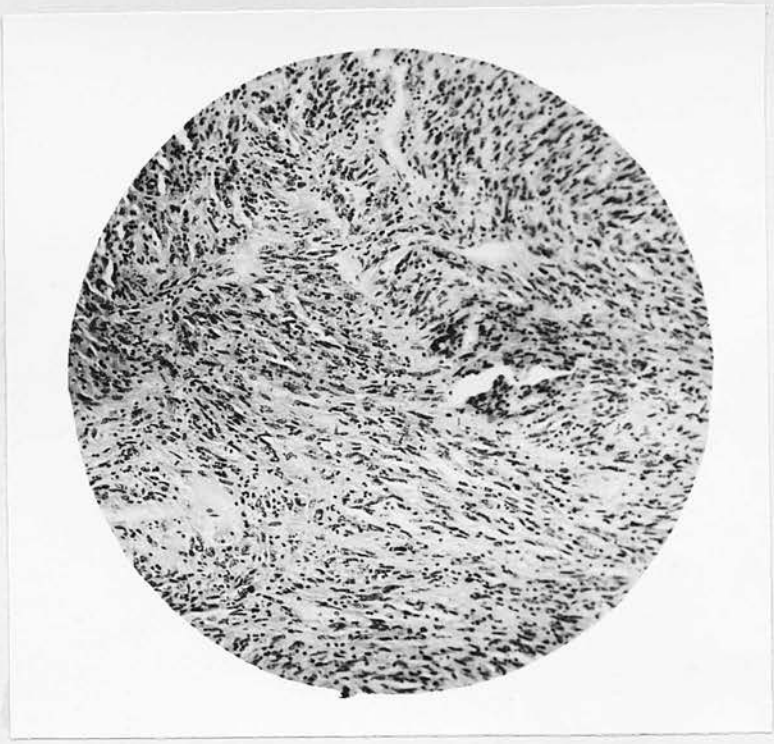


Fig. 119. Sodium-1:2:5:6-dibenzanthracene-9:10-endo-$\alpha\beta$-succinate mouse sarcoma no. 82. x 120.

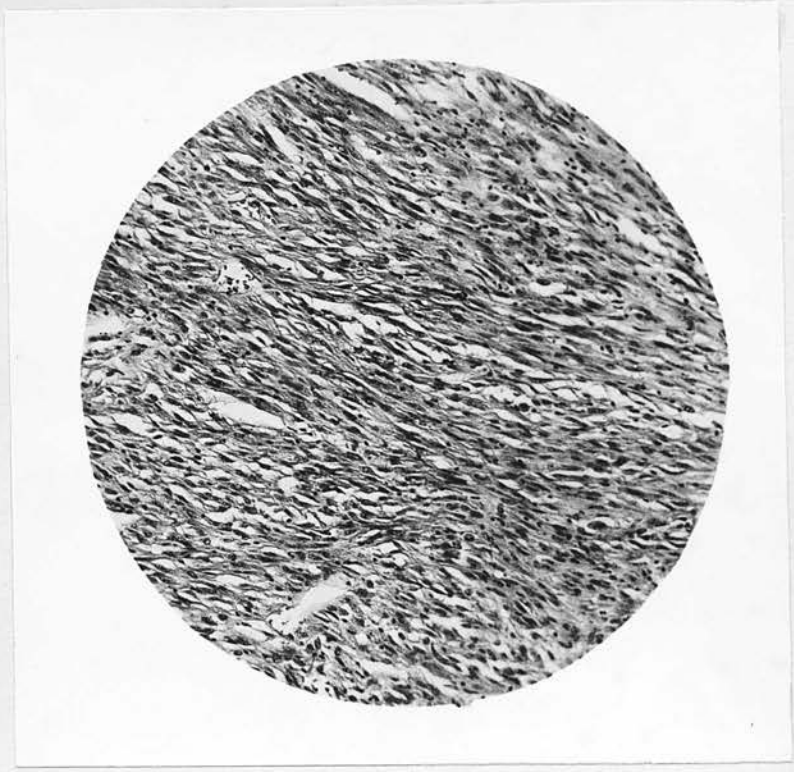


Fig. 120. Sodium-1:2:5:6-dibenzanthracene-9:10-endo-$\alpha\beta$-succinate mouse sarcoma no. 79. x 120.

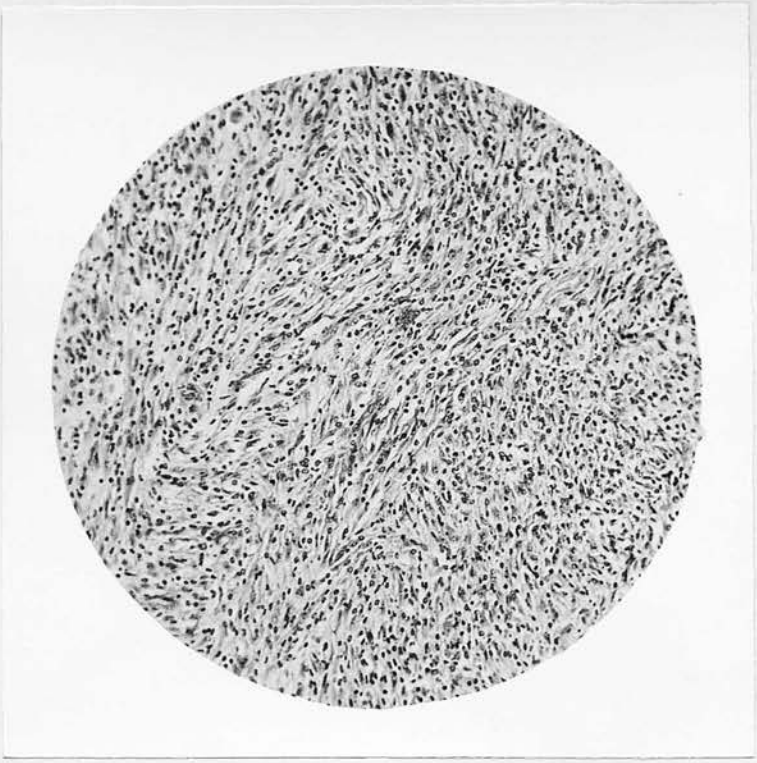


Fig. 121. 3:4-benzpyrene rat sarcoma no. V. x 120.

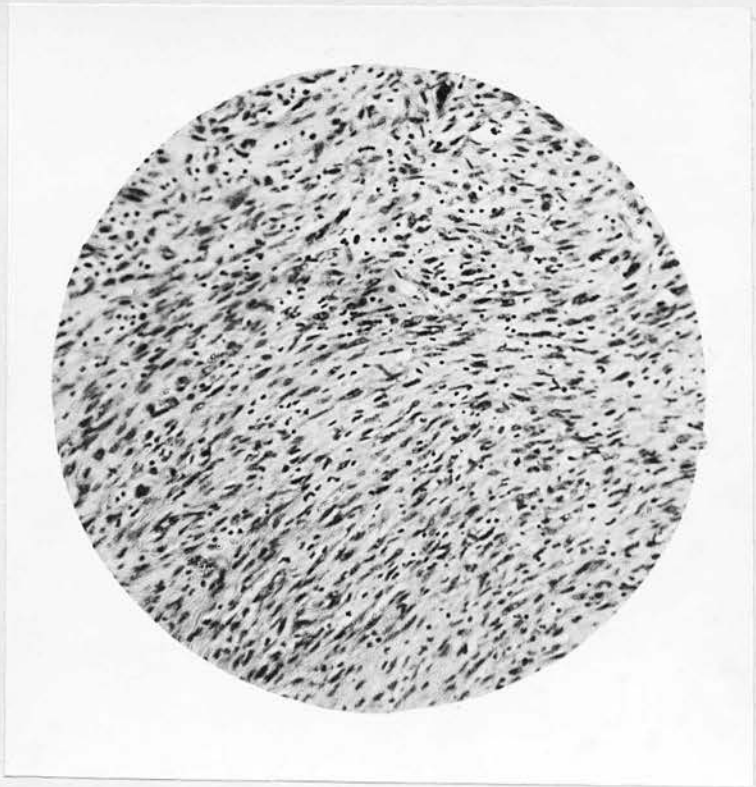


Fig. 122. 3:4-benzpyrene rat sarcoma no. VI. x 120.

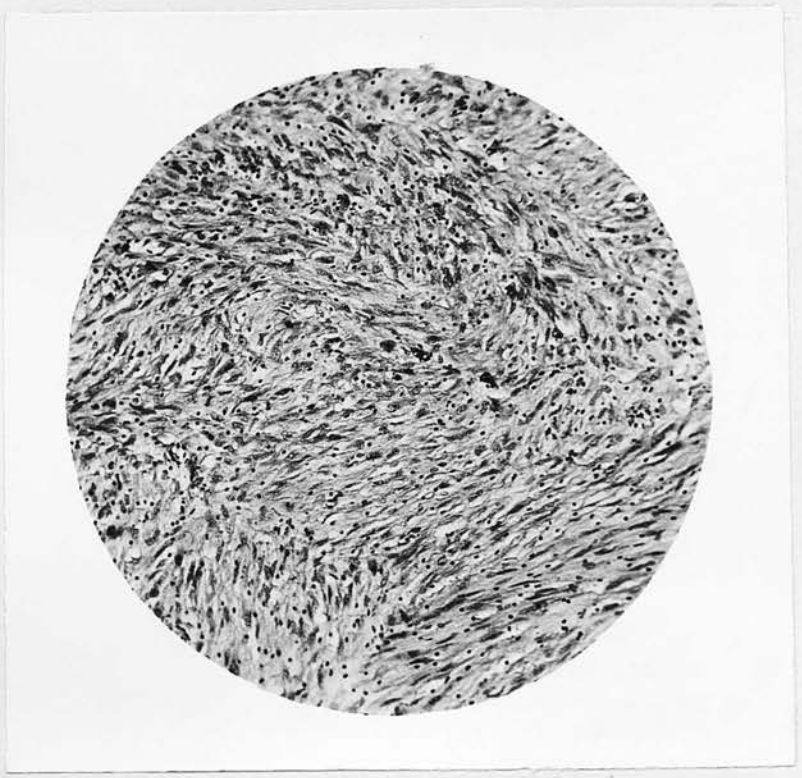


Fig. 123. 3:4-benzpyrene rat sarcoma no. VIII. x 120.

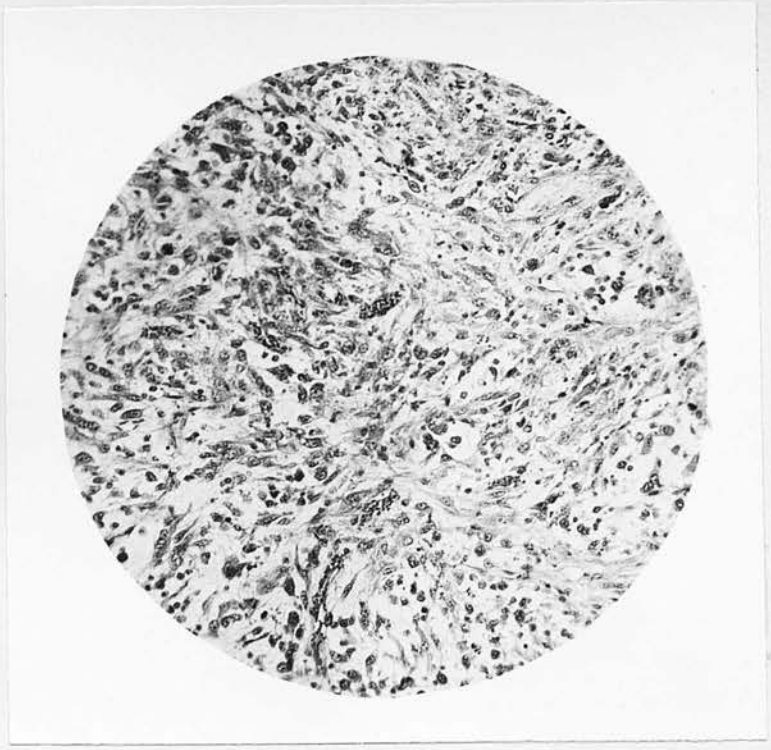


Fig. 124. Cholanthrene rat sarcoma no. III. x 120.

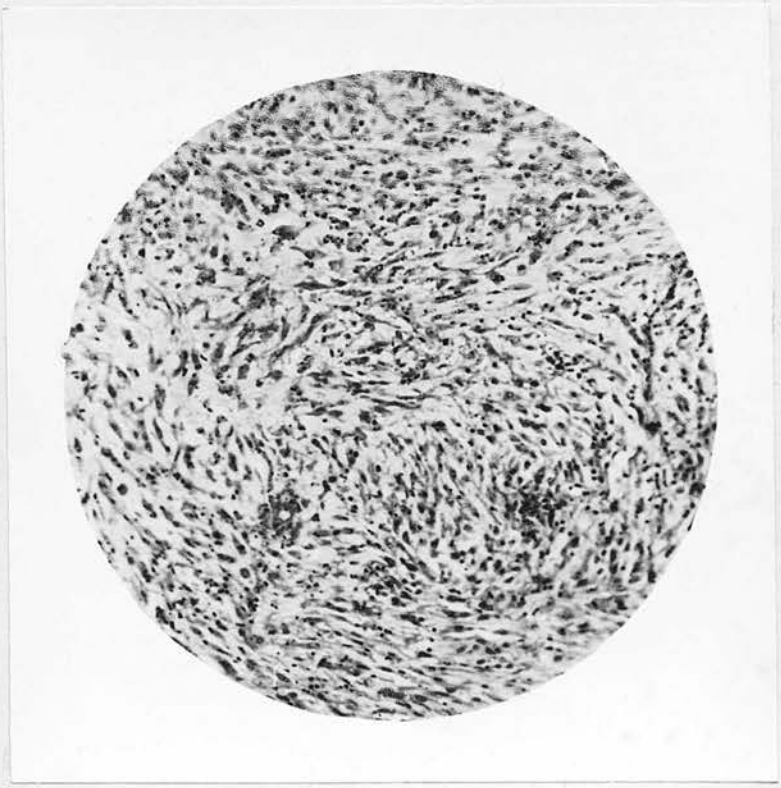


Fig. 125. Cholanthrene rat sarcoma no. IV. x 120.

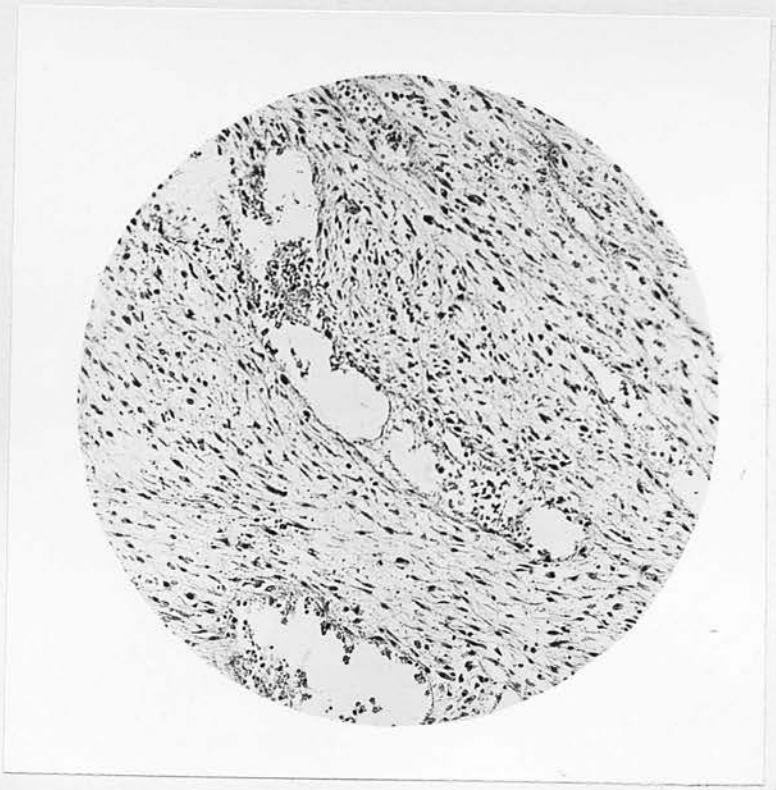


Fig. 126. Cholanthrene rat sarcoma no. V. x 120.

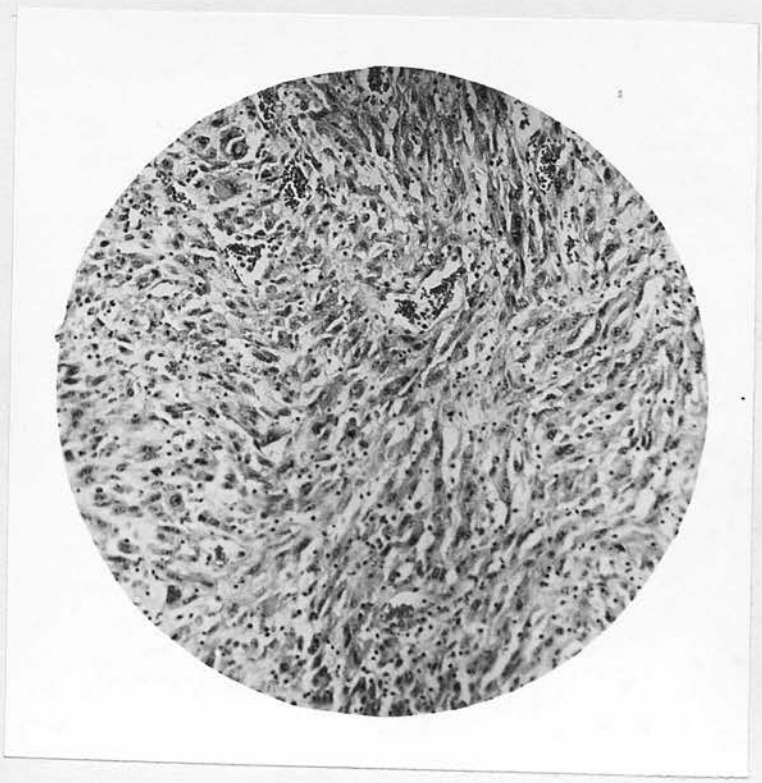


Fig. 127. Cholanthrene rat sarcoma no. VI. x 120.

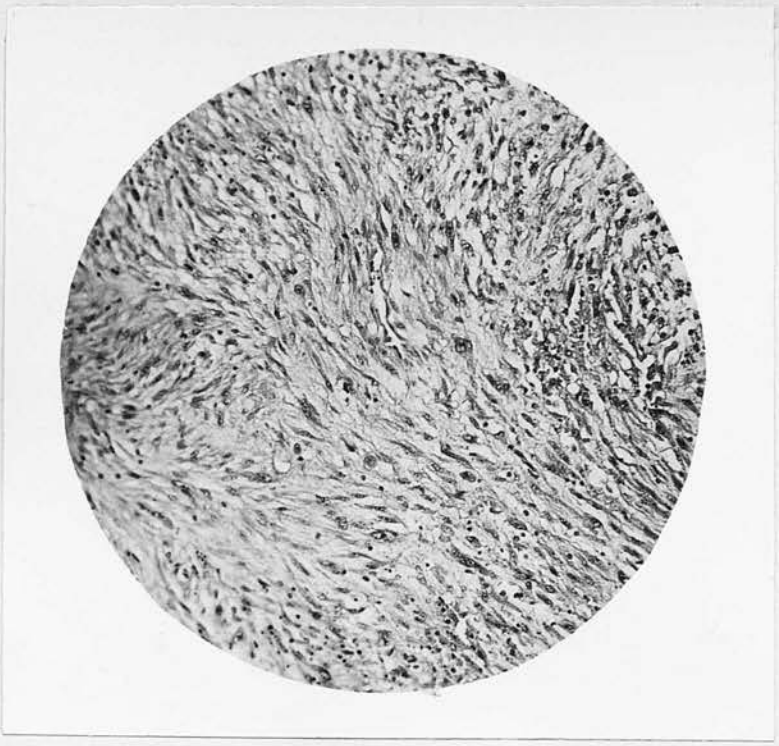


Fig. 128. Cholanthrene rat sarcoma no. VII. x 120.

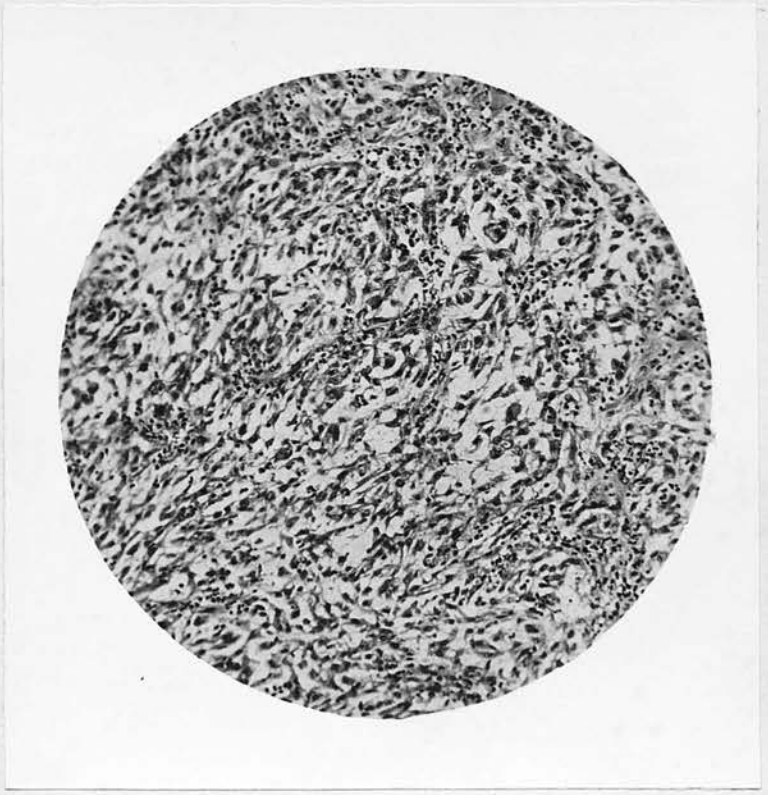


Fig. 129. Cholanthrene rat sarcoma no. X. x 120.

