C E L L U L A R

I N H I B I T I O N

A N D T H E O R I G I N O F

C A N C E R,

W I T H S P E C I A L

R E F E R E N C E T O T H E

M O D E O F A C T I O N

O F T H E

C A R C I N O G E N I C

H Y D R O C A R B O N S.

A T H E S I S

S U B M I T T E D F O R T H E

D E G R E E O F D O C T O R

O F M E D I C I N E O F

T H E U N I V E R S I T Y O F

E D I N B U R G H.

ALEXANDER HADDOW.

MARCH 1937.

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

The work which forms the basis of this thesis was performed in the Department of Bacteriology, University of Edinburgh, during the author's tenure of a Lectureship in that Department and of the Laura de Saliceto Studentship of the University of London. The cost of Section I of the investigation was defrayed by grants from the British Empire Cancer Campaign, with two additional payments from the Roughhead Fund of the University of Edinburgh. Part of this Section was carried out in collaboration with the Research Institute of the Royal Cancer Hospital (Free), and in particular with Dr A.M. Robinson working under the International Cancer Research Foundation. Section II was carried out in collaboration with Dr C.M. Scott and Mrs Scott of the Department of Pharmacology of the University of Edinburgh, and was aided by an expenses grant to the former from the Medical Research Council.

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1st March 1937.

THE INFLUENCE OF VARIOUS POLYCYCLIC HYDROCARBONS ON THE GROWTH-RATE OF TRANSPLANTABLE TUMOURS.

I. INTRODUCTORY.

As a result of considerations which will be discussed to some extent in the present paper, experiments were carried out to test the effect of certain of the carcinogenic hydrocarbons, studied at the Research Institute of the Royal Cancer Hospital (Cook, Hieger, Kennaway and Mayneord 1932; Cook 1932; Barry, Cook, Haslewood, Hewett, Hieger and Kennaway 1935) on the growth-rate of transplantable animal tumours. The work was also extended to include related non-carcinogenic hydrocarbons and other substances. A short preliminary account has already been published (Haddow 1935).

II. EXPERIMENTAL.

(1) Animals.

The majority of the animals used were hooded rats of the Lister Institute strain since previous experience had shown these to be particular-:ly suitable for use with the Jensen and Walker tumours. Wistar rats from the Glaxo laboratories proved susceptible to the Walker tumour and were also used on a few occasions. Batches were selected in which the animals were in perfect condition and of reasonable uniformity of weight (usually 100-150 g.).

(2) Tumour strains and technique of implantation.

The rat tumours studied were the Jensen sarcoma and Walker carcinoma (strains supplied by the Imperial Cancer Research Fund); a sarcoma (LR-10) induced in the Research Institute of the Royal Cancer Hospital by the injection of 1:2:5:6-dibenzanthracene in lard (Burrows, Hieger and Kennaway, 1932) and subsequently maintained by transplantation; and a rat sarcoma, induced by 3:4-benzpyrene, for which the writer is indebted to Dr A.M. Hain of the Institute of Genetics, University of Edinburgh. A few experiments were also carried out with the Rous fowl sarcoma.

Rats were prepared for tumour implantation by the epilation of an area of skin on the right flank. Next day an active tumour of 10-14 days was exposed aseptically. Thin slices were removed from the healthy periphery, placed in sterile saline, and divided into uniform cubes about 2-3 mm. in length. Each rat of the fresh batch was then lightly anaesthetised with ether and, after sterilisation of the skin, a paracentesis cannula containing a fragment of tumour was introduced obliquely through a small incision which was then closed with a Michel clip.

(3) Administration of compounds.

In the early experiments the hydrocarbons were given in aqueous colloidal suspensions (0.03 per cent.) prepared as described by Boyland (1932) or, for higher concentrations, by the use of sodium dispersol (supplied by Imperial Chemical Industries Limited). These preparations were sterilised by boiling or steaming. Intraperitoneal injection was employed throughout, partly to secure adequate absorption and also to exclude all possibility of action by direct contact with the subcutaneous tumour. Colloidal preparations were usually given daily, but later it was found much more convenient, and at least equally satisfactory, to give the compounds in one or two doses dissolved in sesame oil. For this purpose concentrations of 0.5 or 1.0 per cent. were prepared in the hot air oven at 100 C., treatment which served at once to dissolve and to sterilise. One to five c.c. of solution was injected through a small epilated and sterilised area on the abdominal wall.

(4) General design of experiments.