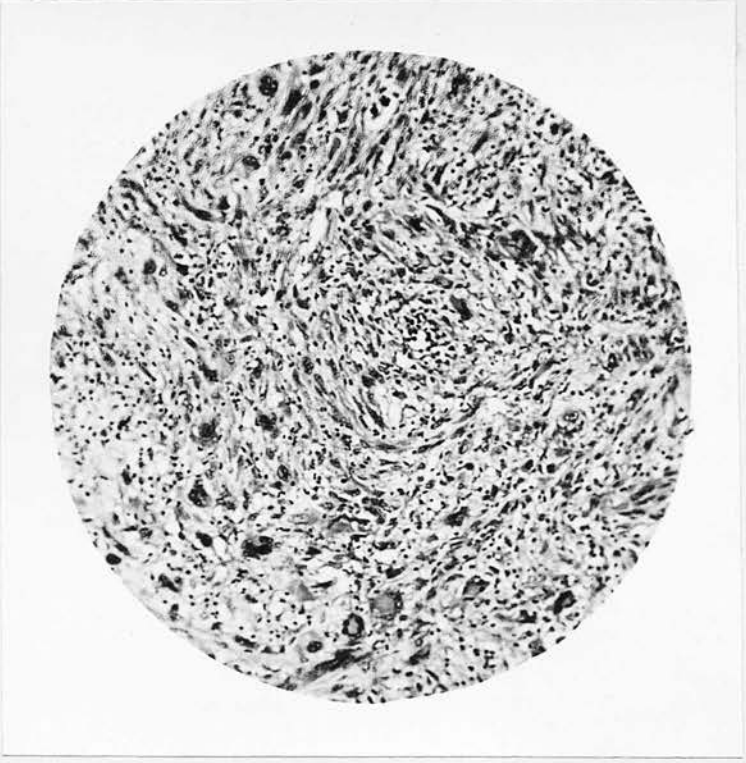


Fig. 130. Cholanthrene rat sarcoma no. XIII. x 120.

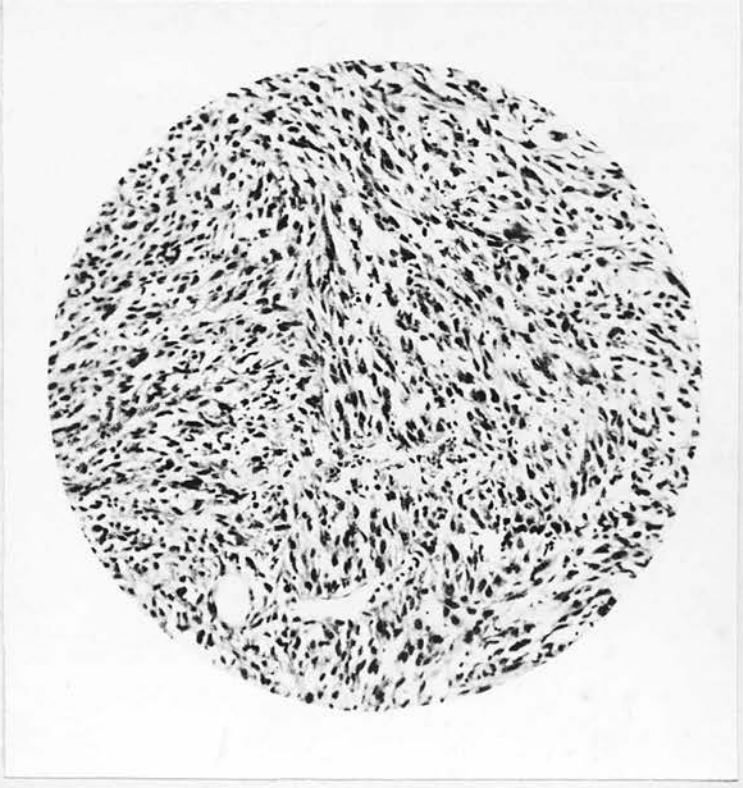


Fig. 131. Cholanthrene rat sarcoma no. XIV. x 120.

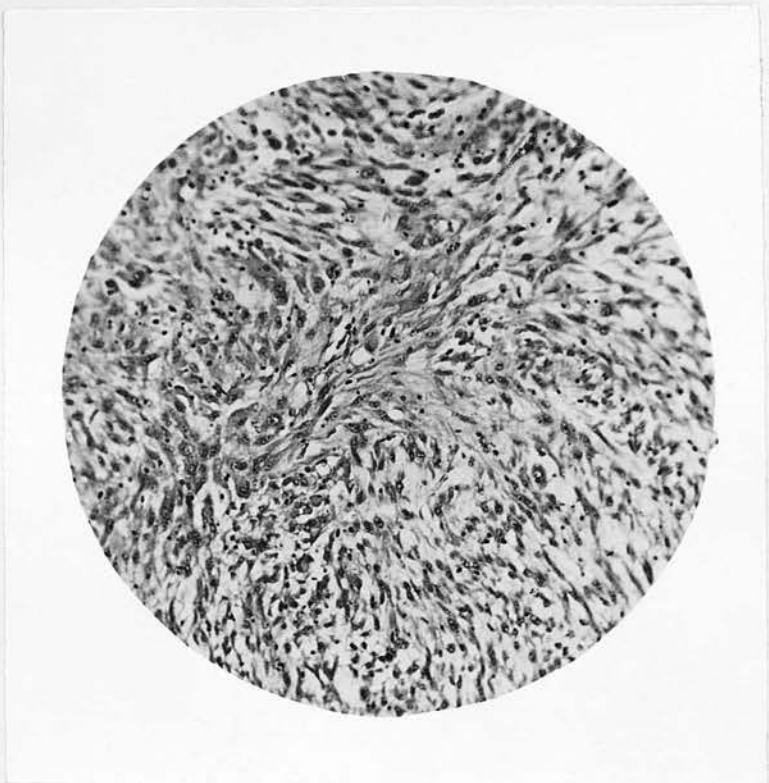


Fig. 132. Cholanthrene rat sarcoma no. B 2. x 120.

Fig. 133. Methylcholanthrene rat sarcoma no. XII. x 120.

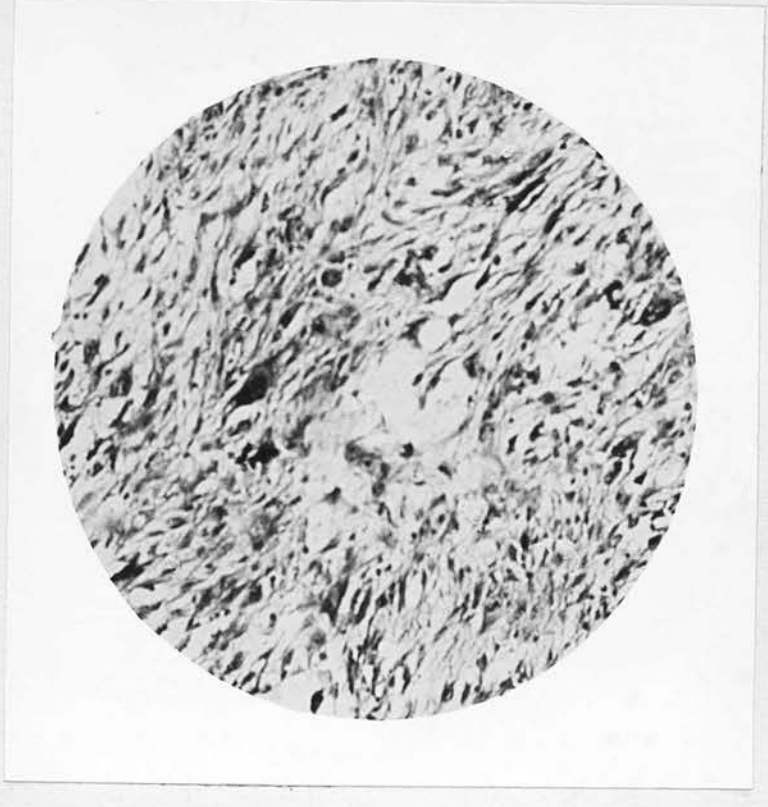


Fig. 134. Methylcholanthrene rat sarcoma no. XV. x 120.

Fig. 135. Methylcholanthrene rat sarcoma no. L. x 120.

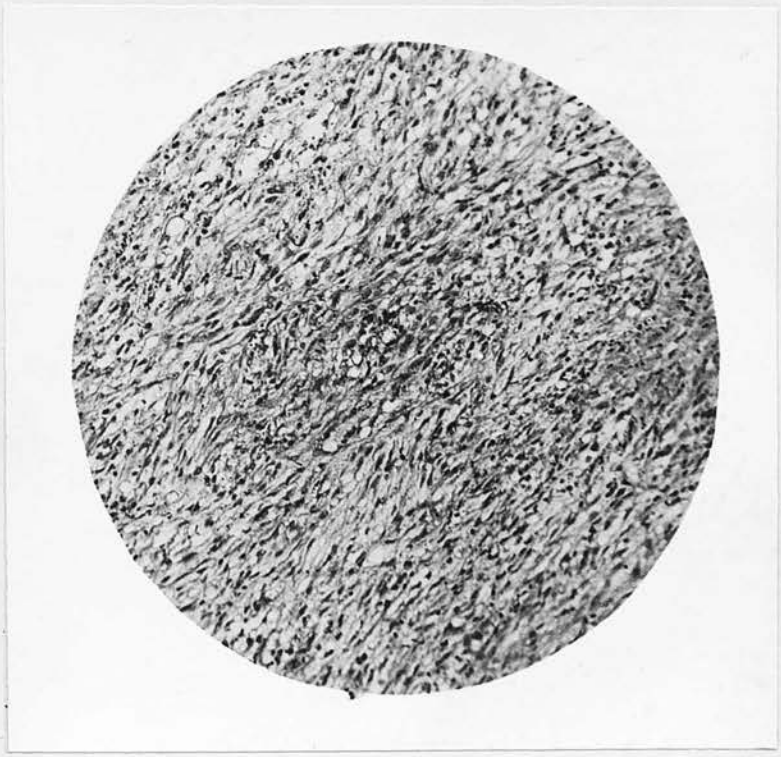


Fig. 136. Methylcholanthrene rat sarcoma grafted and treated with 1:2:5:6-dibenzanthracene. x 120. See Fig. 114 and text.

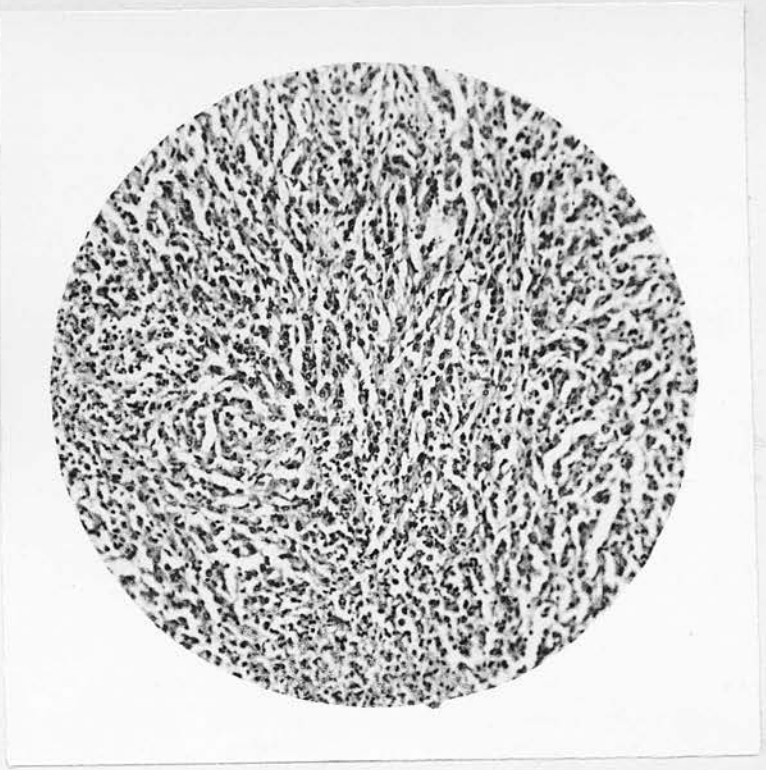


Fig. 137. Methylcholanthrene mouse sarcoma no. H 9. x 120.

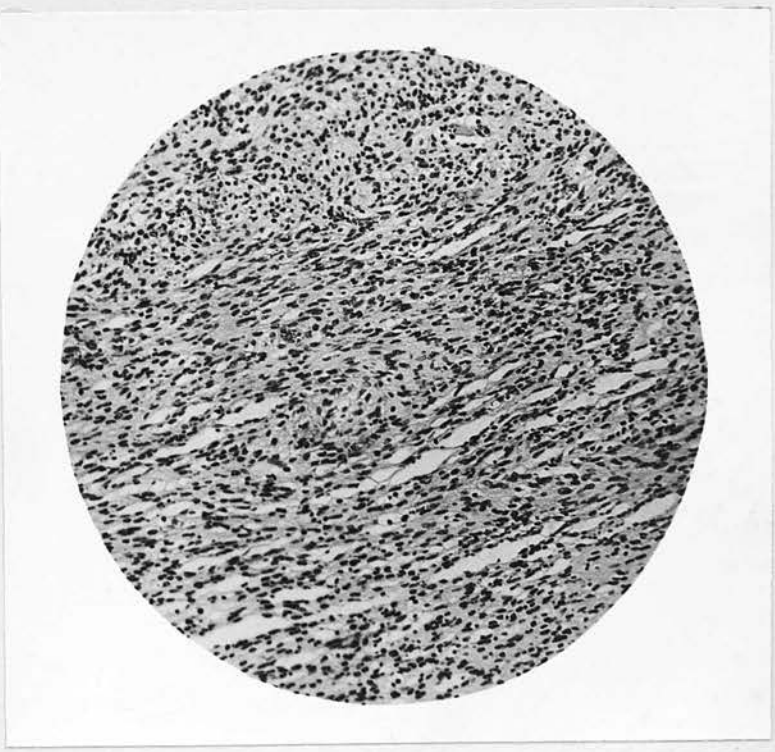


Fig. 138. Methylcholanthrene mouse sarcoma no. H 12. x 120.

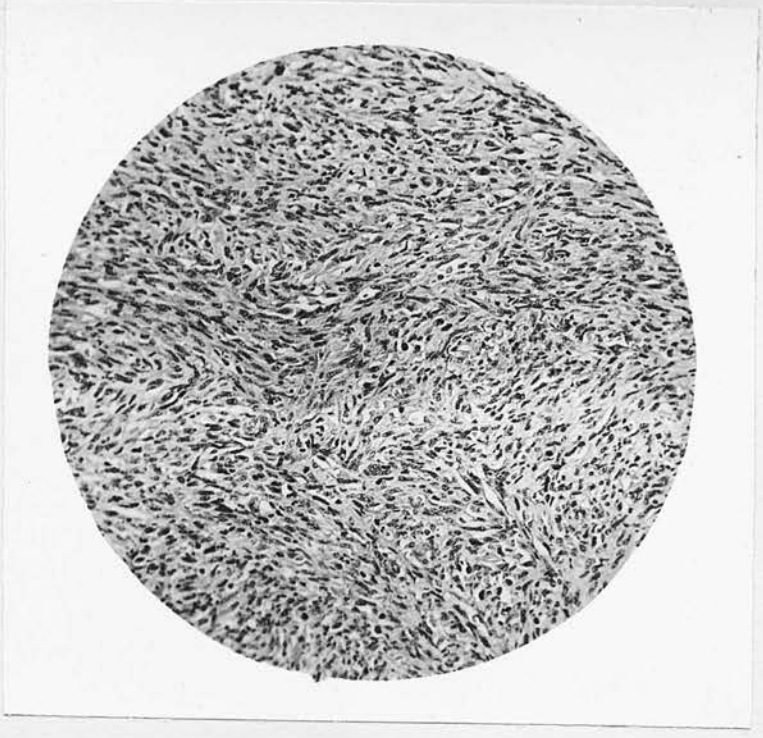


Fig. 139. Methylcholanthrene mouse sarcoma no. H 14. x 120.

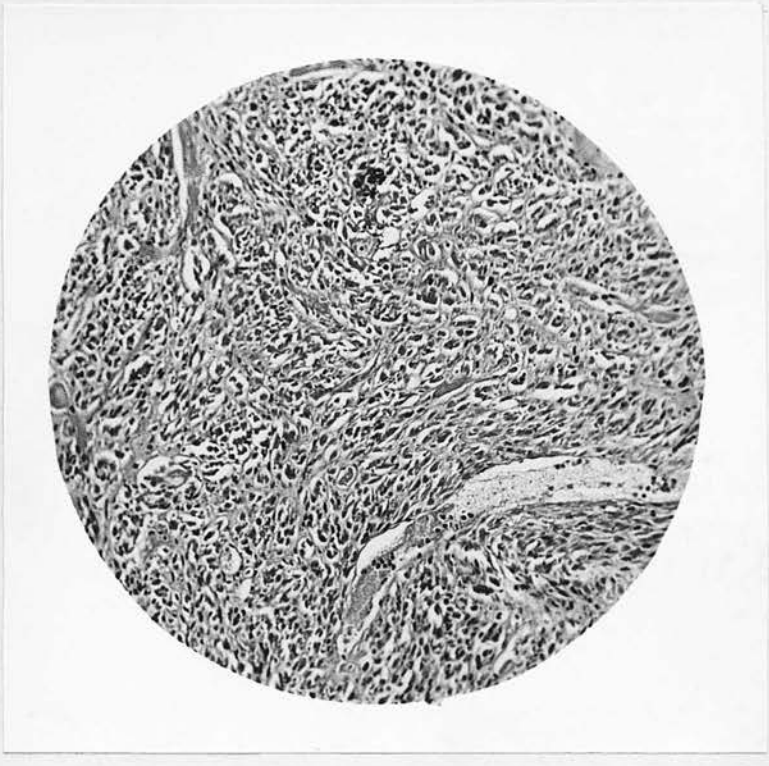


Fig. 140. Sodium-1:2:5:6-dibenzanthracene-9:10-endo-$\alpha\beta$-succinate mouse sarcoma no. 100. x 120.

T A B L E XXII

The response of induced sarcomata to the compound used for induction

Tumour	Inducing and test compound	Test dose	Degree of inhibition
1:2:5:6-dibenzanthracene rat sarcoma B (Fig. 86)	1:2:5:6-dibenzanthracene	30 mg.	moderate
1:2:5:6-dibenzanthracene rat sarcoma V (Fig. 87)	"	2 x 30 mg.	nil
3:4-benzpyrene rat sarcoma VIII (Fig. 88)	3:4-benzpyrene	30 mg.	moderate
3:4-benzpyrene rat sarcoma V (Fig. 89)	"	30 mg.	slight
3:4-benzpyrene rat sarcoma A (Fig. 90)	"	30 mg.	moderate
3:4-benzpyrene rat sarcoma VI (Fig. 91)	"	30 mg.	nil
3:4-benzpyrene rat sarcoma VII (Fig. 92)	"	30 mg.	trace

--- continued over

T A B L E XXII continued.

Tumour	Inducing and test compound	Test dose	Degree of inhibition
cholanthrene rat sarcoma XIII (Fig. 93)	cholanthrene	30 mg.	nil
cholanthrene rat sarcoma X (Fig. 94)	"	30 mg.	slight
methyl cholanthrene rat sarcoma L (Fig. 95)	methyl cholanthrene	25 30 mg.	nil
methyl cholanthrene rat sarcoma XII (Fig. 96)	"	2 x 25 mg.	marked
methyl cholanthrene rat sarcoma A (Fig. 97)	"	25 30 mg.	nil
methyl cholanthrene rat sarcoma X (Fig. 98)	"	30 mg.	nil

T A B L E XXIII

The response of induced sarcomata to compounds other than that used for induction.

Tumour	Inducing compound	Test compound	Test dose	Degree of inhibition
1:2:5:6-dibenz-:anthracene rat sarcoma A (Fig. 99)	1:2:5:6-dibenz-:anthracene	1:2:5:6-dibenz-:acridine	14 mg.	nil
1:2:5:6-dibenz-:anthracene rat sarcoma VII (Fig.100)	"	"	14 mg.	marked
1:2:5:6-dibenz-:anthracene rat sarcoma VI (Fig.101)	"	6:7-dimethyl-1:2-benz-:anthracene	25 mg.	slight
Sodium-1:2:5:6-dibenz-:anthracene-9:10-endo-α/β succinate mouse sarcoma L 1	Sodium 1:2:5:6-dibenz-:anthracene 9:10-endo-α/β succinate	1:2:5:6-dibenz-:acridine	5 mg.	marked
Sodium 1:2:5:6-dibenz-:anthracene-9:10-endo-α/β succinate mouse sarcoma L 2	"	"	5 mg.	trace

--- continued over

T A B L E XXIII continued.

Tumour	Inducing compound	Test compound	Test dose	Degree of inhibition
Sodium-1:2:5:6-dibenz-:anthracene-9:10-endo-α/β succinate mouse sarcoma 100 (Fig. 102)	Sodium-1:2:5:6-dibenz-:anthracene-9:10-endo-α/β succinate	1:2:5:6-dibenz-:acridine	5 mg.	marked
Sodium-1:2:5:6-dibenz-:anthracene-9:10-endo-α/β succinate mouse sarcoma R.L.K.	"	6:7-dimethyl-1:2-benz-:anthracene	12 mg.	moderate
Sodium-1:2:5:6-dibenz-:anthracene-9:10-endo-α/β succinate mouse sarcoma 79	"	1:2:5:6-dibenz-:phenazine	2 x 2.5 mg.	moderate
Sodium-1:2:5:6-dibenz-:anthracene-9:10-endo-α/β succinate mouse sarcoma 82 (Fig. 103)	"	styryl 430	3 x 10 mg.	nil
Sodium 1:2:5:6-dibenz-:anthracene-9:10-endo-α/β succinate mouse sarcoma 83 (Fig. 103)	"	"	4 x 10 mg.	nil

T A B L E XXIII continued.

Tumour	Inducing compound	Test compound	Test dose	Degree of inhibition
cholanthrene rat sarcoma XI (Fig. 104)	cholanthrene	1:2:5:6-dibenz-:anthracene	30 mg.	moderate
cholanthrene rat sarcoma VI (Fig. 105)	"	"	30 mg.	nil
cholanthrene rat sarcoma V (Fig. 106)	"	"	30 mg.	moderate
cholanthrene rat sarcoma IV (Fig. 106)	"	"	30 mg.	slight
cholanthrene rat sarcoma XV (Fig. 107)	"	1:2:5:6-dibenz-:acridine	14 mg.	nil
cholanthrene rat sarcoma IX (Fig. 108)	"	3:4-benz-:pyrene	30 mg.	marked
cholanthrene rat sarcoma B2 (Fig. 108)	"	"	30 mg.	slight
cholanthrene rat sarcoma VII (Fig. 109)	"	methyl-cholanthrene	30 mg.	nil
cholanthrene rat sarcoma III (Fig. 109)	"	"	30 mg.	nil

--- continued over

T A B L E XXIII concluded.

Tumour	Inducing compound	Test compound	Test dose	Degree of inhibition
cholanthrene rat sarcoma XIV (Fig. 110)	cholanthrene	6:7-dimethyl-1:2-benz-:anthracene	25 mg.	marked
methyl-cholanthrene mouse sarcoma H 12 (Fig. 111)	methyl-cholanthrene	"	12 mg.	marked
methyl-cholanthrene mouse sarcoma H 14 (Fig. 112)	"	10-methyl-1:2-benz-:anthracene	10 mg.	nil
methyl-cholanthrene mouse sarcoma H 9 (Fig. 113)	"	1:2:5:6-dibenz-:phenazine	7 mg.	nil

III. Discussion.

1. Present results.

From the results of the present experiments it is apparent that chemically induced tumours, regarded as a class, tend to be considerably less susceptible to the inhibitory action of carcinogenic substances than are transplantable tumours (Haddow and Robinson 1937) or spontaneous tumours of the mouse (Haddow 1938b). Before proceeding to discuss the possible significance of this observation it may be pointed out that the numerous instances of resistance which have been encountered make it likely, on the whole, that retardation of tumour growth-rate, by the hydrocarbons, is due to drug action involving the tumour cells directly although not specifically, and is not an indirect effect due to non-specific interference with the health of the tumour-bearing host.

It is of interest to enquire whether tumours induced by a given hydrocarbon are significantly more resistant to the inhibiting action of the same hydrocarbon than to that of other carcinogenic substances. Table XXIV gives the number of tumours showing (a) resistance, or (b) any degree of inhibition, when tested against the compound used for induction or against other compounds. In this case

cholanthrene was taken as equivalent to methyl-cholanthrene, but the two cases of complete resistance to styryl 430 were included. When arbitrary values rising by one unit (resistance $\equiv$ 1) were allotted to the degrees of response given in Tables XXII and XXIII, scores were obtained as shown in Table XXV. This procedure was judged to be permissible since degrees of sensitivity or resistance are purely relative and continuous with each other.

T A B L E XXIV

	Number of tumours showing...	
	Complete resistance	Sensitivity
Tumours tested with hydrocarbon used for induction.	8	7
Tumours tested with a substance other than that used for induction.	7	14

T A B L E XXV

	Sum of arbitrary degrees of response shown by.....	
	Resistant tumours	Sensitive tumours
Tumours tested with hydrocarbon used for induction.	8	25
Tumours tested with a substance other than that used for induction.	7	57

For Table XXV the value of χ^2 is c. 2.95, so that the more extreme case without correction does not attain the level of even moderate (5 per cent.) significance.

High specificity of resistance would approach a proof of the etiological importance of the inhibitory properties of the carcinogenic hydrocarbons, but as such specificity is absent in the present experiments no such proof can be claimed. It must be pointed out on the contrary that resistance devoid of significant specificity need have no etiological or related meaning of any kind. The radio-resistance exhibited by certain of the most highly differentiated normal cells and tissues obviously implies no fundamental inter-connexion, and is merely a corollary of the fact that radio-sensitivity shows a rough proportionality to normal rate of growth (for a discussion of the radio-sensitivity of tissues see Desjardins 1934). An interesting contribution to this subject was made by Turner and Gomez (1936) who showed that the sensitivity of mammary epithelium to roentgen rays was increased 30-50 per cent. by stimulating more rapid growth of the ducts by oestrogenic hormone.

Although the comparative resistance of chemically induced tumours to the inhibitory action

of carcinogenic substances is not of such a nature as to yield statistical proof of a causal relation, it is equally true that such a relation is far from disproved by the same evidence. In addition there is a body of more circumstantial information, which will now be considered, which indicates that the observed resistance is at least compatible with the etiological views suggested and may even be an essential condition of the proof of the hypothesis. Murray (1931) pointed out that though there are many examples of the greater viability of cancer cells as compared with the normal, there is no clear evidence of a single property in which they are more vulnerable. He regarded the apparent sensitivity to x-radiation as no exception, and thought it probably correct to ascribe the apparently specific effect of radiation in cancer to the delicacy of the indication of action which is provided by the arrest of growth or temporary disappearance following local destructive effects. "Few normal cells provide an indicator of the same kind or order of delicacy, and it is significant that the two tissues which do so, the testis and lymphoid tissues, behave in the same way as do new growths." This somewhat augmented general resistance of the malignant cell is possibly analogous with a similar non-specific increase frequently observed in R bacterial variants

(as for instance in a thirteen-fold increase in percentage survival on slow desiccation of R pneumococci as compared with the corresponding S forms, described by Gay, Atkins and Holden (1931)).

2. The general biology of drug-resistance.

The field of general biology affords innumerable examples of adaptive resistance, both specific and non-specific, fleeting and permanent. Perhaps the simplest are the phenomena of transient refractoriness following stimulation, and the resistance to hormonal or other stimuli which is frequently encountered as a physiological characteristic of cell or tissue. As an illustration of the same principle in general pathology may be cited the description by MacNider (1935, 1936a,b) of the acquired resistance developed by liver cells in response to the toxic action of uranium nitrate. In this case the damage produced by uranium is followed by metaplastic variation and a coincident increase in the resistance of the liver cells not only to uranium nitrate but also to the hepatoxic action of chloroform. Wilson (1922) described experiments which indicated that embryonic mesenchyme cells, cultivated in weak solutions of copper sulphate and sodium arsenite, may develop a heightened tolerance for these two poisons. The above examples represent only a selection of those available, but they serve to show that the cells of higher animals possess "mechanisms which enable living matter to alter itself in such a way that it can come to endure physical or chemical environments normally adverse

to it." (Heilbrunn 1937).

In the case of fixed tissue cells however the capacity for adaptive response shows less freedom, and is certainly less readily studied, than the same property in the unicellular forms of life. It is therefore not surprising that the fundamental characteristics of adaptation are most clearly deciphered in protist and bacterial organisms, where the number of cases investigated is also very much greater. Such organisms not infrequently protect themselves against adverse environmental factors either by the avoidance-reaction (motility) or by the formation of a protective membrane or capsule. But when these responses are not possible (and especially in a closed environment with a uniform concentration of dysgenic influences) the cells may undergo physical or chemical acclimatization of various biological types. In a study of infections with Trypanosoma lewisi Taliaferro (1929; see also Coventry 1925) demonstrated the presence of a reaction product which inhibited the reproduction of the parasites without affecting their viability (see also Dingle 1936 on the growth-inhibitory powers of specific antisera): it is therefore significant that the natural occurrence of serological variations or recidive strains in trypanosomiasis and also in

relapsing fever (see Cunningham, Theodore and Fraser 1934; Russell 1936) is probably to be interpreted as a response to the inhibitory properties of the serum which develop in the course of the infection. Again, according to Pett (1936, 1937) the effect of continuous subculture of yeast in cyanide indicates the possibility of originating a strain which will remain constant with or without cyanide.

By far the most extensively studied case of chemical acclimatization is the trypanosomal drug-resistance originally discovered by Franke and Roehl in Ehrlich's laboratory in 1907, a phenomenon which embraces resistance to many therapeutic substances but particularly to the phenyl radicle present in trypanocidal arsenical drugs. Although any attempt at generalisation must be founded on fact and duly critical, the subject may perhaps be discussed with greater pertinence in the present connexion since Oesterlin (1936, 1937) has propounded the view that the damage to the trypanosome which brings about its destruction in the body of the host, and the damage to the tissue cell which leads to the development of malignancy, may be essentially the same (as perhaps in the case of sarcoma production by the trypanocide styryl 430).

Before reviewing the implications of drug-resistance in trypanosomes it is important to note that arsenic resistance may be a natural characteristic of certain strains (e.g., of T. gambiense) apart altogether from previous contact with arsenical drugs. (see Duke 1933, 1935). However, when suitable strains of T. gambiense are treated with therapeutically ineffective doses of organic arsenicals or antimonials or with acridine dyes, the organisms become resistant to the inhibitory action of all drugs of these classes, although they remain sensitive to arsenious acid, tartar emetic and Bayer 205. According to Yorke (1936), "a strain made resistant to acriflavine is exactly similar to one made resistant to an aromatic compound or arsenic or antimony. While it is impossible to make a normal strain of trypanosomes resistant to tartar emetic, a few doses of this drug to an atoxyl- or acriflavine-resistant strain suffice to make it completely resistant to tartar emetic. The production of a Bayer 205-resistant trypanosome is a long and tedious matter, but such strains can be made, and it is interesting to note that the resistance is specific for Bayer 205." Yorke and his collaborators (see Yorke and Murgatroyd 1935) found that highly resistant strains could be prepared by exposing the trypanosomes to the action of drugs in vitro, and

showed that when resistant strains were examined *in vitro* they were capable of withstanding immensely greater concentrations of trypanocide than sufficed to destroy the normal strain. Schlossberger and Schuffner (1934) also carried out experiments on the drug-resistance of trypanosomes with arseno-pyridine derivatives. In more recent work, King, Lourie and Yorke (1937; see also Browning 1938) described new trypanocidal substances related to synthalin, and found that trypanosomes acquire resistance to undecane diamidine only very slowly if at all, and that trypanosomes completely resistant to aromatic arsenicals and to Bayer 205 exhibit no resistance to undecane diamidine or to synthalin.

The drug-resistance of trypanosomes is however only specific to a very limited extent, and this is the case in similar work with other organisms. Thus Pulst (1902, quoted by Heilbrunn 1937) found that *Penicillium* acclimatized to copper also showed acclimatization to zinc, and *vice versa*. Moreover, if the mould was acclimatized to either of these metals it showed increased resistance to nickel, cadmium and mercury in addition, although acclimatization to nickel did not improve the resistance to cobalt. It is apparent that all these examples lend support to the generalisation that accommodation to non-antigenic poisons tends to

be less specific than that to antigenic substances.

3. Transformation and selection in the induction of resistance.

It is practically certain that the cellular change from normal to malignant involves transformation --- or a series of transformations --- and it is highly improbable that the pathological environment which leads to tumour formation is such as to favour or select rapidly growing cells. The detailed evidence for these statements has been reviewed by the writer (Haddow 1938a), but it is of parallel interest to enquire whether the emergence of resistant strains of micro-organisms is similarly dominated by transformation or is on the other hand due in whole or in part to the effects of selection. In their classical experiments on acclimatization of organisms to poisonous chemical substances, Davenport and Neal (1895) attributed increased resistance in Stentor exposed to mercuric chloride to a change brought about by direct action on the protoplasm and not to selective factors. It was concluded by Yorke (1936) that the drug-resistance which a trypanosomal infection exhibits after repeated

administration of sub-curative doses of a trypanocide is due to a character acquired by the trypanosome itself. According to the same author (see Yorke, Murgatroyd and Hawking 1931) a given population of trypanosomes may show a fourfold difference in susceptibility to arsenic, whereas a resistant strain derived from the same population may withstand a dose four hundred times as great: hence selection alone is an insufficient explanation, and must at least be aided by some factor involving transformation (e.g., unequal fission).

In an important paper by Robertson (1929) on the increased resistance of Bodo caudatus after exposure to acriflavine, this worker pointed out that resistance could be raised considerably above the performance of the best untreated strain, as evidence of the acquisition of a definite heightened capacity; but she also acknowledged the possible criticism that there might be a small undetected group at the extreme upper end of the untreated clone that already had this resistance. However, two observations were made which appeared to render the evidence for modification extremely strong. In the first place, while cultures in a concentration of acriflavine bordering upon the lethal limit were often not viable upon subculture into the same concentration (sometimes not even into drug-free media) in the

early days of development, they were successfully propagated into the drug at a later period. These results were not dependent on the numbers of organisms transferred, and were especially significant since a single organism was sufficient to populate a plate. Secondly, the author described a condition of hypersensitiveness of Bodo caudatus to acriflavine, a phenomenon apparent only within a narrow limit of time and drug-concentration, but one which no selective process of the inhibitory type would suffice to explain. According to Robertson, from these facts "it seems reasonable to suppose that the resistance.....(was) due partly to the effect of the drug upon the organism" during development, and she concluded that the acquisition of drug-fastness in Bodo is brought about by the interaction of selective inheritance and actual modification.

It would therefore appear that the bulk of opinion tends to favour the importance of direct modification, as opposed to selection, in determining the origin of drug-resistant strains, and this forms an accurate parallel to what the writer (1938a) believes most probable in the case of the genesis of the malignant variant.

A further demonstration of biological resistance is given by the emergence of secondary growth, consisting of resistant organisms, when bacterial cultures are exposed to the action of a suitable bacteriophage. Reference is made to the phenomenon since it appears to differ from those already dealt with in depending mainly on selective factors, although even in this case there are differences of opinion. According to White (1937a), "it is moderately clear that there is nothing adaptive about the resistance of the secondary growth. The resistant secondary growth merely represents an unassailable residuum of the original culture in which the specific pabulum (be it polysaccharide or of other nature) of the phage in question is either lacking or mechanically shielded from attack." Yang and White (1934) described the process of roughening in the cholera vibrio as involving loss of the characteristic specific soluble substance of the smooth organism, as in the Salmonella group, and they further found that extremely rough forms isolated from ultrapure cultures without the help of A cholera phage were identical with those obtained by its use and seemed to be invariably resistant. Their attempts to isolate from similar cultures, by the methods of simple selection, variants resistant to A type phage

met with a single but apparently significant success, and these workers were therefore inclined to believe that resistance to A phage is not a modification induced by phage action but that resistant elements are present in such ultrapure cultures and survive lysis. However, Burnet and Lusk (1936), impressed by the ability of a weakly lytic bacteriophage speedily to 'induce' resistance to a more potent phage of the same type, concluded that positive modification under the influence of phage must occur in those cases in which selection of naturally resistant variants could be excluded since there had been insufficient time for selection to operate. White (1937b) admitted that in such cases resistance to the more active agent is not due to selection, but he suggested that the effect might be attributed merely to blockade of the cells ("blockade immunity") and not to any defensive modification of their substance.

4. The cellular change in resistance.

In accepted cases of the true induction of resistant variants by actual modification of the cell substance it is of interest to determine what the mechanism or mechanisms may be. Ehrlich originally advanced the hypothesis that acquired resistance of protozoan strains is due to a loss of binding capacity, and this subject was studied by Yorke, Murgatroyd and Hawking (1931) in their investigations on the nature of trypanosomal drug-resistance. In an important paper Hawking (1937; see however Pedlow and Reiner 1935) investigated the absorption of arsenical compounds by normal and atoxyl-resistant trypanosomes by exposing the organisms *in vitro* at 37° C. to suitable concentrations of these compounds. The parasites were then centrifuged out and the distribution of the arsenical determined, either by measuring the trypanocidal activity of the supernatant fluid for fresh trypanosomes or by chemical estimations of the arsenic content of the supernatant fluid and of the deposited trypanosomes. Hawking found that while normal trypanosomes absorbed all the available drug from suitable concentrations of typical trivalent compounds (reduced tryparsamide, halarsol and novarsenobillon), living resistant

trypanosomes absorbed little or none from similar concentrations of the same compounds, although absorption occurred if stronger concentrations were used or if the trypanosomes were dead. A similar difference in the behaviour of normal and resistant trypanosomes was observed when the parasites were exposed to reduced tryparsamide *in vivo*. Compounds to which the atoxyl-fast trypanosomes showed no resistance, *e.g.*, phenyl-arsenoxide, sodium arsenite or tartar emetic, were absorbed to the same extent by both types of organism. It therefore appears that the comparative failure of atoxyl-fast trypanosomes to absorb typical trivalent arsenical compounds constitutes the explanation of the drug-resistance of these parasites. In a discussion of these results Hawking suggested their further interpretat-:ion on the hypothesis of receptor modification, the change being directed primarily against the side-chains on the benzene ring. In connexion with the underlying basis of resistance it may also be noted that Broom, Brown and Hoare (1936) found differences in susceptibility to organic arsenical compounds in trypanosomes of different charges: thus in one experiment the positively charged variants of *T. evansi* were more susceptible to the negatively charged ion of tryparsamide.

Although drug-resistance in the trypanosomes is one of the best examples of adaptive transformation in the face of dysgenic influences, there are others which probably depend on a similar principle. Thus Neuschlosz (1919, 1920, quoted by Gay 1935) found quinine-fast paramecia capable of destroying 80 per cent. of the drug in solution while non-adapted organisms in the same solution produced a disappearance of less than 5 per cent.

As already indicated, the fact that the comparative resistance of chemically induced tumours to the inhibitory action of the carcinogenic hydrocarbons shows no specificity renders it impossible to assume, on this criterion alone, that the resistant state is of etiological or adaptive significance. However, the biological evidence which has been discussed does not contradict, and tends to support, the view that the emergence of this type of tumour is a response on the part of the affected normal cells to long-continued chemical inhibition.

In search of further evidence it would perhaps be of interest to determine the metabolic behaviour of tumour tissue, induced by specific carcinogenic substances, in the presence of the same inducing substances, and to compare the results with the information already available (e.g., Boyland and Boyland 1934) regarding the effect of such substances

on the metabolism of transplantable and other tumours not so induced.

5. The stability of resistance.

The fundamental etiological problem is the relationship between carcinogenic substances and the cells of the tumours to which they give rise: but it is also pertinent to enquire whether any change takes place in this relationship once the tumour cell is no longer exposed to the direct action of the inducing agent. This raises the question as to how long direct action can be expected to persist. Lorenz and Shear (1936) found that in 59 g. of healthy tumour tissue obtained six to eight months after the treatment of mice with 1:2:5:6-dibenzanthracene in lard solution, 0.8 mg. of the hydrocarbon was still present according to spectrum analysis. After transplantation of these tumours no dibenzanthracene was found in36 g. of tissue from 5th generation or in 148 g. of tissue from 6th generation dibenzanthracene tumours. A second batch of 120 g. from 6th generation dibenzanthracene tumours likewise gave no indication of the presence of the hydrocarbon. In the last cases it was estimated that the amount of

dibenzanthracene, if present at all, was less than 0.01 mg. These results may be compared with other evidence on the rate of disappearance of free and detectable hydrocarbon following injection. Chalmers (1934) determined the quantity of 1:2:5:6-dibenzanthracene remaining in the breast muscle of fowls at short intervals after local injection of the hydrocarbon (1-5 mg.) dissolved in chicken fat or egg-yolk fat. The substance disappeared rapidly from the site of injection, decreasing to less than one-tenth of the original quantity within a few days. Chalmers and Peacock (1936) found that 3:4-benzpyrene and 1:2:5:6-dibenzanthracene were eliminated from chick embryo within a few days of the intramuscular injection in fatty solution of fractions of a milligram of these substances. Minute quantities of the former compound were also removed within a few hours of the intravenous injection of colloidal preparations in both chick embryos and mice. Berenblum and Kendal (1936) estimated the concentration of 1:2:5:6-dibenzanthracene at different times after the intraperitoneal injection of 2 mg. in mice. The greater part disappeared within 3 weeks, and the loss was more complete when the substance was injected dissolved in lard than when it was given as a colloidal solution in water.

Although the present experiments are sufficient to prove the comparative resistance of chemically induced tumours, few data can as yet be offered on the stability or otherwise of such resistance during the course of propagation of the tumour strain by serial implantation. This aspect of the subject is of considerable biological importance, and although the evidence available is quite insufficient to justify any conclusion it may be recalled that the LR-10 rat sarcoma, originally induced by means of 1:2:5:6-dibenzanthracene, was moderately sensitive to the inhibitory action of the same compound when it was tested in the 87th generation of transplantation (Haddow and Robinson 1937). It is recognised that alterations of a genetic character, and involving change by loss, may occur in the tumour cell during the process of transplantation (e.g., Cloudman 1932), and it therefore seems possible that the state of resistance may diminish or fade as the tumour cell becomes more remote from the environmental conditions which led to its emergence. It is obvious that any proof would indicate an important physiological distinction between recently induced tumours and similar tumours after prolonged transplantation.

On the other hand it would appear that the chemo-resistance of parasitic organisms, once

acquired, persists for long periods when the strains are passed through normal untreated animals. Thus Browning (1908) described an atoxyl-resistant strain which was still highly resistant after 140 passages through mice over a period of fourteen months, and a strain resistant to trypan-blue was similarly resistant after eighty passages extending over six months through mice and after five passages through rabbits over a period of five months. While in another strain of atoxyl-fast organisms the resistance was still marked after 67 passages during six months, the resistance had disappeared in the eighty-ninth passage some seven weeks later. Schilling (quoted by Duke 1933) believed that T. brucei may shed acquired abnormalities as a result of passage through Glossina morsitans.