In preliminary series individual tumours served as their own controls, the rate of growth being measured daily for some time before and after administration of the substance to be tested. Later, the growth of a number of treated tumours was compared with that of tumours in control animals, by comparing either the daily measurements or the weight of the tumours after removal at the end of each experiment. In all cases the tumours in a given test were implants from a single tumour of the previous generation, and control and experimental series were comparable in such details as age and weight. Control rats throughout received similar injections of the appropriate material, e.g., the gelatin base used in the preparation of colloid, or sterile sesame oil, and hence were subjected to the same degree of handling and other interference as the corresponding experimental rats.

The majority of the later experiments conformed to a common plan. Injections, usually two, were given shortly after tumour-implantation. In experiments 7, 11, 12, 14, 16, 17, 18, 19, 20, 25, 27, 29, 30, 31, 32, 33, and 34 these injections were given within a period of not more than three days following the graft. After an average period of three weeks the rats were killed and their tumours

removed. A statistical comparison --- rigorous if necessary --- was made of the weights of tumours from the control and experimental series respectively. This arrangement was preferred in most cases since the first purpose of the present work was to test a sufficient number of selected compounds under approximately standard conditions. Nevertheless, some experiments were performed in which the time of administration of the compound --- in relation to tumour-implantation --- differed from the above.

The procedure usually included blood-counts in representative animals at the end of the experiment and the removal of tissues (usually liver, kidney, spleen, suprarenal, thyroid, pituitary, bone-marrow, testis or ovary and the tumour) for histological examination.

A. Carcinogenic hydrocarbons.

Experiment 1: Jensen sarcoma.

1:2:5:6-dibenzanthracene.

Eight rats bearing healthy 10-day-old tumours were divided into two equal groups, of which one received daily 1 c.c. of a 0.03 per cent. aqueous colloidal suspension of 1:2:5:6-dibenzanthracene in 0.5 per cent. gelatin while the controls were given similar injections of the gelatin solution alone. The total dosage of 1:2:5:6-dibenzanthracene

was approximately 8 mg. The growing tumours were measured daily with calipers and the product of the length, breadth and thickness in cm. taken as an index of tumour size, the result of the experiment being expressed in these terms (Table I and Fig. 1). 1:2:5:6-dibenzanthracene retarded the growth-rate considerably. That the result shown in Fig. 1 is characteristic can be seen from the individual data for this experiment given in the Appendix.

T A B L E I

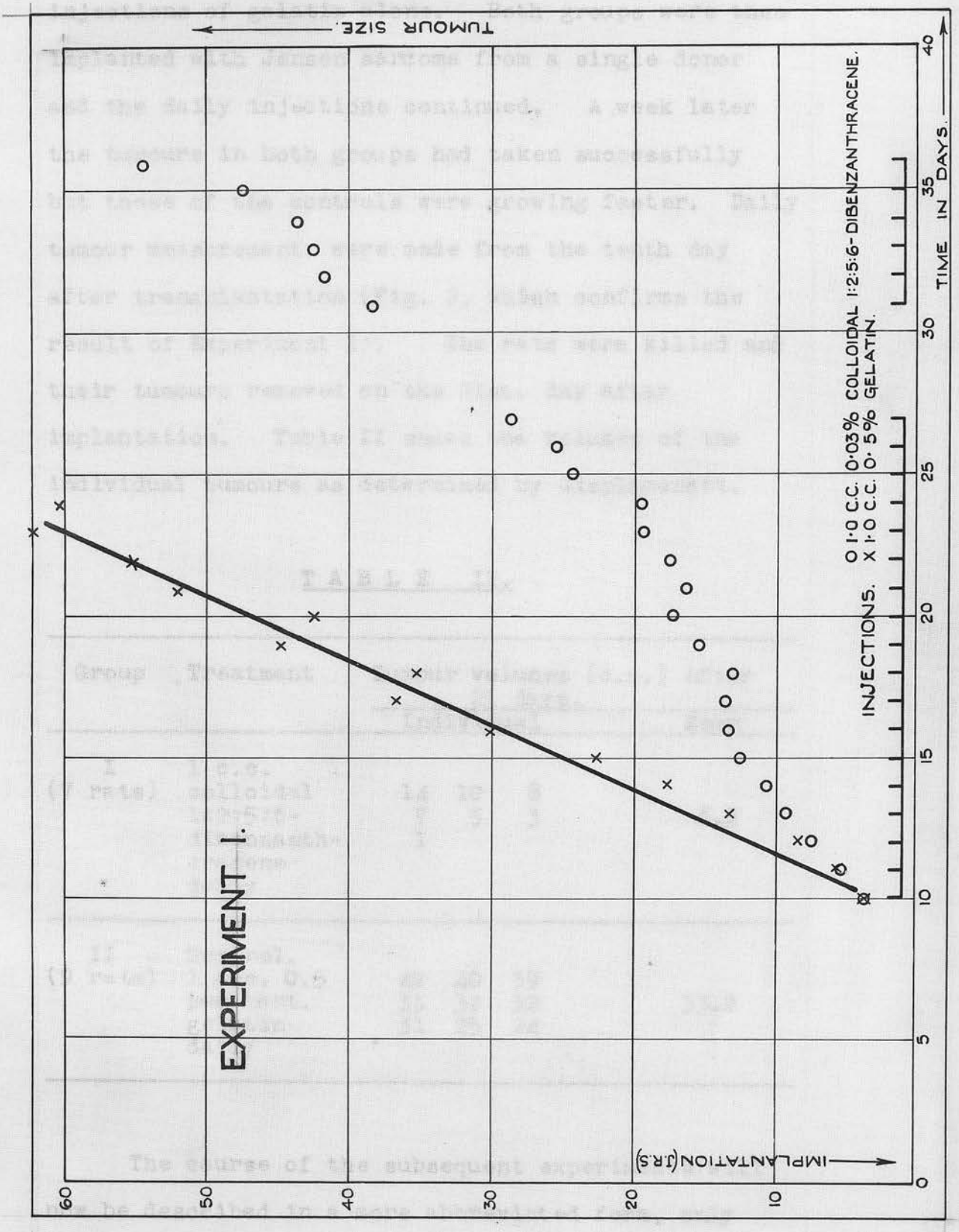
Group	Index of mean tumour size		Percentage increase
	1st day	11th day	
Controls (4)	4.1	37.8	822
Treated (4)	3.9	19.1	490