Murgatroyd and Yorke (1937a) found that T. rhodesiense and T. brucei, which are normally insusceptible to the action of organic arsenicals, became susceptible after animal passage. However, when T. brucei was then made arsenic-resistant it showed no loss of the acquired character on passage.

According to Yorke (1936; see also Murgatroyd and Yorke 1937b) an atoxyl-resistant strain of T. rhodesiense retained unimpaired its original resistance for a period of over 7 years, during which

it was passaged through a series of about 900 mice. Tryparsamide-resistant and acriflavine-resistant strains likewise showed no loss of resistance after maintenance in mice for $5\frac{1}{2}$ and 3 years respectively. A Bayer-resistant strain, however, showed a progressive loss of resistance as it was passaged through normal mice. When resistance was completely developed a dose of 10 mg. per 20 g. of mouse failed to clear the blood of parasites. A year later the resistance had so far decreased that a dose of 0.5 mg. sufficed to clear the blood, and three years later a dose of only 0.025 mg. was sufficient to produce the same result.

Robertson (1929) found that a strain of Bodo caudatus showing mass resistance to acriflavine retained this character substantially, though not entirely, for at least $4\frac{1}{2}$ months of culture upon drug-free media. Such cultures moreover reacquired further fastness, after months of subculture in normal media, with great rapidity and vigour. Nevertheless, when resistant cultures were propagated continuously on drug-free media, there was an appreciable diminution of resistance, probably through loss by the dilution of multiplication over long periods. "The evidence supporting the theory that the loss is due in part to dilution or the wearing down of the modification by multiplication

is upheld by the acceleration of the loss in clones derived from single isolations from the mass-resistant cultures."

6. Recapitulation.

The present paper has been concerned (1) with the facts, so far as they are available, which indicate the relation between the growth-inhibitory action of the carcinogenic hydrocarbons and the cells of tumours induced by these substances, and (2) with the biological principles which may govern such relationships not only in the etiology of cancer but also in other examples of cellular variation in response to chemical substances of a toxic or interfering kind. It has already been pointed out that the reaction of susceptible growing tissues to the inhibitory effects of the carcinogenic hydrocarbons is characterised by unusual prolongation, and it is obvious that when normal cells are repeatedly exposed to such agents, as in the experimental production of new growths, the inhibition must continue without remission. Any degree of cellular recovery is therefore precluded, and it is of the utmost importance that the cell can react to a pathological environment of this special

kind in only two directions, (a) by death, or (b) by adaptation. Such a situation may profitably be contrasted with the pathological effects of pressure. In this case the continuous application of even moderate degrees of pressure results in a uniform atrophy, while if the force is applied intermittently, rapid recovery takes place in the intervals and leads either to re-establishment of the normal state or, if conditions are suitable, to a simple hypertrophy compensatory in type.

It would appear that the carcinogenic hydrocarbons, while they may kill a proportion of the cells with which they are brought into contact, are nevertheless of such a nature that cellular adaptation is the more frequent result. On account of the comparative persistence of the inhibitory effect no simple adjustment, such as is represented by normal recovery, is possible, and it therefore follows that adaptation, if it is to occur, must be so radical in nature as to involve actual transformation of the cellular organisation. It is suggested that inhibition of growth is compensated by a process of dedifferentiation which, since grades of differentiation and characteristic growth-rates show an approximate inverse proportionality, must effect an immediate and automatic increase in the potential growth-capacity of the cell even in

the presence of the hydrocarbon. Apart from adaptation in this broader sense it has been the purpose of the present investigation to determine whether the cells of chemically induced tumours manifest any resistance towards the inhibitory action of the compounds employed. As has been seen, such tumours are very appreciably less sensitive than the transplantable and spontaneous tumours previously studied, but the resistance appears devoid of specificity and is insufficient on that account to justify any assumption of etiological significance.

On the other hand the study of acknowledged cases of drug-resistance shows a similar lack of specificity, a condition which would appear to be characteristic of the response to non-antigenic as opposed to antigenic poisons. While the nature of the resistance exhibited by the cells of chemically induced tumours does not allow a proof that it is necessarily adaptive, the fact of resistance is not opposed to the inhibitory theory and appears consistent with it.

To conclude, it is postulated that the primary biological effect of carcinogenic substances is an interference with normal growth, which, if it is maintained for a sufficient period, frequently leads to cell modification by dedifferentiation. Within

limits, and particularly in the earlier stages of induction, this change may be reversible. If the process is continued, however, a stage is reached after which the transformation is permanent and does not recover even if inhibition be relaxed. The loss in differentiation produces an immediate increase in the potential growth-rate of the variant, roughly proportional to the extent of degradation and probably accompanied by corresponding metabolic alterations. These changes must tend to emancipate the cell from an environmental restraint which would otherwise lead inevitably to death; further, since the process is irreversible, the new cell race is released not only from the local restriction which led to its origin, but also from the integrating forces of the body as a whole, so that it can readily be maintained by serial propagation in the normal tissues of genetically compatible hosts. With due caution, and bearing in mind the provisos which have already been mentioned, it would appear that the cells of chemically induced tumours may, particularly at the time of their emergence and shortly afterwards, manifest a condition of non-specific resistance to the inhibitory influence of the substance which provoked their appearance. Only limited information is available as to the stability or otherwise of the

resistant state during the course of transplantation, but it is possibly impermanent and may prove to be a 'Dauermodifikation' rather than a true heritable change. Robertson (1929) regarded the acquired fastness of Bodo caudatus to acriflavine as a modification not apparently in the nature of a mutation, but representing rather a piling up of changes in a particular direction, and followed, in certain cases where the organisms were subsequently propagated in drug-free medium, by progressive wearing down of the accumulated changes by the dilution involved in rapid fission.

IV. Summary.

1. Sarcomata were induced in rats and mice by means of 1:2:5:6-dibenzanthracene, sodium-1:2:5:6-dibenzanthracene-9:10-endo-$\alpha\beta$-succinate, 3:4-benzpyrene, cholanthrene and methylcholanthrene. When the growth-rate of these tumours had been assessed over a sufficient control period the animals received one or two intraperitoneal injections (in sesame oil) either of the compound used for induction or of another compound (1:2:5:6-dibenzanthracene, 1:2:5:6-dibenzacridine, 3:4-benzpyrene, methylcholanthrene, 6:7-dimethyl-1:2-benzanthracene, 10-methyl-1:2-benzanthracene, 1:2:5:6-dibenzphenazine or styryl 430): all these compounds had previously been shown to possess some degree of growth-inhibitory activity when tested against spontaneous mouse tumours and/or transplant-:able rat tumours.

2. The data indicate that chemically induced sarcomata, regarded as a class, tend to be considerably less susceptible to the inhibitory action of carcinogenic substances than are spontaneous or transplantable tumours. This relative resistance is not however specific, since tumours induced by a given carcinogenic compound were not significantly more resistant to the inhibitory action of the same

compound than to that of other carcinogenic substances.

3. From these experiments it is therefore impossible to derive any proof, such as could be provided by high specificity, that the inhibitory properties of the carcinogenic hydrocarbons are of etiological importance. On the other hand, cases which are acknowledged as true examples of the induction of resistance (e.g., cellular adaptation to organic arsenical or metallic drugs), are frequently characterised by absent or strictly limited specificity, and it is accepted that accommodation to non-antigenic poisons is much less specific than that to antigenic substances: such cases are discussed in detail in relation to the general problem. It is concluded that while the present data afford no proof of an etiological relation between carcinogenicity and inhibitory power, they may perhaps be regarded as circumstantial evidence in favour of such a relation.

V. Appendix.

T A B L E XXVI

Methylcholanthrene rat sarcoma

No. L (see Fig. 95).

Day	Tumour diameters and sum (cm.)
1	2.25, 1.20, 3.45
2	2.35, 0.75, 3.10
3	2.30, 1.20, 3.50
4	2.15, 1.15, 3.30
5	2.40, 1.05, 3.45
6	2.15, 1.15, 3.30
8	2.20, 1.05, 3.25
9	2.20, 1.30, 3.50
10	2.20, 1.25, 3.45
11	2.30, 1.20, 3.50
12	2.60, 1.00, 3.60
13	2.05, 1.15, 3.20
15	2.05, 1.10, 3.15
16	2.25, 1.10, 3.35
17	2.25, 1.05, 3.30
18	2.15, 1.00, 3.15
19	2.40, 1.15, 3.55
20	2.60, 1.30, 3.90
22	2.40, 1.15, 3.55
23	2.55, 1.40, 3.95
24	2.20, 1.10, 3.30
25	2.50, 1.25, 3.75
26	2.65, 1.60, 4.25
29	2.70, 1.55, 4.25
30	3.05, 1.40, 4.45
---	25 mg. methylcholanthrene ---
31	2.75, 1.55, 4.30
32	3.10, 1.65, 4.75
33	3.10, 1.80, 4.90

--- continued over

T A B L E XXVI continued.

Day	Tumour diameters and sum (cm.)
34	2.95, 1.75, 4.70
36	3.25, 1.95, 5.20
37	3.15, 2.00, 5.15
38	3.25, 2.20, 5.45
39	3.30, 2.50, 5.80
40	3.60, 2.55, 6.15
41	3.50, 2.55, 6.05
43	3.80, 2.65, 6.45
44	3.60, 2.55, 6.15
45	3.90, 2.80, 6.70
	--- 30 mg. methylcholanthrene ---
46	3.95, 3.00, 6.95
47	4.00, 3.25, 7.25
48	4.00, 3.20, 7.20
50	4.25, 3.40, 7.65
51	4.25, 3.80, 8.05
52	4.80, 4.35, 9.15
53	4.80, 3.85, 8.65
54	5.05, 4.10, 9.15
55	4.70, 3.80, 8.50

T A B L E XXVII

Cholanthrene rat sarcoma IX

(see Fig. 108).

Day	Tumour diameters and sum (cm.)
1	3.15, 2.80, 5.95
2	3.70, 2.85, 6.55
4	3.90, 3.45, 7.35
5	4.20, 3.40, 7.60
6	4.85, 4.30, 9.15
	--- 30 mg. 3:4-benzpyrene ---
7	5.05, 3.90, 8.95
9	5.45, 4.15, **9.60**
10	5.40, 3.85, 9.25
11	5.40, 4.20, 9.60
13	5.55, 4.30, 9.85
14	5.35, 4.30, 9.65
15	5.75, 4.30,10.05
16	5.75, 4.30,10.05
17	6.00, 4.45,10.45
18	6.00, 4.45,10.45
20	6.00, 4.65,10.65
21	6.65, 4.45,11.10
22	6.50, 4.60,11.10
23	6.90, 4.65,11.55
24	6.95, 5.25,12.20
25	7.10, 5.25,12.35
27	6.80, 4.70,11.50

T A B L E XXVIII

1:2:5:6-dibenzanthracene rat sarcoma No. VII (Fig. 100)

Day	Tumour diameters and sum (cm.)
1	2.30, 2.10, 4.40
2	2.40, 2.25, 4.65
3	2.55, 2.15, 4.70
4	2.50, 2.30, 4.80
6	2.70, 2.45, 5.15
7	2.75, 2.60, 5.35
8	2.90, 2.65, 5.55
9	2.95, 2.75, 5.70
10	3.05, 2.80, 5.85
	--- 14 mg. 1:2:5:6-dibenzacridine ---
11	3.05, 2.80, 5.85
13	3.20, 2.70, 5.90
14	3.10, 2.75, 5.85
16	3.10, 2.85, 5.95
17	2.95, 2.60, 5.55
21	3.10, 2.95, 6.05
22	3.15, 2.90, 6.05
23	3.15, 2.75, 5.90
24	3.15, 2.80, 5.95
25	3.20, 2.65, 5.85
27	3.10, 3.00, 6.10
28	3.20, 3.00, 6.20
29	3.20, 2.80, 6.00
30	3.35, 2.90, 6.25
31	3.35, 2.70, 6.05
32	3.45, 3.00, 6.45
34	3.55, 3.25, 6.80
35	3.60, 2.80, 6.40
36	3.70, 3.20, 6.90
37	3.65, 3.25, 6.90
38	3.75, 3.30, 7.05
39	3.70, 3.40, 7.10
41	3.80, 3.50, 7.30
42	3.80, 3.10, 6.90
43	3.70, 3.35, 7.05
44	3.45, 3.25, 6.70

VI. References.

Berenblum, I. and Kendal, L.P.	1936	Destruction of 1:2:5:6-dibenzanthracene in the mouse. Biochem. Jour., 30, 429-432.
Boyland, E. and Boyland, M.E.	1934	Studies in tissue metabolism. III. The effect of oxidised 1:2:5:6-dibenzanthracene. Biochem. Jour., 28, 244-256.
Broom, J.C., Brown, H.C. and Hoare, C.A.	1936	Studies in micro-cataphoresis. Trans. Roy. Soc. Trop. Med. Hyg., 30, 87-100.
Browning, C.H.	1908	Chemo-therapy in trypanosome-infections: an experimental study. Jour. Path. Bact., 12, 166-190.
Browning, P.	1938	The chemotherapeutic action of synthalin in experimental infections with T. brucei and T. congolense. Jour. Path. Bact., 46, 323-329.

Burnet, F.M. and Lush, D.	1936	Induced lysogenicity and mutation of bacteriophage within lysogenic bacteria. Austral. Jour. Exp. Biol. Med, Sci., 14, 27-38.
Chalmers, J.G.	1934	Role of 1:2:5:6-dibenzanthracene in the production of fowl tumours. Biochem. Jour., 28, 1214-1218.
Chalmers, J.G. and Peacock, P.R.	1936	Further evidence regarding the elimination of certain polycyclic hydrocarbons from the animal body. Biochem. Jour., 30, 1242-1248.
Cloudman, A.M.	1932	A comparative study of transplantability of eight mammary gland tumors arising in inbred mice. Amer. Jour. Cancer, 16, 568-630.
Coventry, F.A.	1925	The reaction product which inhibits reproduction of the trypanosomes in infections with T. lewisi, with reference to its change in titre throughout the course of infection. Amer. Jour. Hyg., 5, 127-144.

Cunningham, J., Theodore, J.H. and Fraser, A.G.L.	1934	Further observations on Indian relapsing fever. Pt. I. Types of spirochaetes found in experimental infections. Ind. Jour. Med. Res., 22, 105-156.
Davenport, C.B. and Neal, H.V.	1895	Acclimatization of organisms to poisonous chemical substances. Arch. f. Entwick. d. Organ., II, 564-583.
Desjardins, A.U.	1934	The radiosensitiveness of cells and tissues and some medical implications. Science, 75, 569-575.
Dingle, J.H.	1936	Growth-inhibiting power of specific antisera. Amer. Jour. Hyg., 23, 1-9.
Duke, H.L.	1933	The typanosomes of man. Their resistance to arsenical drugs. Lancet, ii, 553-557.
-------	1935	Arsenic resistance in trypanosomes. Lancet, i, 903-904.

Gay, F.P., Atkins, K.N. and Holden, M. 1931 Resistance of dehydrated pneumococci to chemicals and heat. Jour. Bact., 22, 295-307.

Haddow, A. 1936 Ann. Rept. Brit. Empire Cancer Campaign, 292-302.

------- 1938a Cellular inhibition and the origin of cancer. Acta, Union internationale contre le cancer, 3, (in press).

------- 1938b The influence of carcinogenic compounds and related substances on the rate of growth of spontaneous tumours of the mouse.

Haddow, A. and Robinson, A.M. 1937 The influence of various polycyclic hydrocarbons on the growth-rate of transplantable tumours. Proc. Roy. Soc., B, 122, 442-476.

Haddow, A., Scott, C.M. and Scott, J.D. 1937 The influence of certain carcinogenic and other hydrocarbons on body growth in the rat. Proc, Roy. Soc., B, 122, 477-507.

Hawking, F. 1937 Studies on chemotherapeutic action. I. The absorption of arsenical compounds and tartar emetic by normal and resistant trypanosomes and its relation to drug-resistance.

Jour. Pharm. Exp. Ther., 59, 123-156.

Heilbrunn, L.V. 1937 An outline of general physiology.

Philadelphia 1937.

King, H., Lourie, E.M. and Yorke, W. 1937 New trypanocidal substances.

Lancet, ii, 1360-1363.

Lorenz, E. and Shear, M.J. 1936 Studies in carcinogenesis. II. The detection of dibenzanthracene in mouse tumours induced by this hydrocarbon.

Amer. Jour. Cancer, 26, 333-343.

MacNider, W. de B. 1935 Acquired resistance of liver cells to the toxic action of uranium nitrate.

Proc. soc. exp. biol. med., 32, 791-793.

---------- 1936a The acquired resistance of fixed tissue cells to chemical injury.

Southern Med. Jour., 29, 1189-1194.

---------- 1936b Acquired resistance of fixed tissue cells.

Jour. Pharm. Exp. Ther., 56, 359-372; 373-382; 383-388.

Murgatroyd, F. and Yorke, W. 1937a Studies in chemotherapy. XIII. The changes observed in T. brucei during five years' maintenance in the laboratory.

Ann. Trop. Med. Parasitol., 31, 145-160.

---------- 1937b Studies in chemotherapy. XIV. The stability of drug-resistance in trypanosomes.

Ann. Trop. Med. Parasitol., 31, 165-172.

Murray, J.A. 1931 29th. Ann. Rept. Imperial Cancer Research Fund.

Oesterlin, M.	1936	Chemotherapie, Fluorescenz und Krebs. Klin. Woch., 15, 1719-1722.
----------	1937	Zusammenhange in der Chemotherapie im Hinblick auf das Carcinom-Problem. Klin. Woch., 16, 1598-1601.
Pedlow, J.T. and Reiner, L.	1935	On the mechanism of chemotherapeutic action. XII. Comparison of the binding of chemotherapeutic agents by normal and resistant trypanosomes. Jour. Pharm. Exp. Ther., 55, 179-199.
Pett, L.B.	1936	Studies on yeast grown in cyanide. II. Biochem. Jour., 30, 1438-1445.
-------	1937	Observations on yeast grown in cyanide. Report of proceedings, IInd. Internat. Congress for Microbiol., London. 475-476.

Robertson, M. 1929 The action of acriflavine on Bodo caudatus. Parasitology, 21, 375-416.

Russell, H. 1936 Observations on immunity in relapsing fever and trypanosomiasis. Trans. Roy. Soc. Trop. Med. Hyg., 30, 179-190.

Schlossberger, H. and Schuffner, R. 1934 Drug-resistance experiments with arsenopyridine derivatives on trypanosomes. Jour. Chemother., 11, 81-92.

Taliaferro, W.H. 1929 The immunology of parasitic infections. New York 1929.

Turner, C.W. and Gomez, E.T. 1936 The radiosensitivity of the cells of the mammary gland. Amer. Jour. Roentgenol., 36, 79-93.

Robertson, M.	1929	The action of acriflavine on Bodo caudatus. Parasitology, 21, 375-416.
Russell, H.	1936	Observations on immunity in relapsing fever and trypanosomiasis. Trans. Roy. Soc. Trop. Med. Hyg., 30, 179-190.
Schlossberger, H. and Schuffner, R.	1934	Drug-resistance experiments with arsenopyridine derivatives on trypanosomes. Jour. Chemother., 11, 81-92.
Taliaferro, W.H.	1929	The immunology of parasitic infections. New York 1929.
Turner, C.W. and Gomez, E.T.	1936	The radiosensitivity of the cells of the mammary gland. Amer. Jour. Roentgenol., 36, 79-93.

White, P.B. 1937a Resistance developed against bacteriophage.

Report of proceedings, IInd. Internat. Congress for Microbiol., London, 378.

-------- 1937b Lysogenic strains of V. cholerae and the influence of lysozyme on cholera phage activity.

Jour. Path. Bact., 44, 276-278.

Wilson, J.L. 1922 Tolerance and acquired tolerance of the mesenchyme cells in tissue cultures for copper sulphate and sodium arsenite.

Bull. Johns Hopkins Hosp., 33, 375-377.

Yang, Y.N. and White, P.B. 1934 Rough variation in the cholera vibrio and its relation to bacteriophage.

Jour. Path. Bact., 38, 187-200.

Yorke, W. 1936 In Discussion on pharmacological action.

Proc. Roy. Soc., B, 121, 601-605.

Yorke, W. and Murgatroyd, F. 1935 Biological problems in chemotherapy. Trans. Roy. Soc. Trop. Med. Hyg., 28, 435-457.

Yorke, W., Murgatroyd, F. and Hawking, F. 1931 Studies in chemotherapy. V. Preliminary contribution to the nature of drug-resistance. Ann. Trop. Med. Parasitol., 25, 351-358.

ON SECONDARY COLONY DEVELOPMENT IN BACTERIA

AND AN ANALOGY WITH TUMOUR PRODUCTION IN HIGHER FORMS

On secondary colony development in bacteria and an analogy with tumour production in higher forms.

I. Introduction.

II. Secondary colony formation.

III. Possible interpretations.

1. Contamination hypothesis.
2. Primary selection hypothesis.
2a. Biochemical changes involved, in relation to primary selection.
3. Transformation and secondary selection.
3a. Specificity of transformation.

IV. Theories concerning the mechanism involved in transformation.

1. Hybridisation.
2. Segregation of characters in non-equational fission.
3. Dedifferentiation, dissociation, adaptive reduction.
4. Bacterial mutation.
5. The side-chain theory.

On secondary colony development in bacteria and an analogy with tumour production in higher forms.