Experiment 2: Jensen sarcoma.

1:2:5:6-dibenzanthracene.

Ten rats were given four consecutive daily injections of 1 c.c. 0.03 per cent. colloidal 1:2:5:6-dibenzanthracene in gelatin while a control group of nine rats received the same number of

FIGURE 1


TUMOUR SIZE.
EXPERIMENT I.
IMPLANTATION (T.R.S.)
0
10
20
30
40
50
60
0
5
10
15
20
25
30
35
40
TIME IN DAYS.
INJECTIONS. O 1·0 C.C. 0·03% COLLOIDAL 1:2:5:6-DIBENZANTHRACENE.
X 1·0 C.C. 0·5% GELATIN.

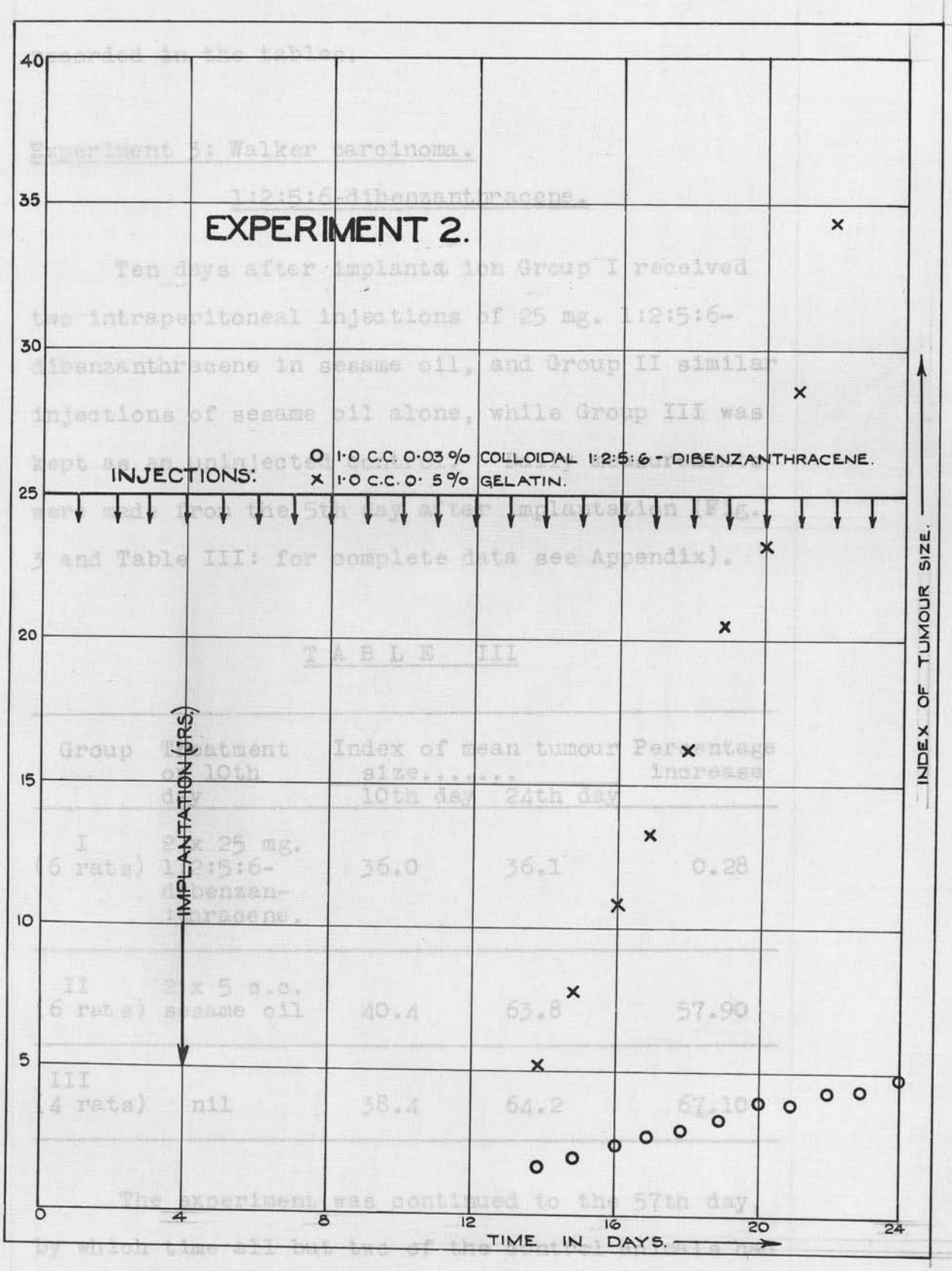
injections of gelatin alone. Both groups were then implanted with Jensen sarcoma from a single donor and the daily injections continued. A week later the tumours in both groups had taken successfully but those of the controls were growing faster. Daily tumour measurements were made from the tenth day after transplantation (Fig. 2, which confirms the result of Experiment 1). The rats were killed and their tumours removed on the 21st. day after implantation. Table II shows the volumes of the individual tumours as determined by displacement.