I. Introduction.

In a recent review (Haddow 1938) and in other papers (see Haddow 1936, Haddow and Robinson 1937) the author propounded the theory that the cancer cell represents a discontinuous and irreversible variant of the corresponding somatic cell, differing from the latter in the characters of fission and metabolic behaviour. It was suggested that the problem of the origin of cancer might therefore be regarded as a special case in the origin of cellular variation, and suggestions were made as to the fundamental nature of the environmental changes leading to tumour production. In particular, it was postulated that these changes are frequently inhibitory in nature, and that the increase in growth-rate which marks the emergence of malignancy is due in many cases not to any primary stimulation of growth but represents the emancipation of potentially variable cells from chronic inhibition. Thus, evidence was presented to show that a number of the carcinogenic hydrocarbons exhibit growth-inhibitory properties not possessed by a considerable number of related non-carcinogenic substances. It was

further found (Haddow, Scott and Scott 1937) that the inhibitory effect thus produced differed from that manifested by diverse toxic substances in its relative prolongation even after a single administration and in its primary independence of toxic action in the non-specific sense. The stages in the production of malignancy by the carcinogenic hydrocarbons (and possibly by other tumour-producing agents such as x- and gamma-radiation) were therefore conceived to be (1) the enforcement of a sustained retardation in the rate of growth of the treated normal cells; and (2), an irreversible dedifferentiation of the affected but viable cells accompanied by permanent metabolic alterations, increase in growth-rate, and functional release from environmental inhibition.

It may be asked whether there is any other instance of the operation of such a principle of variation, and it is the object of the present paper to draw attention to such a case. In the author's opinion the environmental origin of organic change can nowhere be so clearly deciphered, or studied with such a degree of experimental control, as in the bacteria, and although the investigation of variation in bacteria possesses inherent disadvantages as compared with certain aspects of the same subject in other living forms, these are partly absent in the present example. The phenomenon of secondary colony

formation has long been recognised by bacteriological workers, e.g., by Günther 1895, Hartmann 1903 in Torula colliculosa n. sp., Preisz 1904 and Eisenberg 1906. But it is almost certainly not familiar to the generality of investigators in other fields of biology, and it is clear that relatively little attention has been concentrated on its wider significance.

II. Secondary colony formation.

The growth of a bacterial colony is in every case limited by the quantity of available foodstuff in the surrounding medium, and in certain cases it is also retarded by the local accumulation of inhibitory products of bacterial metabolism. Ordinarily therefore the increase of such a colony is brought to a close by the operation of these factors in differing degrees, the major influence being mostly a decrease in available nutriment to such a level as to render protoplasmic synthesis --- and cell division --- no longer possible. In normal circumstances no further colonial change of any significance takes place. It is however a matter of old-standing observation that for certain

bacterial strains the colony evolution is more complex than the above, since in these cases development is accompanied or followed by the appearance of so-called papillae or secondary colonies (daughter colonies, Knöpfe), which apparently arise from the substance of the primary or mother colony. It is, further, of the greatest interest that the cells of these secondary colonies can frequently be shown to metabolise some constituent of the medium which the cells of the primary colony are unable to attack. An important factor is obviously the inherent capacity for variation of any given strain, and it seems clear that "an organism which has no capacity for spontaneous variation to a carbon compound is not caused to vary to it by cultivation in contact with the compound no matter how long the contact may be prolonged" (Lewis 1934). It is nevertheless probable that daughter colonies may be observed in cultures of all species of bacteria, a view which was supported by Enderlein.

Klotz (1906) showed that a non-lactose-fermenting organism, B. perturbans, if grown on solid media containing lactose, produced colonies which developed lactose-fermenting papillae. A further and important example of this phenomenon was provided in a strain of B. coli studied by Neisser (1906) and Massini (1907) and referred to by them

as Bact. coli mutabile. When this organism was grown on solid media containing lactose and a suitable indicator such as neutral red, it formed primary colonies which were unable to split the available lactose and which therefore remained pale. During development, however, secondary colonies appeared which frequently possessed the property of fermenting lactose. The work of Massini was repeated by Burk (1908), and the succeeding years saw a number of papers confirming and extending these observations. Thus Muller (1909, 1911; see also Benecke 1909) described the production of papillae by certain strains of coliform bacilli grown in the presence of arabinose and by B. typhosus when grown on rhamnose or iso-dulcite. Similarly, Burri and Duggeli (1909) and Burri (1910) described a coliform bacillus (Bact. imperfectum) not fermenting saccharose but yielding a saccharose-fermenting race (B. perfectum) after growth in medium containing this sugar. Other studies at that time included those by Kowalenko (1910) with single-cell technique, Jacobsen (1910), Thaysen (1911), Penfold (1911, 1912), Bernhardt and Markoff (1912), Mandelbaum (1912), Dobell (1912-13) and Baerthlein (1913), while among more recent contributions may be mentioned those of Stewart (1926, 1927, 1928), Haag (1927), Hadley (1927), Ramchandani (1929), Kennedy, Cummings and Morrow

(1932), Lewis (1933, 1934), Kritschewski and Ponomarewa (1934), Hall (1935), Dulaney and Michelson (1935) and Deere, Dulaney and Michelson (1936).

Lewis (1933) classified his variable strains --- of B. mycoides --- in three groups as follows: (1) those obtaining carbon principally from nitrogen-containing compounds but capable of variation in relation to sucrose; (2) those giving secondary colonies on agar containing protein alone, variation apparently being related to some unused fraction of the split protein; (3) strains giving secondary growth both in the presence and absence of sucrose but varying to sucrose in preference to protein. The addition of non-utilisable carbon compounds in respect of which the organism lacked the capacity to vary (organic acids as well as carbohydrates and alcohols) was without effect in all groups, nitrogen metabolism proceeding unhindered. The same author (1934) used eleven strains of colon bacteria variable to lactose or sucrose, two strains of B. paratyphi B variable to raffinose, and three strains of B. typhi variable to rhamnose, and his work is of special importance as showing that secondary colony formation may occur in synthetic media containing lactose as the sole source of carbon. According to Hall (1935) --- in Bact. coli mutabile --- "the

'mutant' type rarely, if ever when pure, reverts to the parent type, and, as a rule, is indistinguishable from it morphologically, culturally or serologically, except by its more rapid fermentation of lactose, and by its inability to form characteristic multiple papillae or secondary colonies."

Figs. 141 and 142 show the appearance of secondary colony formation in strains of B. paratyphosus B in which the secondary variant possessed the property of fermenting lactose. Muller (1909) discovered that B. paratyphosus B (although not B. aertrycke) produces secondary colonies when grown in the presence of raffinose, and Figs. 143-154 trace the development of a single colony of another strain of the same organism on raffinose-neutral-red-bile-salt-agar.

Figs. 141 and 142. Secondary colony formation in strains of B. paratyphosus B on neutral-red-bile-salt-lactose-agar. Fig. 141 x 30, Fig. 142 x 10.

Fig. 143.
12 hours.

Fig. 144.
18 hours.

Fig. 145.
24 hours.

Fig. 146.
30 hours.

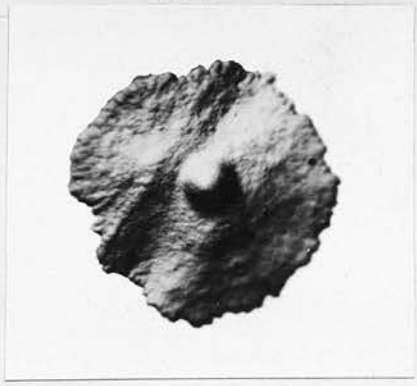


Fig. 147. 42 hours.

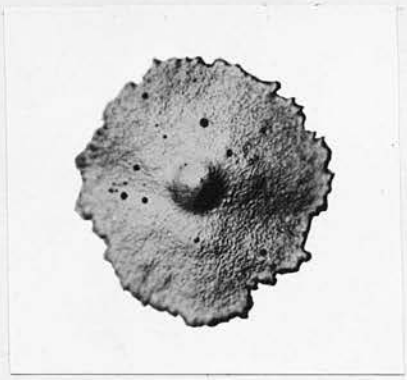


Fig. 148. 54 hours.

Fig. 149. 66 hours.

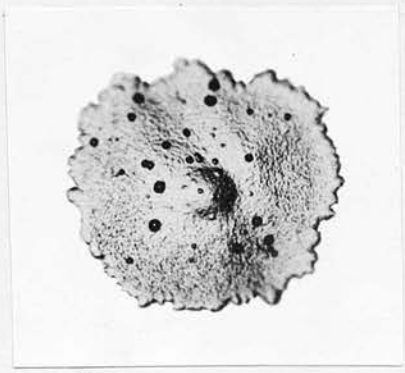


Fig. 150. 78 hours.

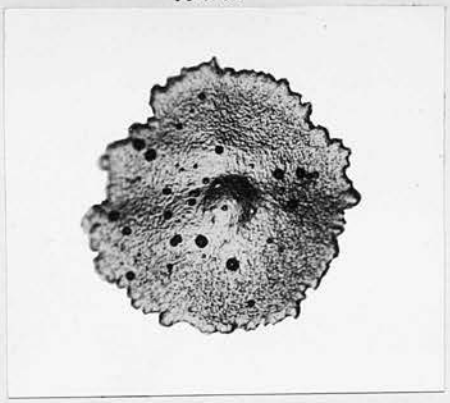


Fig. 151. 90 hours.

Fig. 152. 114 hours.

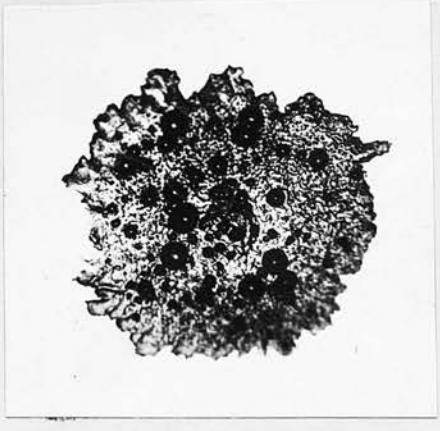


Fig. 153. 162 hours.

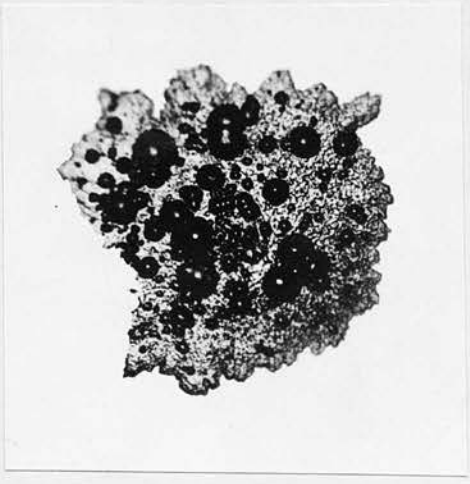


Fig. 154. 222 hours.

III. Possible interpretations.

1. Contamination hypothesis.

It is clear therefore that there is no doubt of the reality of the phenomenon as one affecting many strains of a considerable number of bacterial species. It must however be realised that several interpretations are theoretically possible, and these may now be examined. It has been suggested in the first place that the appearances might depend on a simple contamination of the culture by the organism appearing in the papillae. There is however a considerable body of evidence to show that no ordinary form of the contamination hypothesis is sufficient to account for the facts observed. In particular it must be stressed that not infrequently the cells of the secondary colonies can only be shown to differ from those of the primary colony in one biological character, that of utilising a constituent of the medium which the primary is unable to attack. This at least tends to indicate genetic relationship between the two forms. Again, secondary colony formation in a suitable strain is very commonly found to occur in every colony in each of repeated platings, and possesses a definite relation in time --- as will be shown --- to the development of the primary colony. Further, admixture of discrete colonies of the two

types is not ordinarily met with: hence purification from the alleged contaminant is not possible. It is therefore apparent that contamination in the usual sense is hardly an acceptable explanation.

2. Primary selection hypothesis.

Even when the close relationship of the two forms be admitted, there is however the alternative possibility that they co-exist in a natural mixture or a symbiotic relationship, and that preponderance of one over the other is brought about by certain selective factors in the environment. But any simple selection hypothesis is greatly weakened by the fact that the behaviour of strains such as Bact. coli mutabile as described above, remains unchanged even after long-continued purification (as by repeated plating of broth cultures derived from single colonies). It is further disproved by the results obtained from single cell isolations (e.g., Kowalenko 1910; Penfold 1912, p. 215) which Lewis (1933) regarded as excluding selection in many cases.

The problem is not unlike that in the work of Stephenson and Stickland (1933), in which experiments

were conducted to test whether the hydrogenlyases produced during the 20-hour period of a culture were the result of selection. It was shown that when formate is introduced into a growing culture of Bact. coli the enzyme appears in full strength before the lapse of a single generation, i.e., before any demonstrable increase in the total or viable count. In this as in the case under discussion, it seems that the phenomenon is a transformative rather than a selective process, and that the cause is represented by environmental changes in the medium. In any discussion of the possible selective origin of secondary colony forms it must be remembered that these variants can almost always be shown to possess a metabolic advantage in that they are capable of utilising a constituent of the medium not utilisable by the primary form. If their origin were purely selective, therefore, one might hope to detect signs of a continuous utilisation of the substance in question (e.g., lactose) from the beginning of the culture. As will be seen subsequently in another connexion, this probably does not occur. While evidence is thus strongly against simple or primary selection as an explanation of secondary colony formation, there is no possible doubt that once variation has occurred the altered environmental circumstances are such as must exert powerful

selective forces, leading to a relative preponderance of the variant and in many cases to overgrowth of the primary colony.

2a. Biochemical changes involved, in relation to primary selection.

At this stage some attention must be devoted to the evidence which is available regarding the biochemical changes involved in secondary colony formation. In the first place it is obvious that one is not justified in assuming that carbohydrates are not utilised simply because the medium fails to become acid. This was pointed out by Merrill (1930) and, in addition, Jones, Orcutt and Little (1932), working with strains of atypical colon bacilli, concluded from quantitative determinations that lactose in their experiments was utilised from the beginning of culture, although the lactose broth remained alkaline for periods varying from 3 or 4 days to 2 or 3 weeks. It is a possibility therefore, as has been pointed out by several workers, that both the parent and variant strains of Bact. coli mutabile are able to utilise lactose and that the difference in their behaviour is due to

a difference in type of sugar metabolism. In 1934 Lewis remarked "much has been written on the problem of whether the specific sugar or alcohol is attacked from the beginning of a culture or only after a period of contact with it, but no very definite experimental evidence is available and opinions differ." In 1927 however Stewart had investigated the same problem by a method employing B. coli communis as an indicator of the presence of lactose. He found that before the emergence of the secondary form Bact. coli mutabile consumed either no lactose or only minute quantities, e.g. less than 0.0325 percent. in four days. On the other hand the primary form varied to the secondary form in a discontinuous manner, leading to an abrupt and steep increase in sugar consumption. In 1936 moreover Deere, Dulaney and Michelson carried out experiments, using the official gravimetric method for lactose determination of the Association of Agricultural Chemists, to determine whether the primary form of Bact. coli mutabile utilises lactose from the beginning of growth or only after the appearance of the secondary form. The results showed that the parent form of the organism uses only very small quantities of lactose, if any, before the secondary variant can be detected. On the other hand, the primary form showed similar ammonia production and

pH changes whether grown in plain broth or lactose broth, indicating that it uses nitrogenous compounds as its source of energy. Further, the primary and secondary forms when grown in plain broth produced like changes in ammonia production and pH. These authors pointed out however that their results did not eliminate the possibility that minute amounts of lactose might be utilised by Bact. coli mutabile, since the lactose determination was not sufficiently accurate to estimate the disappearance of traces from a concentration of one per cent. It is of interest that for E. coli Hershey and Bronfenbrenner (1936) found no indication of a lactose-fermenting mechanism other than preliminary hydrolysis to the constituent monosaccharides. Real changes in lactose fermentation may be attributed to variation in the cellular activity of lactase, an enzyme which Hershey and Bronfenbrenner found to be intracellular and not liberated to any appreciable extent after death and autolysis of the bacteria.

3. Transformation and secondary selection.

Quite apart from the simple selection hypothesis already considered, the view was put forward by Smith (1913) and others that variation occurs in the absence of any specific sugar and that the sugar when present merely acts as a selective agent favouring the "spontaneous" variant. Stewart (1927) however thought there was no basis for any suggestion that variation of this type is constantly occurring without the appropriate stimulus. As has already been mentioned, there seems little doubt that selection must operate immediately transformation has taken place, so that the next phase of the problem concerns the relation of the sugar to the induction of variation. Writing in 1933 Lewis thought there could be little or no difference of opinion that enhancement of fermentative power takes place only when the organism is cultivated in contact with the specific compound with which the variation is associated....."ability to attack unused nutrients is acquired through variation due to the specific stimulus exerted by the substance concerned." But this author later modified his opinion (1934), and described experiments which he claimed showed that variation in certain coliform strains occurred in the absence of lactose, the variant cells being however so few as to escape

detection by conventional methods of plating. He then regarded the evidence as conclusive "that variation of mutabile strains to a given specific carbon compound occurs spontaneously in media from which the compound is absent", and looked on sugars and alcohols as acting as acting as specific selective agents rather than as stimulators of variation. While Lewis still correlated the occurrence of variation with limitation of growth due to depletion of the medium, he felt himself obliged to abandon the theory of a specific inciting stimulus to variation in mutabile strains of bacteria. In the writer's opinion this problem of the relation between the carbon compound and the induction of variation is still not solved, and there appears to be a need for further critical experiments with purified synthetic media containing no trace of the substance in question.

3a. Specificity of transformation.

It is of interest that the study of training and adaptation in bacteria gives considerable support to the theory that primarily non-utilisable compounds in the substrate may influence the induction of variation in a more or less specific fashion. A good account of this subject is given by Knight (1936), according to whom "the enzymic constitution of bacteria and yeasts can change under the stimulus of changed composition of the media in which the cells are grown, and can respond to different substrates by production of the appropriate enzyme." Among examples of this type of change may be quoted the work of Dienert (1900) who showed that a yeast usually unable to ferment galactose became able to do so after being grown in the presence of this sugar. Similarly Twort (1907; see also Penfold 1911, 1912) carried out important experiments on the induction of the property of lactose-fermentation (normally absent) in B. typhosus. Twort found that the long-continued propagation of this organism in a lactose-containing substrate might be followed by the appearance of variants capable of fermenting this sugar, and in addition he described a number of similar variations in carbohydrate fermentation when other organisms were grown for various periods of time in the presence of

different sugars. Prolonged growth under such circumstances was thus shown to tend to the production of variants which fermented the particular substances with which the strain was cultured. Although this process was referred to as "training", Penfold suggested that the modus operandi of the sugar might again be largely selective. Penfold also studied the fermentation of dulcitol by B. typhosus. Late fermentation of dulcitol is however recognised as a frequent attribute of this organism, and this author found that of several different strains of B. typhosus grown in litmus-dulcitol-peptone-water some formed acid in one or two days, although the majority did not do so till the fourth or fifth day or even later. When such cultures were plated out daily on agar containing dulcitol and neutral red, the proportion of red colonies to white showed a gradual increase from the second to about the fifth day, reaching a maximum when the peptone water culture had become acid. Arkwright (1930) regarded the history of this variation as "a typical case of the transformation of a culture which is unable to ferment a certain sugar or alcohol into one capable of rapidly fermenting it." In such a case the opportunities for the operation of natural selection are obvious in so far as a few individual bacteria capable of fermenting the alcohol are afforded an excess supply

of available foodstuff as compared with the non-fermenters, a preferential situation which is likely to result in the overgrowth of the latter by the former. But the critical point in the underlying biology is once more the question of the precise mode of emergence of the fermenting strain, and in this connexion it is of importance that in this case also the white colonies of the original culture on dulcitol-neutral-red-agar in the course of development produced red papillae composed of active dulcite-fermenters.

Karstrom (1937) investigated the relation between the presence of specific carbohydrates in the substrate and the development of the corresponding carbohydrases in bacteria. As a result he classified the cell enzymes as (1) constitutive enzymes produced constantly and independently of the composition of the media in which the cells were grown; and (2) adaptive enzymes produced only in the presence of, and in response to, specific substrates in the culture medium. According to Virtanen (1934), "in general it seems that the metabolic enzymes proper are always constitutive, whereas the enzymes (especially hydrolases) which convert the nutrients into suitable form for the actual metabolic reactions may be adaptive." The apparently adaptive production of hydrogenlyases by organisms of the

colon group was examined by Stephenson and Stickland (1932, 1933) and Yudkin (1932). The last author discussed the alternative hypotheses of selection and adaptation as possible explanations of the phenomenon, and decided in favour of the latter. Similar evidence was provided by Stephenson and Stickland, who showed that while the addition of formate to Bact. coli growing in tryptic casein-digest medium produced no simple selective action in either direction, adaptive production of formic hydrogenlyase occurred more quickly than would be expected on a purely selective basis. In a later study of the formation of galactozymase by Saccharomyces, Stephenson and Yudkin (1936) further weakened the selection hypothesis by demonstrating that specific and adaptive enzyme production occurred in the complete absence of cell growth or multiplication. According to Knight (1936), "the training of an exacting strain to become non-exacting involves a change in the enzymic constitution of the organisms. An exacting strain which requires, for example, tryptophan as an essential nutrient, when it is trained and becomes non-exacting and able to use ammonia as sole source of nitrogen, must have acquired enzymes to enable it to synthesise tryptophan using ammonia as nitrogen source. In general, in the training of exacting strains to become non-exacting, enzymes required for

the synthesis of protoplasm from the simpler nutrients have to be produced. The suggestion is, therefore, that the new enzymes are produced as a direct reaction to the chemical stimulus of the new nutrients, in the absence of the normal nutrients." In a study by Gladstone (1937) of the nutrition and nitrogen requirements of Staphylococcus aureus, the great majority of strains grew well on media of known chemical composition which included 16 amino-acids. Initial differences in amino-acid requirements were found to exist among these strains, but as the organisms were adapted to utilise fewer amino-acids such differences tended to disappear. Finally, by a process of training, strains were produced which could grow on a medium from which all amino-acids were excluded, and in which the main source of nitrogen was ammonia.