T A B L E II.

Group	Treatment	Tumour volumes (c.c.) after 21 days. Individual	Mean
I (7 rats)	1 c.c. colloidal 1:2:5:6-dibenzanthracene daily	14 10 8 7 5 3 1	6.8
II (9 rats)	Control. 1 c.c. 0.5 per cent. gelatin daily	42 40 39 33 33 32 31 25 24	33.2

The course of the subsequent experiments will now be described in a more abbreviated form, only those details being set down which cannot well be

FIGURE 2


EXPERIMENT 2.
INJECTIONS:
O 1·0 C.C. 0·03 % COLLOIDAL 1:2:5:6 - DIBENZANTHRACENE.
× 1·0 C.C. 0·5 % GELATIN.
IMPLANTATION (JRS.)
INDEX OF TUMOUR SIZE.
TIME IN DAYS.
0
4
8
12
16
20
24
5
10
15
20
25
30
35
40

recorded in the tables.

Experiment 3: Walker carcinoma.

1:2:5:6-dibenzanthracene.

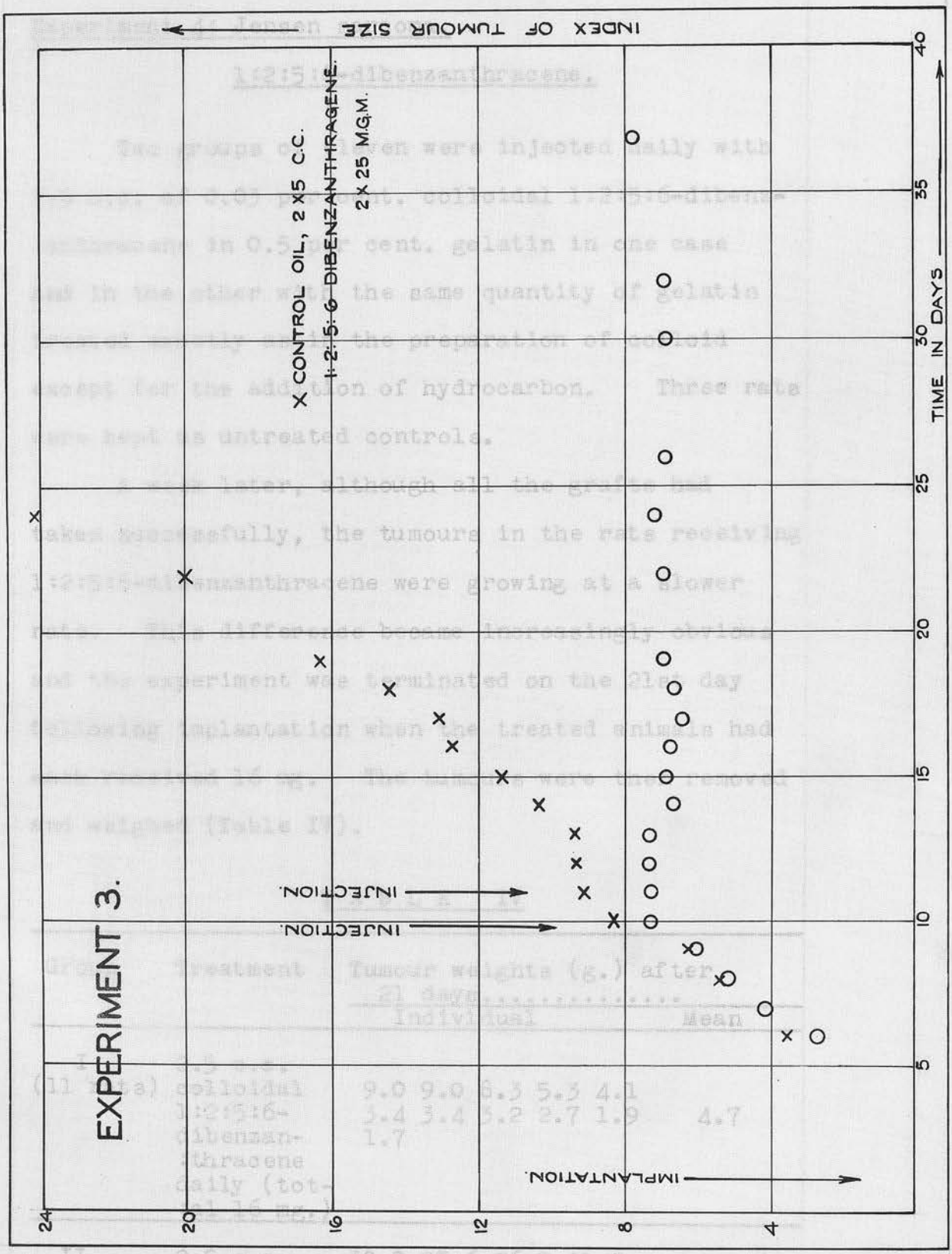
Ten days after implantation Group I received two intraperitoneal injections of 25 mg. 1:2:5:6-dibenzanthracene in sesame oil, and Group II similar injections of sesame oil alone, while Group III was kept as an uninjected control. Daily measurements were made from the 5th day after implantation (Fig. 3 and Table III: for complete data see Appendix).

T A B L E III

Group	Treatment on 10th day	Index of mean tumour size..... 10th day	24th day	Percentage increase
I (6 rats)	2 x 25 mg. 1:2:5:6-dibenzan-:thracene.	36.0	36.1	0.28
II (6 rats)	2 x 5 c.c. sesame oil	40.4	63.8	57.90
III (4 rats)	nil	38.4	64.2	67.10

The experiment was continued to the 57th day, by which time all but two of the control animals had died with extremely large tumours. Meantime the treated tumours were quiescent or increasing very slowly.

FIGURE 3


INDEX OF TUMOUR SIZE
TIME IN DAYS
X CONTROL OIL, 2 X 5 C.C.
+ 2:5:6 DIBENZANTHRAGENE. 2 X 25 M.G.M.
EXPERIMENT 3.
INJECTION.
INJECTION.
IMPLANTATION.
24
20
16
12
8
4
5
10
15
20
25
30
35
40

Experiment 4: Jensen sarcoma.

1:2:5:6-dibenzanthracene.

Two groups of eleven were injected daily with 2.5 c.c. of 0.03 per cent. colloidal 1:2:5:6-dibenz-:anthracene in 0.5 per cent. gelatin in one case and in the other with the same quantity of gelatin treated exactly as in the preparation of colloid except for the addition of hydrocarbon. Three rats were kept as untreated controls.

A week later, although all the grafts had taken successfully, the tumours in the rats receiving 1:2:5:6-dibenzanthracene were growing at a slower rate. This difference became increasingly obvious and the experiment was terminated on the 21st day following implantation when the treated animals had each received 16 mg. The tumours were then removed and weighed (Table IV).