It must be mentioned that in most cases of the adaptive production of enzymes by bacteria the alterations are of the nature of fluctuational modifications, withdrawal of the specific substrate leading to atrophy or disappearance of the appropriate enzyme system. But the information they yield is sufficient at least to suggest that similar specific relationships may exist between organism and substrate in the induction of irreversible and heritable changes. In the view of Hershey and Bronfenbrenner (1936)

phenomena suggesting the specific elaboration of enzymes corresponding to a defined substance present in the medium indicate no more than the realisation of a potential and perhaps latent physiological capacity of the bacterial species in question. This may be compared with the earlier suggestion by Grey (1924) that the bacterial enzymes concerned in the decomposition of carbohydrates are few compared with the number of substances which may be attacked. Grey postulated five systems which in various combinations might cause the degradation of such substances: an enzyme removing hydrogen, an enzyme removing oxygen, and enzymes splitting off groups of 1, 2 and 3 carbon atoms respectively, the last three being interchangeable or at any rate of common origin.

IV. Theories concerning the mechanism involved in transformation.

From the evidence detailed above it is reasonable to conclude that the phenomenon of secondary colony development involves biological transformation, and that the main role of selection is confined to the period following emergence of the variant. It now becomes necessary to consider the various possibilities concerning the mechanism of change. "In order that two differentiated halves may be produced, some event must take place by which a chemical distinction between the two halves is effected." (Bateson 1913). Morphological appearances alone are of little or no value in deciding this question. The component organisms of secondary colonies may present no apparent structural divergence from the normal, although the writer has frequently found evidence of pronounced disintegrative changes in such cells. In a study of impression films of the secondary colonies produced by B. paratyphosus B (Schottmuller) on raffinose-agar, Kritschewski and Ponomarewa (1934) described and depicted an extraordinary series of large-celled variants of spindle- and trypanosome-like morphology. But such changes are irregular in occurrence and difficult of interpretation, and the mechanism of variation can

best be studied from physiological rather then morphological data.

1. Hybridisation.

Attention must first be directed to the hypothesis which posits a sexual process in bacteria and hybridisation as the source of such variants. While the study of variation in bacteria is undoubtedly subject to certain disadvantages, it has been widely assumed that since such cells divide as a rule by asexual binary fission with a complete absence of the fundamentally non-essential influence of sex and conjugation, variation by hybridisation can well be excluded. But although one must always entertain the possibility of an autogamous or even syngamous fusion in bacteria as the lowest order of sexuality, it is curious to find that the mere occurrence of discontinuous and heritable variation has been regarded as evidence of sexual reproduction in these forms, a view dismissed by Henrici (1928) as a quite unwarrantable assumption. From time to time however certain authors have attempted to describe or interpret the phenomena of bacterial variation in the terms employed for higher forms. Thus in a study of diphasic variation in the Salmonella, White (1925)

thought there could be no doubt that specific and non-specific qualities were in general alternatives comparable almost to a pair of Mendelian allelomorphs. Stewart (1926, 1927, 1928) is however perhaps the only author to attempt a description of secondary colony formation in Mendelian terms. He chose the paracolon bacilli and the colon bacilli as examples of stable and true-breeding non-lactose-fermenters and lactose-fermenters respectively, with Bact. coli mutabile as an unstable non-lactose fermenting organism regularly giving off a fermenting daughter-race; and he brought forward evidence which he interpreted as showing that these three groups are Mendelian variants of one species, of which paracolon is the homozygous dominant, mutabile the heterozygote, and colon the homozygous recessive. Since the positive character for fermentation is recessive he regarded the dominant factor --- for Bact. coli mutabile probably correctly as will be seen later --- as an inhibitor, and claimed that while all three groups have the power of fermenting lactose, the dominant and heterozygote also carry a factor which inhibits this property. Representing the inhibitory factor as I, its absence as i, and the factor for lactose fermentation as F, he described the constitution of the three groups as follows: paracolon IIFF, Bact. coli mutabile IiFF, colon iiFF. The regularly

recurring variation of mutabile Stewart represented as Mendelian variation brought about by the segregating of allelomorphs in a heterozygote, IiFF dividing to IIFF, IiFF and iiFF. The most powerful objection to such a hypothesis is the fact that the IIFF form is not found among the descendants of the papillae of mutabile in culture, a difficulty which Stewart suggested might be explained by an overgrowth of this form by the IiFF form, or by the association of a lethal factor with the IIFF combination. In discussing the objection that hybridisation must depend on gametic union, Stewart pointed out the lack of positive evidence that bacteria do not in fact conjugate and the possibility that, even if they do not, a heterozygote may arise by mutation affecting one member only of an allelomorphic couple. As a result of his studies Stewart drew up the following as the laws governing variation in native heterozygous bacteria: "1. A bacterium heterozygous in a given character (e.g., power of fermenting lactose), if exposed to the appropriate stimulus, will give descendants of two kinds: (a) heterozygous like the parent, (b) homozygous recessive (fermenting lactose). Since the positive character is recessive to the negative, the formulae of the two must be written IiFF/L for the heterozygote, and iiFF/L for the recessive. The homozygous

dominant, IIFF/L, a paracolon, has not yet been found among the offspring of native heterozygotes. 2. The homozygous recessive or red race breeds strictly true, even after many subcultures on media indifferent for the character concerned. 3. If a race is heterozygous for two or more characters (e.g., fermentation of lactose and dulcite), and if it is exposed to the stimulus appropriate for one character it will segregate in this character only. 4. Segregation will only take place in answer to a definite stimulus and in adaptation thereto. There is no basis for the suggestion that variation is constantly taking place without the appropriate stimulus, but that the varying forms only survive when selected by appropriate surroundings, and that in consequence the variation falsely appears adaptive."

Stewart's hypothesis has not received any degree of support, although it represents a very necessary and complete examination of one of the more important possible mechanisms involved in this type of variation. Henrici (1934) however thought there was no justification for regarding these variations as evidence of sexual reproduction, and opinion is general that the facts can be interpreted on a simpler basis. According to Henrici "we have ample evidence that

variations similar to those exhibited by bacteria occur in other organisms known not to exhibit sexual reproduction. We may observe variants arising in secondary colonies or in sectors, in cultures of moulds and yeasts and unicellular green algae. In moulds, little localised tufts of aerial mycelium differing from the parent type in colour or spore formation may be considered homologous with secondary colonies. Now when moulds and yeasts reproduce sexually they form typical sexual spores, ascospores or zygospores, which may be readily observed and recognised. Cultures of moulds and yeasts may be found to exhibit all of the types of variation encountered in colonies of bacteria, without exhibiting any sign of sexual spores. The author has observed a dissociation of the yeast Torula pulcherrima into red and white strains and into smooth and rough forms without any trace of ascospore formation. Moreover, it has been possible to demonstrate typical variations of this type in cultures of a fungus (one of the smut parasites) which was started from a single spore known to be in the haploid state. Here there could be but one sex in the culture, yet permanent variations occurred." It is of further interest that Erikson (1935), in a study of the aerobic streptothrices, and Sherman and Wing (1937), for the coli-aerogenes group, were unable to obtain any evidence of a sexual mode of reproduction.

2. Segregation of characters in non-equational fission.

Topley and Ayrton (1923-24) pointed out that if the variations of bacteria are to be interpreted on the basis of genetics, the facts may be fitted to the theory more readily if we assume that a segregation of characters has occurred during a non-equational fission, rather than assuming that they have resulted from a re-combination of characters in a sexual fusion of cells (see Henrici 1934). "The biological factors discussed must have a material basis, and the suggestion that the variations observed are due to an unequal distribution of the substances concerned, at the moment when division of the bacillus occurs, is so obvious that it may well be provisionally accepted as a working hypothesis. Such acceptance does not imply any specific view as regards the structure or mode of reproduction of the bacilli. We know that multiplication by binary fission occurs, and this form of growth gives ample opportunity for such a segregation as we have suggested."

Variation by non-equational division is probably best exemplified by the phenomenon of colonial sector-formation. It has long been observed that whole sectors of a colony may show obvious variation in one or more of a number of

characters (e.g., microscopic or gross structure, opacity or consistency; biochemical activity such as pigment production or specific fermentations). A general study of the subject was given by Nirula (1928) and a mention of sector-formation in Ascomycetes by Dodge (1936), while Punkari and Henrici (1935) described interesting variations in a chromogenic asporogenous yeast (T. pulcherrima) involving both secondary colony formation and sectoring. It is of some interest that the angulation of the sector, and the distance of its apex from the centre of the colony, give a measure of the time of emergence of the variant in relation to the growth of the colony as a whole. It is also striking that such sector-formation in colonial organisms is the simplest possible example of modification within the "Woodger's cell-cone" of developmental biology (see Woodger 1931; and Needham 1936, Fig. 13 and p. 63). Fig. 155 illustrates the appearance of lactose fermenting sectors in a non-lactose-fermenting organism, and other examples have been observed by the writer from time to time. Thus when certain plate-cultures of B. paratyphosus B (Tidy) were examined under the Leitz Ultropak at a magnification of about 50 diameters and in conditions of dull illumination, it was found that the colonies, which under other

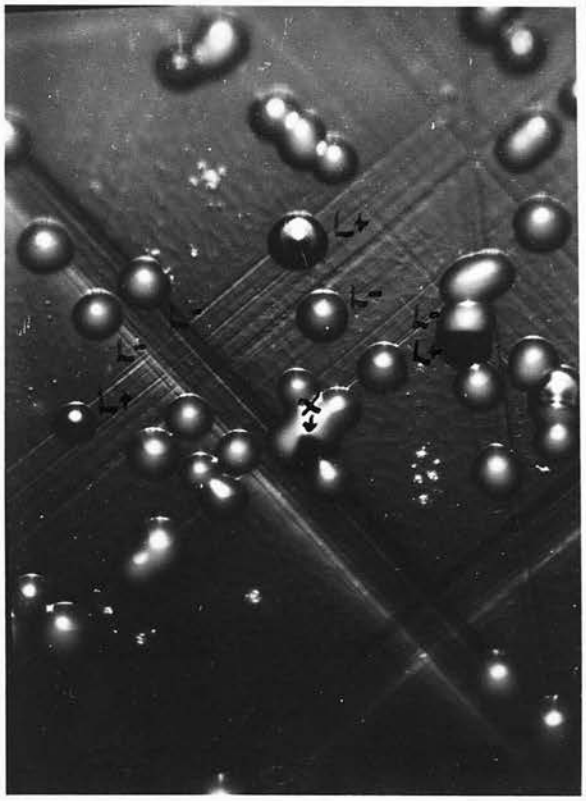


Fig. 155. Sector formation and appearances suggesting variation by non-equational fission in an organism growing on neutral-red-bile-salt-lactose-agar. Note lactose-fermenting sector at X. x 30.

conditions of magnification and illumination appeared perfectly uniform, were sharply divided into two types according to colour. While some were dull white, others possessed a delicate olive-green colour, and in addition a number of colonies showed remarkably beautiful sector-variation in these two colours. Such appearances were probably dependent on differences in the surface structure of the two colonial types.

It is found as a rule that the cells of the sector breed true, while the bulk of the colony gives rise to further colonies which may show sector-formation in turn. That is, the relation between primary cell and variant is the same as in the case of secondary colony formation. This physiological similarity is of interest in the study of secondary colonies themselves, since the possible influence of selection in the induction of these variants can be discounted much more readily in sector-formation, for obvious reasons, than in the case of secondary colony formation. It is also of importance that Nirula's studies on sectoring were carried out with isolated cells obtained by a modification of the Burri single-cell technique.

3. Dedifferentiation, dissociation, adaptive reduction.

It is probable from the relation between normal and variant strains both in secondary colony formation and in sectoring that the variant is derived from the parent by a process involving dedifferentiation or loss. Since the change results in the manifestation of a character (e.g., lactose-fermentation) previously apparently absent, it follows that the material lost must be regarded as an inhibitor of the reaction or enzyme-system concerned. Such changes are usually discussed under the heading of "bacterial dissociation", a term however which Gardner (1935) regarded as inept, and to which he preferred "adaptive reduction." If the change under discussion is indeed a metabolic dedifferentiation which permits the utilisation of an otherwise inaccessible substrate, it may be compared with the metabolic reversions described by Fildes (1934). "It appears possible to assume that when an organism adjusts itself to a simple substrate with which normally it cannot deal, it is because at one time in the history of the species it had been in the habit of using that substrate, that the process is a process of atavistic reversion," (as in the synthetic adjustment of the typhoid bacillus in the presence of ammonia, with which it cannot normally

deal, but absence of tryptophan, on which it normally is dependent).

It is important that more than one degree of dedifferentiation may be shown. Thus Stewart (1927) described a typhoid race "Bucknall" which achieved full dulcite-fermenting capacity by two discontinuous steps, each of which took place in papilla formation on dulcite. The race thus formed pale red papillae from which came white and pale red colonies. The pale red colonies in their turn formed dark red papillae, which gave pale red and dark red colonies on subculture. The white, pale red and dark red forms were quite distinct and remained so after growth on dulcite-free media. Stewart interpreted the phenomenon as due to the successive segregation of two inhibitory factors. Lewis (1934) also noted tertiary colony formation on continued ageing.

4. Bacterial mutation.

The question now arises whether the emergence of secondary growth can justifiably be referred to as bacterial mutation. Although the term has been freely employed in bacteriological literature, note must be taken of certain objections and qualifications to its use. Jollos was among the first to point out that the term "mutation", coined for sudden changes occurring in the forms of life with sexual reproduction (metaphyta and metazoa), is not applicable to asexual bacteria in which testing of the mutation process by crossing experiments is impossible. Again, in the opinion of Haldane (1933) a given difference between two somatic cell types cannot be proved or disproved, by genetical methods, to be due to chromosomal aberration or gene mutation, since such cells do not reproduce sexually and it is only by sex-reproduction that the geneticist can distinguish nuclear from plasmatic changes. "The proof that a change is due to a gene mutation can only be given if the altered gene segregates from its normal allelomorph at meiosis according to Mendel's laws....." (Haldane 1934). But according to the same author (1932) "there is no reason to think that bacterial mutation is a phenomenon essentially different from mutation in higher organisms, and it is not even clear that it is commoner."

Much of the difficulty is due to the use of the term without adequate definition, and confusion has arisen through insufficient appreciation of, or respect for, its technical use, with all its added implications derived from plant and animal genetics. Further, many geneticists appear disinclined to allow the word in any but its artificial or technical sense. The situation is exactly similar to that in the description of the malignant cell as a somatic mutation (see Haddow 1937). It may be noted on the other hand that a definition of mutation sufficiently wide to include micro-organisms (and somatic cells) was given by Gates (1915). According to Bernhardt (1915) the term "mutation" must be used in bacteriology in a sense other than that of de Vries, and as implying that the descendants show new heritable characters, regardless of the means by which their hereditary constitution has been influenced, or of the extent of the change.

Assuming that the conception of mutation is valid in the present example, the simpler mechanisms may briefly be reviewed. The possibility of mutation by factorial loss (and especially by the loss of an enzyme-inhibitor in non-equational fission) has already been considered as a probable interpretation in some of the cases at least. It is naturally of

especial importance in circumstances where dedifferentiation or degradation of various kinds (e.g., serological) have been experimentally demonstrated, and it permits less scope for reversibility than other explanations. But apart from the loss (or gain) of individual factors, variation may be due to the temporary inhibition of one by another, a conception referred to by Toenniessen (1921) as "Valenzwechsel der Erbfaktoren." The changes occurring in the cell were also likened (by Gates) to the shift of a polygon of forces moving to a new position of stability. Lastly, according to Henrici (1934) the assumption of a tautomeric structure of the living protoplasm of bacteria may explain the changes occurring in the genetic elements by means of mutation, and the resultant permanent modification forms or mutants. Thus Gotschlich (1929) considered it possible that the genes behave in a manner similar to tautomeric forms which exist in equilibrium while their substitution forms are unequivocal and definite.

5. The side-chain theory.

Muller (1911) regarded the type of variation under discussion, and particularly that shown by B. typhosus in the presence of iso-dulcite, as an adaptation to a substance which otherwise inhibits growth. Further, he sought to interpret such changes on the basis of Ehrlich's side-chain theory. According to this view B. typhosus possesses receptors which anchor iso-dulcite: growth is therefore inhibited since the bacillus is not able to use these receptors for assimilation. There is considerable likelihood that this conception is fundamentally correct, and experimental evidence is available to support it. Thus Knight (1936) discussed the inhibition of growth in nitrifying bacteria (Nitrosomonas, Nitrobacter) produced by organic compounds such as glucose, urea and asparagine, with special reference to the work of Winogradsky on inhibition of growth and of Meyerhof on inhibition of respiration. In such experiments respiration and growth are inhibited in parallel, growth-inhibition resulting from a respiratory inhibition which is due in turn to displacement of the normal respiratory substrate (in this case ammonia or nitrite) or to a block of the respiratory catalyst (as by glucose when applied in the absence of normal substrate). Similarly, Sobotka, Holzman

and Reiner (1936) found that non-utilisable pentoses, and particularly xylose, have a retarding effect on the rate of fermentation of hexoses by brewer's yeast, an effect due mostly to competitive diffusion. Muller's hypothesis, that certain non-utilisable compounds in the substrate may inhibit growth through interference with the assimilation of utilisable compounds, is therefore seen to be an entirely reasonable one. Assuming its correctness he postulated that the mutation consists in the emergence of a race capable of overcoming this receptor-block.

V. Cellular inhibition and the origin of discontinuous variation.

1. The general concept.

There can be no doubt that the cellular changes under discussion are intimately bound up with antecedent or coincident changes in the bacterial environment. In this regard Muller's application of Ehrlich's hypothesis is of much greater interest than any of the theories already discussed, since it suggests both cause and mechanism, while the others are concerned with alternative mechanisms alone. The mere proof of the correctness of any one of these alternatives (e.g., simple segregation, sexual segregation, or the various possibilities included under bacterial mutation) might in no way elucidate the real problem, viz., the nature of the force which induces such change. To the writer, the side-chain hypothesis seems of particular interest as representing a special case of what may be suggested as a principle of wider application, namely, that cellular inhibition of various kinds and degrees is the initial process in the induction of dedifferent-:iating, irreversible and therefore heritable changes in asexual cells. The source of inhibition appears to be mainly if not exclusively environmental in origin, so that knowledge of the nature of the

significant environmental alterations gives an opportunity to correlate these with the resultant biological change. In this connexion Table XXIX shows the development of raffinose-fermenting secondary colonies in B. paratyphosus B (Tidy) --- on 1 per cent. raffinose-neutral-red-bile-salt-agar --- in relation to the growth of the primary (five colonies). The aggregate of these data is presented in Fig. 156, and data based on Figs. 143-154 are shown graphically in Figs. 157 and 158. Lemon (1933) suggested that the maximum expansion rate in a bacterial colony be indicated by plotting the actual increase in unit intervals of time. If for each period of time (T) the bacterial count or area is N_0, N_1, N_2, ...at times t_0, t_1, t_2, ..., the following formula is used:

$$\frac{(N_1 - N_0)}{(t_1 - t_0)_{\frac{1}{2}}(T_1)}, \quad \frac{(N_2 - N_1)}{(t_2 - t_1)_{\frac{1}{2}}(T_2 - T_1)\ T_1}, \text{ ... etc.,}$$

where the suffix of the denominator indicates the mid-point of the period during which the increase has occurred. Fig. 158 shows the advantage of this method in demonstrating the relation of the emergence of secondary colonies to inhibition of the growth of

T A B L E XXIX

Development of raffinose-fermenting secondary colonies in B. paratyphosus B in relation to the growth of the primary colony.

Hours after inoculation	Mean diameter of primary col. in micrometer units					Number of secondary colonies				
col.-	1	2	3	4	5	1	2	3	4	5
17	9	10	8	10	5	0	0	0	0	0
24	20	20	21	19	16	0	0	0	0	0
28	26	25	24	25	23	0	0	0	0	0
40.5	37	35	34	41	40	0	0	0	0	0
48	43	40	40	47	46	0	0	0	0	0
52	42	40	40	50	50	?	?	?	0	?
65	43	42	40	58	55	11	18	20	11	2
72	43	42	40	60	63	14	27	20	12	4
78.5	43	43	40	65	65	22	30	25	14	6
91	48	50	46	75	74	32	48	39	35	33
100	50	45	45	78	70	45	52	39	41	47
118	50	48	45	78	93	65	79	68	91	76
137	50	50	50	80	85	100	102	115	148	108
160	53	52	50	100	95	95	134	115	160	130

the primary colony.

For the successful induction of discontinuous and permanent variation in any cellular character two main factors thus appear to be necessary: (1) an environmental source of interference with, or inhibition of, the given character, of such a nature as to be long-continued but nevertheless admit of the survival of the majority of the affected cells; (2) an organism possessing the inherent capacity for variation in respect of the character in question. The general concept has been well expressed by Noel Paton (1926): "If changes in environment lead to changes in the chemical processes in the living units they may so alter the conditions that continued existence is impossible, or may lead to adaptation to the chemical changes, provided that the modification is not detrimental to existence under the altered conditions." In the theoretical case of an organism growing in a completely unimpeded manner in a medium to which it is ideally adapted, no variation of any kind will be expected to occur. Such a state is most nearly approached in the logarithmic phase of bacterial growth, and Winslow (1935) has shown that if one transfers a culture in this phase of growth to a fresh medium of the same kind, the cell characters remain strikingly constant. It has already been suggested that depletion of

foodstuff is the most important factor in the inhibition of growth of a bacterial colony growing in a limited medium, and it is certainly significant that the influence of increased density of inoculation is to accelerate secondary colony formation, and the effect of frequent subculture to postpone both papillation and variation. "If subcultures are made daily on to fresh media, the change which initiates papilla formation can be deferred indefinitely in the bacteria carried on, for variation does not appear in them even if the appropriate sugar is continuously present." (Stewart 1927). Papillation may also be prevented by the addition of utilisable substrate: thus Lewis (1933) described a protein-sparing effect in an interesting strain of B. mycoides in which, although secondary colonies appeared on agar containing protein alone, the variation apparently being related to some unused fraction of the split protein, no secondary growth occurred on the addition of sucrose.