T A B L E IV

Group	Treatment	Tumour weights (g.) after 21 days..... Individual	Mean
I (11 rats)	2.5 c.c. colloidal 1:2:5:6-dibenzan-:thracene daily (tot-:al 16 mg.)	9.0 9.0 8.3 5.3 4.1 3.4 3.4 3.2 2.7 1.9 1.7	4.7
II (11 rats)	2.5 c.c. 0.5% gelatin	30.0 27.6 26.0 21.5 20.8 19.1 14.5 9.4 8.7 7.1 3.4	17.1
III (3 rats)	nil	28.0 25.5 14.5	22.7

Experiment 5: Jensen sarcoma.

1:2:5:6-dibenzanthracene.

Group I received daily from the day of grafting 5.0 c.c. colloidal 1:2:5:6-dibenzanthracene and Group II the same of gelatin which had been treated with acetone as in the preparation of colloid. The tumours were removed on the 18th day, when each rat in Group I had received about 30 mg. dibenz-:anthracene. (see Table V; rats which proved resist-:ant to the graft are omitted).

TABLE V

Group	Treatment	Tumour weights (g.) after 18 days..... Individual	Mean
I (8 rats)	5 c.c. colloidal 1:2:5:6-dibenzan-:thracene daily (total 30 mg.)	3.4 2.4 2.2 1.7 1.5 0.9 0.8 0.4	1.6
II (5 rats)	5 c.c. 0.5% gelatin daily	20.0 15.5 10.2 7.7 7.0	12.0

Experiment 6: Jensen sarcoma.

1:2:5:6-dibenzanthracene.

Two groups of twelve were used and treatment began on the day of grafting. Tumours were removed after 14 days, when the experimental rats had received about 12 mg. (Table VI).

T A B L E VI

Group	Treatment	Tumour weights (g.) after 14 days..... Individual	Mean
I (9 rats)	2.5 c.c. colloidal 1:2:5:6-dibenzan-:thracene daily (total 12 mg.)	4.0 2.0 2.0 2.0 1.5 1.5 1.0 0.5 0.5	1.7
II (10 rats)	2.5 c.c. 0.5% gelatin daily	10.0 10.0 7.0 6.0 6.0 6.0 5.0 4.0 2.0 2.0	5.8

Experiment 7: Walker carcinoma.

1:2:5:6-dibenzanthracene.

Thirty rats were grafted and fifteen of them then received two doses of 5.0 c.c. of a 0.5 per cent. solution of 1:2:5:6-dibenzanthracene in sesame oil on successive days, while the remainder were given the same quantity of oil alone. Table VII and Fig. 4 show the end-result, which represents the most intense inhibitory effect so far obtained in these experiments.

T A B L E VII

Group	Treatment	Tumour weights (g.) after 21 days..... Individual				Mean
I (15 rats)	2 x 25 mg. 1:2:5:6-dibenzan-:thracene in oil	2.2	1.4	1.3	1.3	1.05
		1.1	1.1	1.0	1.0	
		1.0	0.9	0.9	0.7	
		0.7	0.6	0.6		
II (15 rats)	2 x 5 c.c. sesame oil	49.6	46.6	38.0		30.90
		37.5	35.7	33.2		
		29.7	29.7	29.2		
		29.0	28.5	22.9		
		20.5	17.4	15.7		

A B

10 cm.

Fig. 4: Experiment 7; Walker carcinoma
1:2:5:6-dibenzanthracene 50 mg.

Tumours after 21 days.

A: Controls...mean weight 30.90 g.

B: Treated.... " " 1.05 g.

Experiment 8: Rous fowl sarcoma.

1:2:5:6-dibenzanthracene.

Twenty-four Brown Leghorn fowls of about 500 g. were grafted in the breast muscle. Half of them were then given three doses of 30 mg. 1:2:5:6-dibenzanthracene in oil on successive days by intramuscular injection on the opposite side while the rest received sesame oil alone. There was considerable mortality in both groups. The survivors were killed on the 18th day and their tumour tissue dissected out and weighed. A significant inhibition was produced, similar in degree to that obtained in the foregoing experiments with rat tumours (Table VIII).