2. The nature of the inhibitory process and adaptive response.

Speaking of the induction of heritable variation in general terms, it seems probable that identical end-results may be produced through inhibitory effects of different kinds. It may be regarded as certain that the gradual inhibition in growth-rate of a developing bacterial colony is not dependent on intrinsic causes, as was assumed by Stewart (1927; cf. Winslow 1935, above). The effect appears to be conditioned entirely by environmental changes, and the nature of these may now be discussed.

The physiological results of these changes constitute senescence. In relation to the induction of variation it is therefore important that secondary colony formation is closely dependent on the ageing of the primary colony, and that papillae appear first on the older or more central portions, where inhibitory changes are likely to be more intense. Inhibition of growth is almost certainly dependent on a metabolic inhibition, and it is of interest that Wooldridge, Knox and Glass (1936), in a study of the effect of age on bacterial enzymes, found that the activity of

most bacterial dehydrogenases appears to increase at first, to reach a maximum --- usually within 24 hours --- and subsequently to diminish. The same close relation between enzyme activity and phase of growth was further studied by Wooldridge and Glass (1937; see also the data given by Bach 1936 for decline in activity of various dehydrogenases in B. proteus in the first 96 hours of culture).

Lewis (1933) recognised the arrest of growth as due not to intrinsic but to extrinsic factors, the chief being depletion of nutrients. But he also described experiments to show that metabolic changes in the medium, quite apart from simple depletion, influence secondary colony formation. Lewis regarded it as improbable that any general law could be applied to all cases but thought that different extrinsic factors might cause arrest of growth in different organisms. In some cases for example retardation is probably assisted by alkaline changes in the medium --- a circumstance which must imply an additional advantage to the acid-producing organisms of the secondary colonies --- while in others inhibition is known to be due in part to accumulation of peroxides. Gause (1934) found that the growth of populations of Saccharomyces cerevisiae ceased before exhaustion of the energy sources of the medium, the effect being due to the establishment

of a definite ratio between the concentrations of alcohol and sugar. Brown (1937) has recently discussed the production of growth-inhibiting substances by fungi. For a general account of the causes of inhibition of bacterial growth, whether by depletion or intoxication of the medium or by physical crowding, see Gay (1935).

In an interesting study of a luminous organism in relation to bacterial nutrition on agar Cruickshank (1934) investigated the question whether growth is limited by the accumulation of toxic substances or is the result of exhaustion of the nutrient substances of the medium. The activity of light production appeared to bear a direct relation to the state of growth (see also Harvey 1928, Shoup 1929 and Johnson 1936 on oxidation intensity and luminescence in bacteria. Harvey found exact agreement in the oxygen consumption of luminous bacteria as determined by the Thunberg micro-respirometer and by the time which elapses before the luminescence of an emulsion of the bacteria in sea water begins to dim, when over 99 per cent. of the dissolved oxygen has been consumed). By using the light production of the colonies, recorded photographically, as a measure of metabolic activity, Cruickshank found that diffusible food substances are rapidly consumed from the agar in the

neighbourhood and subsequently from a distance. It is of interest that the rate of diffusion of essential food substances was found to correspond approximately to that of haemoglobin in agar. Since the same principle appeared to apply in the case of other organisms, it was concluded that the important factor in determining the final amount of growth is the volume of agar from which the organisms may ultimately draw their food supply. Experiments were also described to show that whereas luminosity declined with exhaustion of the medium, it became restored following replenishment by agar patching. Cruickshank found it difficult therefore to explain his findings on the basis that arrest of growth is due to inhibition by metabolic products, but concluded that from the time that growth commences there is set up a gradient of concentration of nutritive material, and that with continued growth the concentration throughout the agar surrounding the colony progressively falls. He recognised the important fact that although nutrition might fall below the level adequate for cell-division, it might still be sufficient for some measure of metabolic activity.

In a study of factors influencing bacterial populations Cleary, Beard and Clifton (1935) found

that growth is first restrained by the changing ratio of total cells to available building material. This view was supported by Clifton, Cahen and Morrow (1936), who concluded that "growth-rate decreases as the concentration per cell of materials essential for growth decreases in cultures of bacteria in which a relatively high population has been established." In a similar study Damboviceanu and Roth (1936) also attributed arrest of development to disappearance of foodstuff, and were unable to detect the presence of inhibitory substances. From experiments described by Hershey and Bronfenbrenner (1937) it appears that under the usual conditions of cultivation, rates of growth and respiration of Bact. coli are limited by the rate at which oxygen can reach the cells, and that this limitation prevents rapid exhaustion of the nutrient materials. If however oxygen is available in excess, growth soon ceases as a result of oxidative removal of foodstuffs. They also stated that their work did not lend support to the view that accumulation of growth-inhibitory metabolic products is responsible for cessation of growth in bacterial cultures.

But although inhibition is very largely due to the negative influence of depletion or deprivation, there is no doubt that the same result may also be

produced to some extent, as has already been indicated, by the positive effects of various harmful substances in the medium. These may be classified as (1) toxic substances foreign in nature, i.e., possessing no natural relation to the metabolism of the cells in question; (2) toxic products of metabolism (for the mathematical aspects of intoxication of a medium by catabolic substances see Kostitzin 1935, 1937); (3) potentially nutrient substances which are however not utilisable but cause inhibition by competitive interference with assimilation of the normal substrate. Similar to substances in class (3) are compounds which, while possessing high affinity for specific enzymes, cannot be attacked and therefore inhibit (e.g., the competitive inhibition of succinic dehydrogenase by malonic acid). And it is probably of related interest that in at least a number of cases (e.g., xanthine oxidase, Dixon and Thurlow 1924) the velocity of enzyme activity may be appreciably retarded by even moderate increase in the concentration of specific substrate.

Whereas acute inhibition or injury is followed either by death or by recovery, the type of mild yet chronic inhibition described in the present paper is such as does not cause death in any high proportion of the affected cells, at least for a considerable time.

The conditions are probably similar to those in an ageing population of Paramecium caudatum (see Gause 1934, Fig. 5), where decline in reproduction-rate is accompanied by increasing environmental resistance, increase in the intensity of competition, and decrease in the capacity to realise potential growth. Secondly, gradual recovery is impossible on account of the uniform persistence of the inhibition. In the case of a bacterial culture allowed to age without renewal of medium or removal of inhibitory substances, it is obvious that inhibition must continue without remission, provided the organism itself does not vary. But the cell may undergo adaptive variation if such a process is inherently possible for any given strain, and there can be little doubt of the relative physiological advantages of such a change as is exemplified by the formation of secondary colonies. In the face of a condition of gradually increasing stasis we find the multicentric development of areas showing vigorous proliferation, and these proceed to increase both in size and in numbers at a time when the growth of the primary colony continues to be retarded or has altogether ceased.

Since an inhibition of the kind suggested may be produced either by the prolonged non-lethal action of certain "toxic" substances or by the constant presence of non-utilisable substances competing with

the normal substrate, it is probable that the corresponding adaptive reactions are (1) a change whereby absorption of the toxic substance is lessened, the cell thereby becoming relatively refractory to its action; and (2) a change enabling the cell to metabolise the previously non-utilisable inhibitor and so emancipate itself from the inhibition due to this cause. Secondary colony formation is an ideal example of the latter mechanism, and it is of the greatest possible interest that proven examples of the former are also available. It has long been recognised for instance that the exposure of pathogenic trypanosomes to non-curative concentrations of various trypanocidal drugs frequently leads to the appearance of new strains exhibiting a more or less specific drug-resistance (see Schlossberger and Schüffner 1934; Yorke and Murgatroyd 1935a,b; von Jancso and von Jancso 1935), and Ehrlich originally advanced the hypothesis that acquired resistance of protozoan strains is due to a loss of binding capacity. In an important paper Hawking (1937; see however Pedlow and Reiner 1935) investigated the absorption of arsenical compounds by normal and atoxyl-resistant trypanosomes by exposing the organisms <u>in vitro</u> at 37° C. to suitable concentrations of these compounds. The parasites were then centrifuged out and the distribution of the arsenical determined,

either by measuring the trypanocidal activity of the supernatant fluid for fresh trypanosomes or by chemical estimations of the arsenic content of the supernatant fluid and of the deposited trypanosomes. Hawking found that while normal trypanosomes absorbed all the available drug from suitable concentrations of typical trivalent compounds (reduced tryparsamide, halarsol and novarsenobillon), living resistant trypanosomes absorbed little or none from similar concentrations of the same compounds, although absorption occurred if stronger concentrations were used or if the trypanosomes were dead. A similar difference in the behaviour of normal and resistant trypanosomes was observed when the parasites were exposed to reduced tryparsamide *in vivo*. Compounds to which the atoxyl-fast trypanosomes showed no resistance, *e.g.*, phenyl-arsenoxide, sodium arsenite or tartar emetic, were absorbed to the same extent by both types of organism. It therefore appears that the comparative failure of atoxyl-fast trypanosomes to absorb typical trivalent arsenical compounds constitutes the explanation of the drug-resistance of these parasites. In a discussion of these results Hawking suggested their further interpretation on the hypothesis of receptor modification, the change being directed primarily against the side-chains on the benzene ring.

Although drug-resistance in the trypanosomes is one of the best examples of adaptive transformation in the face of a dysgenic influence, there are others of considerable interest. Thus Neuschlosz (1919, 1920, quoted by Gay 1935) found quinine-fast paramecia capable of destroying 80 per cent. of the drug in solution while non-adapted organisms in the same solution produced a disappearance of less than 5 per cent. Again, according to Pett (1936, 1937), the effect of continuous subculture of yeast in cyanide indicates the possibility of developing a strain which will remain constant with or without cyanide.

The principles above defined are strikingly exemplified by the methods used to induce dedifferentiation --- frequently irreversible or associated with renewed power of growth --- in bacteria and protozoa generally. These include prolonged ageing, starvation, or the growth of an organism in its own antiserum or in suitable concentrations of bacteriostatic dyes, and are patently such as result in a profound interference with metabolism and growth. The mechanism of the bacteriostatic action of dyes was studied by Huddleson (1937) in the case of the Brucellae.

" When a dye is added to culture media, a chemical or

physical union occurs between the dye and certain food substances in the media necessary for bacterial growth. When united with certain dyes in the proper concentration, the food substances cannot be utilised for growth energy by Brucella. Thus, there follows no visible growth on the medium.....The mechanism of dye bacteriostasis in the case of Brucella would appear to lie in inherent differences in the capacities of certain of the species to assimilate for growth energy the necessary food substances that are combined with certain dyes in the proper concentration." It is of interest that H ⟶ O changes similar to those produced by weak concentrations of antiseptics have also been described in cultures grown under conditions of deprivation, as in dilute broth or on agar with a diminished concentration of nutritive substances. The essential principle governing bacterial variation has been well expressed by Rettger and Gillespie (1935) on the basis of their observations of the process in B. megatherium. "The factors which stimulate cellular variation are apparently unfavourable to continued normal growth. When they accumulate very rapidly in a culture, development ceases before variation can take place. In other words, variation is possible only when favourable and unfavourable influences are so balanced as to

permit slow growth in the face of untoward circumstances." It is also important that the natural occurrence of serological variations or relapse strains in trypanosomiasis and in relapsing fever (see Cunningham, Theodore and Fraser 1934; Russell 1936) is probably to be interpreted as a response to the inhibitory properties of the serum which develop in the course of the infection. In a study of infections with Trypanosoma lewisi Taliaferro (1929; see also Coventry 1925) demonstrated the presence of a reaction product which inhibited the reproduction of the parasites without affecting their viability (see also Dingle 1936 on the growth-inhibitory powers of specific antisera).

VI. The analogy between secondary colony formation in bacteria and tumour production in higher forms.

The appearances described above, and in particular the generation of a vigorously growing strain of cells in a senescent colony, seemed to the writer to present a striking resemblance to the emergence of a malignant variant in the cells of ageing animal tissues. Such a comparison raises the question as to how far one can be justified in any attempt to transfer principles governing the behaviour of bacterial cells to a study of the somatic cells of higher forms. In discussing another aspect of the same problem White (1926) wrote: "It is a very far cry from the genetic phenomena of the higher animals and plants to the serology of the Salmonella types. But when the same type of explanation, which has been found to hold good in the case of the former, is found readily applicable to the latter, criticism based simply on grounds of complexity is devoid of intrinsic force." There is little doubt that certain cellular attributes are so fundamental that the principles which govern them must apply throughout the whole range of living matter. This seems most true of the laws pertaining to growth and its variations,

and if so the change to which we allude as cancer in man and animals must be more basic than sexual differentiation itself. Again, it is a notable circumstance that various important functions in different forms, as for instance the fermentation of carbohydrate by yeast and sugar-utilisation by mammalian tissues, may show chemical similarity or even identity (see Kluyver 1932). It is probable then that certain laws, and especially those relating to growth, operate in a continuous fashion throughout the whole field of biology. As a further case may be chosen the similarity or identity (logistic law of Verhulst) of such varied examples as the sigmoid curve of growth of a bacterial colony in a restricted medium (Figs. 156, 157), the curve of human population growth (Pearl 1925), the curve of human body growth as estimated by increase in height (see Kostitzin 1937 p. 181), that of the growth of the blue whale as measured by increase in length (see Report on the progress of the Discovery Committee's investigations p. 18: Colonial Office 1937), and the general statement that "populations of the most diverse kinds, ranging from bacteria and yeast to man, are found statistically to follow in their growth the logistic curve" (Pearl 1927).

If it be allowed that secondary colonies represent tumours of the primary colony, then we

assume in the latter some element of unity or primitive organisation. It is interesting that Willmer (1935) postulated such colony-wholeness in tissue cultures of metazoan cells, drawing attention to "....the suggestion that a tissue culture should not be regarded simply as a group of cells growing independently in a medium away from their natural position, but rather that it should be compared to a primitive organism, with a certain regulative capacity, that is to say, with a tendency to behave as a whole." "It might be natural to suppose that the cells of a culture grow outwards into the medium because, by so doing, they move to areas of higher oxygen tension, lower CO_2 tension, and a weaker concentration of metabolites. This in a solid medium would tend to give the culture a spherical form, which in practice it does assume when embedded in the medium far away from any surface, and it would seem possible to imagine other fairly simple forces, such as those caused by the proximity of the glass surface of the coverslip or flask, which might tend to flatten the sphere into the disk shape characteristic of well-grown cultures; but the necessity for an explanation of the curious distribution of the layers of cells away from the glass and the evidence obtained from a study of the repair of injured cultures indicate that other more

complex forces enter into the situation, and that the culture must be regarded not as a collection of individual cells but rather as a colony which shows evidence of polarity, and of the existence of definite axes." A similar principle is probably represented, in its very simplest form, by the isolated bacterial colony. Conversely, the fundamental nature of the cancer process is shown by the fact that, although the countless cells of the mammalian organism are constrained or aligned to play their part in the division of labour within the cell-state, they still retain sufficient individuality to enable them to shed their differentiation in the face of local adverse changes.

As the most constant and striking feature of cancer is rapid and continuous or uncontrolled growth of the malignant cells, so the outstanding characteristic of bacterial papilla formation is the more vigorous growth of the secondary variant. In both cases we have the apparent paradox that, in circumstances which lead to ageing or inhibition of the organism as a whole, there may appear a new cell type with a permanently increased rate of growth and, probably, with other physiological properties which enable it to resist, or to escape from, the increasing stranglehold brought about by

environmental changes. It is important to emphasise that the alteration does not consist in the acquisition of a new function so much as in variation in the property of growth which is normally possessed by all cells excepting those of extreme differentiation. There is no doubt that different strains of malignant cells have genetically characteristic rates of growth, and these are probably the reflection of a similar condition in the corresponding normal somatic cells. Again the same principle probably applies for bacteria: thus Mason (1935), as the result of a comparison of the meximal growth rates and generation times of various bacteria under optimal conditions, looked on growth rate as often characteristic of the genus.

Aside from the general aspect, the argument may now be briefly recapitulated and the analogy continued in points of detail. It is a remarkable fact that the theories which from time to time have been evolved to deal with these phenomena are strikingly similar, or even identical, in their application. So we find that selection hypotheses (for selection in the origin of cancer see Fischer 1936, 1937), various aspects of the mutation theory, and even cellular hybridisation, have all been invoked in attempts to elucidate the origin of variations of this kind. Even from the beginning

therefore it must be acknowledged that papilla-formation and tumour-formation have much in common, since they both represent a similar type of variation in an asexual cell. Again, it is clear that the cellular change in both cases is of the nature of a dedifferentiation which is mostly irreversible. It has been mentioned that variable bacteria may undergo more than one dedifferentiation, giving rise to secondary and tertiary colonies in succession, and similar changes have been shown to occur in the cancer cell. Thus Bittner (1931) showed that hereditary genetic changes might occur in the tumour cell during the process of transplantation, and that the cell might deviate from the genetic constitution of the individual which gave rise to it. Cloudman (1932) also demonstrated that a tumour in the course of propagation might change its character so as to become transplantable in a larger proportion of a mixed population, and found that in these circumstances one or more genes less were required for susceptibility in the host. Such changes are obviously in the direction of further dedifferentiation or change by loss.

As regards causation, it is the writer's belief that the origin of a malignant race of cells is not to be attributed to any process of direct stimulation of growth, as is so often assumed. From present

knowledge it appears improbable that any primary stimulatory process will produce an increased amount of growth for any period of time much longer than the duration of its action. On the contrary, the increase in rate of growth of malignant tissue is maintained quite indefinitely, and long after it has escaped from the pathological environment, localised in time as well as in space, in which it was engendered. When the application of a carcinogenic hydrocarbon is followed some months later by the appearance of a rapidly growing neoplasm, the result may suggest some process of direct stimulation. But such a view is not necessarily correct, and it seems less likely that the continued proliferation of malignant cells is due to any formative stimulus than that it represents the unmasking or release, in an adaptive dedifferentiation, of the capacity for growth which they always possessed, albeit in an inhibited sense, in their differentiated or integrated state.. In the earlier history of cellular pathology a somewhat similar problem centred round the nature of inflammatory response, and according to Welch (1897; see Welch 1937), "the doctrine of Virchow was long accepted without question, that inflammatory cell-growth is the result of the action of external stimuli, the so-called inflammatory irritants, upon the cells, which are thereby directly

incited to grow and multiply. The attack upon this doctrine has been vigorously led by Weigert, who denies absolutely the power of any external agencies to stimulate directly cells to proliferation. He considers that to concede such a bioplastic power to external agents is equivalent to the acceptance of a kind of spontaneous generation of living matter. Weigert's views upon this subject have undoubtedly had a most powerful influence upon pathology. It has been such an influence as a good working hypothesis, whether finally demonstrated to be true or not, has often had in the development of science. In putting to the test of actual observation Weigert's hypothesis, we have been led to recognise the frequency and the importance of primary injuries to cells inflicted by external agencies. Not only various degenerations and necroses of entire cells, but more subtle and partial damage of cytoplasm and nucleus have been made the subject of special study. It has been recognised that our older methods of hardening tissues reveal often only very imperfectly the finer structure of cells, and new and better methods have been introduced which enable us to detect more delicate lesions of cell-substance which formerly escaped attention.....Weigert's postulate of some primary injury to the tissues as the immediate effect of mechanical, chemical and

other external agencies, which were formerly regarded as the direct stimuli of cell-growth and multiplication, has been fulfilled in many instances where such damage had previously been overlooked or unsuspected. It is his belief that in cases where we cannot now detect such primary injury more thorough search and better methods will enable us to do so."

With regard to malignant variation the present suggestion is that the adaptation is an irreversible one in response to chronic interfering changes in the environment of the type defined above, and it is a striking fact that agents such as x-rays and the gamma rays of radium, which the majority of workers now regard as inhibitory in their biological effects under all conditions, should rank next to the carcinogenic hydrocarbons in tumour-producing activity.

Pursuing still further the analogy between the formation of tumours and colonial papillae, attention must be directed to certain physiological similarities. First is the important circumstance that in both cases the variant cells show permanently altered metabolic properties which must confer distinct survival value and which probably furnish the additional energy demanded by the increased rate of growth. Secondly, both types of variation give indications of the importance of relative degrees

of cell-stability (probably genetically determined) in the face of dysgenic changes. The significance of this conception has already been demonstrated for experimental carcinogenesis (e.g., Kreyberg 1936), and it is probably equally valid in the induction of mutation in bacteria. Thus the environmental changes which induce secondary colony formation in the case of certain cultures are quite without this effect in the great majority of bacterial strains, indicating the essential importance of cellular instability or an intrinsic capacity for variation of this particular type. Whereas adverse circumstances mostly produce a continuous cellular modification which is reversible in its earlier stages but later ends in death, the same changes produce in mutable strains a discontinuous alteration in cell characters with the emergence of a strain of increased relative viability.

Much discussion has centred round the question whether malignant change occurs initially in a single cell or in a number of cells almost simultaneously, and of considerable general interest is the fact that while secondary colony formation may affect but a single focus within the primary colony, it is more commonly multicentric in origin, and within a short time not infrequently affects several hundreds of foci in a colonial area of say 5-15 sq. mm. (Figs.