TABLE VIII

Group	Treatment	Weight of tumour tissue (g.) after 18 days.....				
		Individual				Mean
I (7 fowls)	3 x 30 mg. 1:2:5:6-dibenzan-:thracene in oil	14 3	11 2	10 2	4	6.6
II (9 fowls)	sesame oil alone	53 35 13	46 28	46 22	42 14	33.2

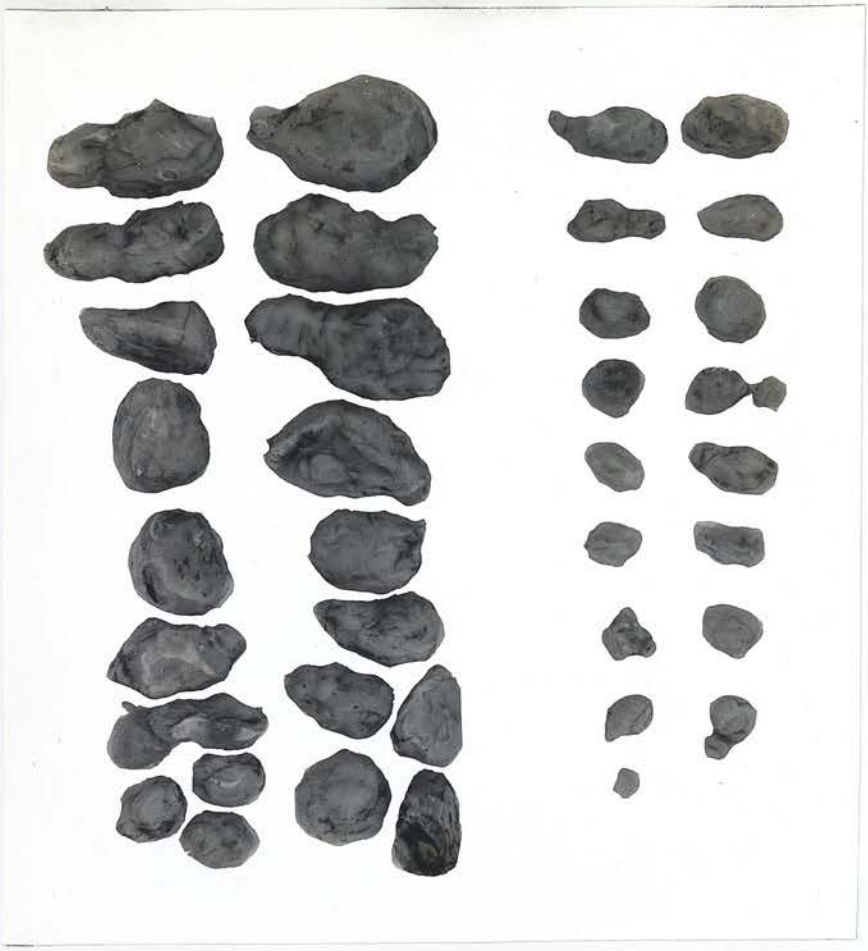
Experiment 9: Jensen sarcoma.

5:6-cyclopenteno-1:2-benzanthracene.

Forty rats were grafted. Twenty then received daily 0.03 per cent. colloidal 5:6-cyclopenteno-1:2-benzanthracene in 0.5 per cent. gelatin, ten received the control solution of gelatin alone and ten remained untreated. Of Group I, one died and two proved resistant to the graft. The rest were killed on the 18th day, when those in the first group had each received 15 mg. (Table IX and Fig. 5).

TABLE IX

Group	Treatment	Tumour weights (g.) after 18 days..... Individual				Mean
I (17 rats)	2.5 c.c. colloidal 5:6-cyclopenteno-1:2-benzanthracene daily (total 15 mg.)	8.1	7.9	6.4	4.1	
		4.1	4.0	3.4	3.3	3.5
		2.8	2.5	2.4	2.3	
		2.3	2.0	1.7	1.6	
		0.4				
II (10 rats)	2.5 c.c. 0.5% gelatin daily	33.4	29.7	29.5	24.8	
		15.9	15.9	13.2	8.9	18.2
		6.4	4.7			
III (10 rats)	nil	49.1	40.4	34.6	34.2	
		21.0	14.7	14.6	14.6	24.9
		13.4	12.5			



A B C

10 cm.

Fig. 5: Experiment 9; Jensen sarcoma.

5:6-cyclopenteno-1:2-benzanthracene
15 mg.

Tumours after 18 days.

A: Injected controls...mean weight 18.2 g.
B: Uninjected controls.. " " 24.9 g.
C: Treated " " 3.5 g.

Here one could compare the controls subjected to daily injection etc. with those not so handled, as regards the mean weight of the tumours in each group. Although the mean tumour weight was higher in the latter group, the application of the t method (Fisher 1932) showed that the difference was not significant, the value of P being 0.2-0.3. On the other hand the difference between the means of Groups I and II in Table IX was highly significant, ($P < 0.01$), as was the case for all the experiments so far described (see also Table XXXVI).

Experiment 10: Jensen sarcoma.

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

Sodium-1:2:5:6-dibenzanthracene-9:10-endo-alpha-beta-succinate was tested in view of its special interest as a water-soluble carcinogenic compound (Burrows and Cook 1936).