147-154, Table XXIX). The outcome must obviously be the resultant of the regional distribution of inhibition and the degree of homogeneity of the affected bacterial population. Although inhibitory conditions at any given time must very from point to point in the colony, and in general be more intense in the centre than at the periphery, the individual cells of the primary colony must exhibit a relatively high degree of biological uniformity. Assuming an essential similarity between bacterial papillae and tumours, and disregarding whether the exact analogy is between a single tumour and an individual papilla or the mass which may result from the fusion of several, it seems probable that while animal tumours may arise from a focus so small as to involve only a single cell, multicentric development is at least a possibility in cases where identical environmental conditions affect a number of biologically comparable cells in a small volume of tissue.

It is of interest to find to what extent bacteriologists have been impressed by this general analogy. Massini himself acknowledged the resemblance (1907, p. 264 and Fig. 1): in the words of Marie's abstract-review (1907)....."à la facon d'une tumeur maligne, ces granules traversent les différentes couches de chaque colonie ou bien se développent dans le voisinage du point ou celle-ci

a offert trop de résistance." While the present paper was in course of preparation the writer was interested to learn through Sir John Ledingham that Dr W.J. Penfold had many years ago been impressed by the resemblance between the formation of secondary colonies and tumours. In a subsequent correspondence Dr Penfold was kind enough to provide full details. Penfold took the analogy seriously as early as 1912 --- when he first drew attention to the subject --- and still later in 1922, when he published a paper (Penfold 1922) embodying his main views. After describing papilla-formation in B. coli when grown on monochloracetate-agar and in B. typhosus on iso-dulcite agar, he pointed out that the causative substances can generally be classified in two groups, potential food substances on the one hand, and growth-inhibitory poisons on the other. In view of the discussion of possible mechanisms of variation, it must be noted that Penfold was fully aware of the importance of the corresponding types of adaptive response, viz., acquisition of the capacity to metabolise a previously non-utilisable foodstuff, and, secondly, development of resistance to a poison to which the cells were formerly susceptible. Penfold apparently recognised the organisms of the secondary colonies as variants derived from the original bacteria, although in his

interpretation he probably attributed undue primary importance to the role of selection. Further, he traced the fundamental resemblances between a bacterial colony and a composite tissue, and between the nodules appearing as secondary colonies in the one and as neoplasms in the other. Lastly, while interested mainly in extrinsic factors in the causation of tumours and bacterial papillae, he also indicated the significance of intrinsic (cellular) factors as influencing the variation process in each case. Penfold concluded with a short account of preliminary and projected experiments on the influence of aniline, toluidine, benzidine and naphthylamine on secondary colony formation in B. coli, B. dysenteriae Flexner and other species.

This concludes the general discussion of evidence on which the general thesis, of biological analogy or even homology between secondary colonies and metazoan tumours, is based. It need hardly be pointed out that the basic phenomena of growth, senescence and variation can be studied with greater accuracy in the bacterial colony than in almost any other living material, and the developing colony is particularly suited to the study of the changes brought about by depletion and other inhibitory influences. Technical conditions are such as could scarcely be realised under any circumstances in the

culture of differentiated tissues, and the main advantages are due to the high rates of growth and metabolism exhibited by many bacterial species growing in standard media, to the relative ease with which cultures can be manipulated in a pure condition, and to the facility with which the composition of the medium can be experimentally controlled.

VII. Summary.

The development of our knowledge of secondary colony formation is summarised. Various possible interpretations are surveyed, and it is concluded that the phenomenon is usually an irreversible dedifferentiation and represents an adaptation to unfavourable or inhibitory conditions. Inhibition may be due (1) to gradual depletion of the medium, (2) to the action of toxic substances in the environment, and (3) --- possibly --- to the competitive effect of non-utilisable substances in the substrate. It is suggested that the respective substances may impress some degree of specificity on the transformation in cases (2) and (3), and that selection must play an important part in case (3) once the variation has appeared. After a description of the nature of the adaptive response, attention is drawn to the analogy between secondary colony formation and tumour production in higher forms, and this is discussed in points of detail as well as on general grounds.

VIII. References.

Arkwright, J.A.	1930	Variation. In A system of bacteriology. (Medical research council). London 1930.
Bach, D.	1936	L'evolution des dehydrogenases du Proteus vulgaris. C. rend. soc. de biol., 122, 1068-70.
Baerthlein, K.	1913	Untersuchungen uber Bact. coli mutabile. Centr. f. Bakt., I, 66, 21-35.
Bateson, W.	1913	Problems of genetics. New Haven 1913.
Benecke, W.	1909	see Zeits. f. indukt. Abstammungs. u. Vererbungs-:lehre, 2, 215-218.
Bernhardt, G.	1915	Uber Variabilitat pathogener Bakterien. Zeits. f. Hyg., 79, 179-248.
Bernhardt, G. and Markoff, W.N.	1912	Ueber Modifikationen bei Bakterien. Beitrag zur Frage der sogenannten "Mutation" bei Bakterien. Centr. f. Bakt., I, 65, 1-4.
Bittner, J.J.	1931	A genetic study of the transplantation of tumors arising in hybrid mice. Amer. Jour. Cancer, 15, 2202-2247.

Brown, W.	1937	The production of growth-inhibiting substances by fungi. Report of proceedings, IInd. Internat. Congress for Microbiol., London, 37-38.
Burk, A.	1908	Mutation bei einem der Koligruppe verwandten Bakterium. Arch. f. Hyg., 65, 235-242.
Burri, R.	1910	Über scheinbar plotzliche Neuerwerbung eines bestimmten Gärungsvermogens durch Bakterien der Coligruppe. Centr. f. Bakt., II, 28, 321-345.
Burri, R. and Duggeli, M.	1909	Beiträge zur Systematik der Coli-aerogenes-Gruppe nebst Beschreibung einer neuen Methode zur Untersuchung der Gärungsgase. Centr. f. Bakt., I, 49, 145- 174.
Cleary, J.P., Beard, J.P. and Clifton, C.E.	1935	Studies of certain factors influencing size of bacterial populations. Jour. Bact., 29, 205-213.
Clifton, C.E., Cahen, S.F. and Morrow, G.	1936	Metabolic activities of Escherichia coli in a synthetic medium. Proc. soc. exp. biol. med., 35, 40-44.

Cloudman, A.M. 1932 A comparative study of transplantability of eight mammary gland tumours arising in inbred mice.

Amer. Jour. Cancer, 16, 568-630.

Coventry, F.A. 1925 The reaction product which inhibits reproduction of the trypanosomes in infections with T. lewisi, with reference to its change in titre throughout the course of infection.

Amer. Jour. Hyg., 5, 127-144.

Cruickshank, J. 1934 A study of a luminous organism in relation to nutrition on agar.

Jour. Path. Bact., 39, 141-148.

Cunningham, J., Theodore, J.H. and Fraser, A.G.L. 1934 Further observations on Indian relapsing fever. Pt. I. Types of spirochaetes found in experimental infections.

Ind. Jour. Med. Res., 22, 105-156.

Damboviceanu, A. and Roth, H. 1936 Contribution à l'étude du métabolisme des bactéries (milieux vaccinés, milieux épuisés).

C. rend. soc. de biol., 123, 61-63.

Deere, C.J., Dulaney, A.D. and Michelson, I.D. 1936 The utilisation of lactose by Escherichia coli-mutabile.

Jour. Bact., 31, 625-633.

Dienert, F.	1900	Sur la fermentation du galactose et sur l'accoutumance des levures a ce sucre. Ann. Inst. Past., 14, 139-189.
Dingle, J.H.	1936	Growth-inhibiting power of specific antisera. Amer. Jour. Hyg., 23, 1-9.
Dixon, M. and Thurlow, S.	1924	Studies on xanthine oxidase. II. The dynamics of the oxidase system. Biochem. Jour., 18, 976-988.
Dobell, C.	1912 -13	Some recent work on mutation in micro-organisms. Jour. Genetics, 2, 324-350.
Dodge, B.O.	1936	Reproduction and inheritance in Ascomycetes. Science, 83, 169-175.
Dulaney, A.D. and Michelson, I.D.	1935	A study of B. coli-mutabile from an outbreak of diarrhoea in the new-born. Amer. Jour. Publ. Health, 25, 1241-1251.
Eisenberg, P.	1906	Über sekundare Bakterien-kolonien. Centr. f. Bakt., I, 40, 188-194.
Erikson, D.	1935	The pathogenic aerobic organisms of the actinomyces group. Medical Research Council Spec. Report no. 203. London 1935.

Fildes, P.	1934	Some medical and other aspects of bacterial chemistry. Proc. Roy. Soc. Med., 28, 1-12.
Fischer, A.	1936	Kraeftcellen som en ubikvitaer Varietet af normale Vaevsceller. Hospitalstidende, 79, 1064-1070.
-------	1937	The theory of the developmental physiology of malignant tumours. Amer. Jour. Cancer, 31, 1-20.
Gardner, A.D.	1935	See Brit. Med. Jour., i, 1318.
Gates, R.R.	1915	The mutation factor in evolution. London 1915.
Gause, G.F.	1934	The struggle for existence. Baltimore 1934.
Gay, F.P.	1935	Agents of disease and host resistance. London 1935.
Gladstone, G.P.	1937	The nutrition of Staphylo-:coccus aureus: nitrogen requirements. Brit. Jour. Exp. Path., 18,
Gotschlich, E.	1929	Allgemeine Morphologie und Biologie der pathogenen Mikroorganismen. In Handbuch der pathogenen Mikroorganismen. Jena 1929.

Grey, E.C.	1924	The latent fermenting powers of bacteria. I-III. Proc. Roy. Soc., B, 96, 156-170.
Gunther, C.	1895	Einfuhrung in das Studium der Bakteriologie. Leipzig 1895.
Haag, F.E.	1927	Der Milzbrandbacillus, seine Kreislaufformen und Varietaten. Arch. f. Hyg., 98, 271-321.
Haddow, A.	1936	Ann. Rept. Brit. Empire Cancer Campaign, 292-302.
-------	1938	Cellular inhibition and the origin of cancer. In press.
Haddow, A. and Robinson, A.M.	1937	The influence of various polycyclic hydrocarbons on the growth rate of transplantable tumours. Proc. Roy. Soc., B, 122, 442-476.
Haddow, A., Scott, C.M. and Scott, J.D.	1937	The influence of certain carcinogenic and other hydrocarbons on body growth in the rat. Proc. Roy. Soc., B, 122, 477-507.
Hadley, P.	1927	Microbic dissociation. Jour. Infect. Dis., 40, 1-312.
Haldane, J.B.S.	1932	The causes of evolution. London 1932.
----------	1933	Genetics of cancer. Nature, 132, 265-266.

Haldane, J.B.S.	1934	See Jour. Path. Bact., 38, 507-508.
Hall, I.C.	1935	Metabolic "mutation" and colonial dissociation in the genus Bacterium. Jour. Bact., 29, 13.
Hartmann, M.	1903	Eine rassenspaltige Torula-Art, welche nur zeitweise Maltose zu vergaren vermag (Torula colliculosa n. sp.). Woch. f. Brauerei, 20, 113-114.
Harvey, E.N.	1928	The oxygen consumption of luminous bacteria. Jour. Gen. Physiol., 11, 469-475.
Hawking, F.	1937	Studies on chemotherapeutic action. I. The absorption of arsenical compounds and tartar emetic by normal and resistant trypanosomes and its relation to drug-resistance. Jour. Pharm. Exp. Ther., 59, 123-156.
Henrici, A.T.	1928	Morphologic variation and the rate of growth of bacteria. Baltimore 1928.
----------	1934	The biology of bacteria. New York 1934.

Hershey, A.D. and Bronfenbrenner, J.B.	1936	Dissociation and lactase activity in slow lactose-fermenting bacteria of intestinal origin. Jour. Bact., 31, 453-464.
----------	1937	On factors limiting bacterial growth. Proc. soc. exp. biol med., 36, 556-561.
Huddleson, I.F.	1937	The mechanism of the bacteriostatic action of dyes on the growth of the Brucellae. Report of proceedings, IInd. Internat. Congress for Microbiol., London, 35-37.
Jacobsen, K.A.	1910	Mitteilungen uber einem variablen Typhusstamm (Bact. coli mutabile). Centr. f. Bakt., I, 56, 208-216.
Jancso, N. von and Jancso, H. von	1935	The role of the natural defence forces in the evolution of the drug-resistance of trypanosomes. II. The rapid production of Germanin-fast T. brucei strains in animals with paralysed defence. Ann. Trop. Med. Parasitol., 29, 95-109.
Johnson, F.H.	1936	Oxidation intensity and luminescence in bacteria. Jour. Cell. Comp. Physiol., 8, 439-463.

Jones, F.S., Orcutt, M. and Little, R.B.	1932	Atypical (slow) lactose-fermenting B. coli. Jour. Bact., 23, 267-279.
Karstrom, H.	1937	The problem of adaptation in bacteriology. Report of proceedings, IInd. Internat. Congress for Microbiol., London, 473-474.
Kennedy, J.A., Cummings, P.L. and Morrow, N.M.	1932	Atypical lactose-fermenters belong to the genus Bacterium (Bergey); Cultural and biochemical reactions. Jour. Infect. Dis., 50, 333-343.
Klotz, -.	1906	See Jour. Infect. Dis., 2, (supp.) 35.
Kluyver, A.J.	1932	Microbial metabolism and its bearing on the cancer problem. Science, 76, 527-532.
Knight, B.C.J.C.	1936	Bacterial nutrition: material for a comparative physiology of bacteria. Medical Research Council Special Report no. 210, London 1936.
Kostitzin, V.A.	1935	Sur l'intoxication d'un milieu par les produits cataboliques d'une population. C. rend. Acad. sci., 201, 516-518.

Kostitzin, V.A.	1937	Biologie mathématique. Paris 1937.
Kowalenko, A.	1910	Studien uber sogenannte Mutationserscheinungen bei Bakterien unter besonderer Berucksicht-:igung der Einzellen-:kultur. Zeits. f. Hyg., 66, 277-290.
Kreyberg, L.	1936	Genetic factors in the development of spontaneous and induced tumours in animals. Repts. of the IInd. Internat. Congress against Cancer, Brussels, I, 115-121.
Kritschewski, I.L. and Ponomarewa, I.W.	1936	On the pleomorphism of bacteria. I. On the pleomorphism of B. paratyphi B. Jour. Bact., 28, 111-126.
Lemon, G.C.	1933	An interpretation of bacterial growth-rate curves. Jour. Hyg., 33, 495-496.
Lewis, I.M.,	1933	Secondary colonies of bacteria with special reference to Bac. mycoides. Jour. Bact., 25, 359-386.
--------	1934	Bacterial variation with special reference to behaviour of some mutabile strains of colon bacteria in synthetic media. Jour. Bact., 28, 618-638.

Mandelbaum, M.	1912	Ueber das Bact. metatyphi. Centr. f. Bakt., I, 63, 46-53.
Marie, A.	1907	See Bull. Inst. Pasteur, 5, 791-793.
Mason, M.M.	1935	A comparison of the maximal growth rates of various bacteria under optimal conditions. Jour. Bact., 29, 103-110.
Massini, R.	1907	Uber einen in biologischer Beziehung interessanten Kolistamm (Bacterium coli mutabile). Ein Beitrag zur Variation bei Bakterien. Arch. f. Hyg., 61, 250-292.
Merrill, M.M.	1930	Carbohydrate metabolism of organisms of the genus Mycobacterium. Jour. Bact., 20, 235-286.
Müller, R.	1909	Ueber mutationsartige Vorgange bei Typhus-Paratyphus- und verwandten Bakterien. Centr. f. Bakt., I. Ref. 42, Bhft. 57-58.
-------	1911	Mutation bei Typhus- und Ruhrbakterien. Mutation als spezifisches Kulturmerkmal. Centr. f. Bakt., I, 58, 97-106.
Needham, J.	1936	Order and life. London 1936.

Neisser, M.	1906	Ein Fall von Mutation nach de Vries bei Bakterien und andere Demonstrationen. Centr. f. Bakt., I, Ref. 38, 98-100.
Nirula, R.L.	1928	Saltations in bacteria. Ann. Bot., 42, 431-476.
Paton, D. Noel	1926	The physiology of the continuity of life. London 1926.
Pearl, R.	1925	The biology of population growth. New York 1925.
------	1927	The growth of populations. Quart. Rev. Biol., 2, 532-548.
Pedlow, J.T. and Reiner, L.	1935	On the mechanism of chemotherapeutic action. XII. Comparison of the binding of chemotherapeutic agents by normal and resistant trypanosomes. Jour. Pharm. Exp. Ther., 55, 179-199.
Penfold, W.J.	1911	Studies in bacterial variation with special reference to the chemical functions of the members of the coli-typhoid group. Jour. Hyg., 11, 30-67.
----------	1912	On the specificity of bacterial mutation. Jour. Hyg., 12, 195-217.

Penfold, W.J.	1922	A contribution to a discussion on the causation of cancer. Med. Jour. Australia, 1, 460-462.
Pett, L.B.	1936	Studies on yeast grown in cyanide. II. Biochem. Jour., 30, 1438-1445.
-------	1937	Observations on yeast grown in cyanide. Report of proceedings, IInd. Internat. Congress for Microbiol., London. 475-476.
Preisz, H.	1904	Studien uber Morphologie und Biologie des Milzbrandbacillus. Centr. f. Bakt., I, 35, 280-293.
Punkari, L. and Henrici, A.T.	1935	Further studies on spontaneous variations of Torula pulcherrima. Jour. Bact., 29, 259-267.
Ramchandani, J.C.	1929	Saltations in bacteria. II. Bacillus prodigiosus. Ann. Bot., 43, 579-586.
Rettger, L.F. and Gillespie, H.B.	1935	Bacterial variation: an inquiry into the underlying principles governing the cell morphology of Bac. megatherium. Jour. Bact., 30, 213-232.

Russell, H.	1936	Observations on immunity in relapsing fever and trypanosomiasis. Trans. Roy. Soc. Trop. Med. Hyg., 30, 179-190.
Schlossberger, H. and Schüffner, R.	1934	Drug-resistance experiments with arseno-:pyridine derivatives on trypanosomes. Jour. Chemother., 11, 81-92.
Sherman, J.M. and Wing, H.U.	1937	Attempts to reveal sex in bacteria: with some light on fermentative variability in coli-aerogenes group. Jour. Bact., 33, 315-321.
Shoup, C.S.	1929	The respiration of luminous bacteria and the effect of oxygen tension upon oxygen consumption. Jour. Gen. Physiol., 13, 27-45.
Smith, J.H.	1913	On the organisms of the typhoid-colon group and their differentiation. Centr. f. Bakt., I, 68, 151-165.
Sobotka, H., Holzman, M. and Reiner, M.	1936	Selective fermentation. III. Fermentation of hexose-pentose mixtures. Biochem. Jour., 30, 933-940.
Stephenson, M. and Stickland, L.H.	1932	Hydrogenlyases: Bacterial enzymes liberating molecular hydrogen. Biochem. Jour., 26, 712-724.

Stephenson, M. and Stickland, L.H.	1933	Hydrogenlyases. III. Further experiments on the formation of formic hydrogenlyase by Bact. coli. Biochem. Jour., 27, 1528-1532.
Stephenson, M. and Yudkin, J.	1936	Galactozymase considered as an adaptive enzyme. Biochem. Jour., 30, 506-514.
Stewart, F.H.	1926	Mendelian variation in the paracolon-mutabile-colon group, and the application of Mendel's principle to the theory of acquired virulence. Jour. Hyg., 25, 237-255.
----------	1927	Segregation and autogamy in bacteria: a contribution to cellular biology. London 1927.
----------	1928	The life cycle of bacteria. Alternate asexual and autogamic phases. Jour. Hyg., 27, 379-395.
Taliaferro, W.H.	1929	The immunology of parasitic infections. New York 1929.
Thaysen, A.C.	1911	Studien uber funktionelle Anpassungen bei Bakterien. Centr. f. Bakt., I, 60, 1.

Toenniessen, E.	1921	Ueber die Variations-:formen der Bakterien und ihre Uebereinstimmung mit den Variationsformen der Metazoen. Centr. f. Bakt., I, 86, 353-380.
Topley, W.W.C. and Ayrton, J.	1923 -24	The segregation of biological factors in B. enteritidis (Aertrycke). Jour. Hyg., 22, 305-313.
Twort, F.W.	1907	The fermentation of glucosides by bacteria of the typhoid-coli group and the acquisition of new fermenting powers by Bacillus dysenteriae and other micro-organisms. Proc. Roy. Soc., B, 79, 329-336.
Virtanen, A.I.	1934	On the enzymes of bacteria and bacterial metabolism. Jour. Bact., 28, 447-460.
Welch, W.H.	1937	Adaptation in pathological processes. Baltimore 1937.
White, P.B.	1925	In An investigation of the Salmonella group, with special reference to food poisoning. Medical Research Council Special Report no. 91. London 1925.
--------	1926	Further studies of the Salmonella group. Medical Research Council Special Report no. 103. London 1926.

Willmer, E.N.	1935	Tissue culture: the growth and differentiation of normal tissues in artificial media. London 1935.
Winslow, C.-E. A.	1935	What do we mean by a bacterial life-cycle? Science, 81, 314-315.
Woodger, J.H.	1931	The concept of organism and the relation between embryology and genetics. Quart. Rev. Biol., 6, 178-207.
Wooldridge, W.R. and Glass, V.	1937	Variability in the activity of bacterial enzymes. II. Factors associated with viability and growth. Biochem. Jour., 31, 526-531.
Wooldridge, W.R., Knox, R. and Glass, V.	1936	Variability in the activity of bacterial enzymes. I. The effect of the age of the culture. Biochem. Jour., 30, 926-931.
Yorke, W. and Murgatroyd, F.	1935a	Biological problems in chemotherapy. Lancet, i, 191-193.
----------	1935b	Biological problems in chemotherapy. Trans. Roy. Soc. Trop. Med. Hyg., 28, 435-457.
Yudkin, J.	1932	Hydrogenlyases. II. Some factors concerned in the production of the enzymes. Biochem. Jour., 26, 1859-1871.