Twenty-four rats were grafted and divided into two equal groups. One received daily the substance in partial solution in sterile water while the other received the solvent only. On the 18th day the treated rats had received about 100 mg. of succinate (Table X). At autopsy, the treatment appeared to have produced some irritation of the peritoneum, an effect not observed with the compounds studied thus far.

TABLE X

Group	Treatment	Tumour weights (g.) after 18 days..... Individual				Mean
I (12 rats)	Na-1:2:5:6-dibenzanthr-:acene-9:10-endo-alpha-beta-succinate, c. 100 mg.	9.5 6.3 3.9	7.2 5.3 3.7	7.1 4.7 2.9	6.5 3.9 1.8	5.2
II (12 rats)	control	36.4 27.2 17.1 8.6	31.8 17.9 14.6 8.3	29.3 17.2 10.0 6.4		18.7

Experiment 11: Walker carcinoma.

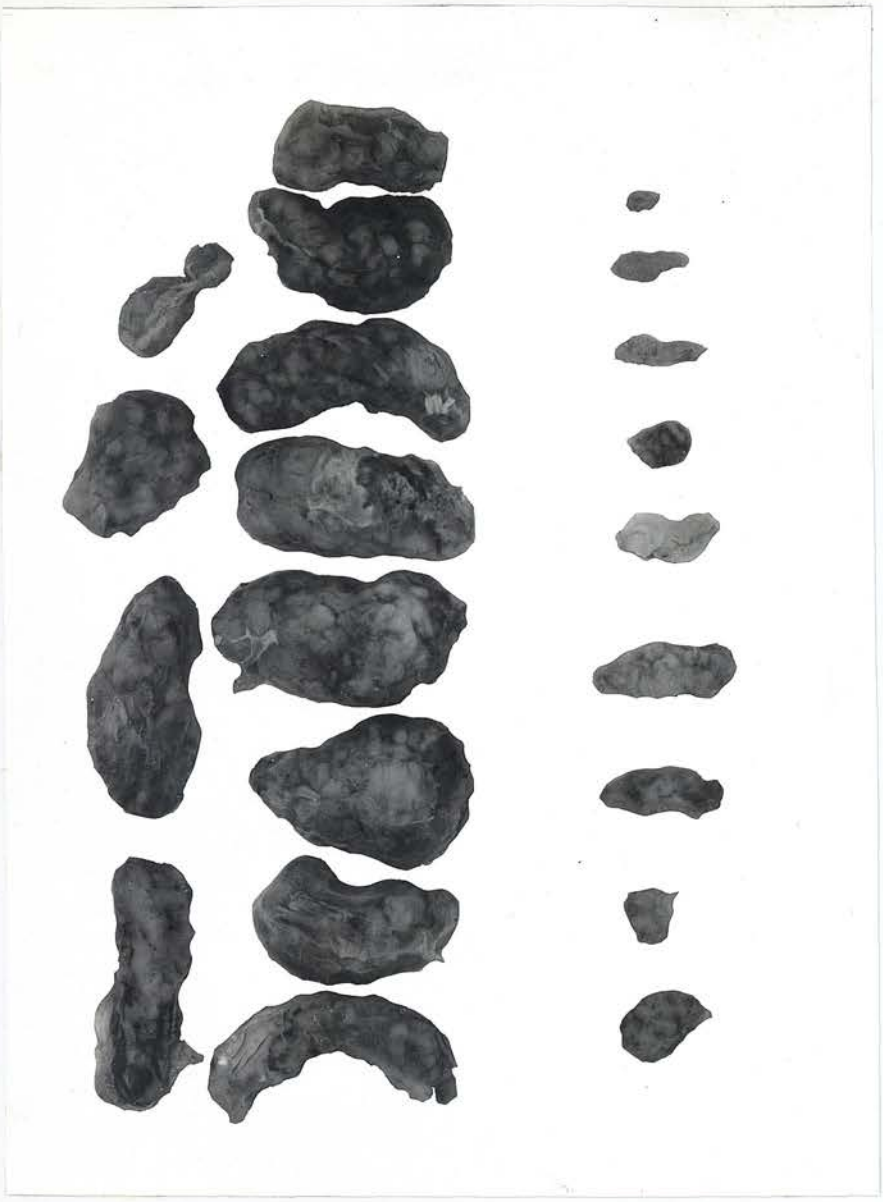
3:4-benzpyrene.

Twenty-five rats were grafted. Group I were then given two injections of 25 mg. 3:4-benzpyrene in sesame oil on successive days, while the rest received oil alone. Three treated animals died during the period of 28 days (Table XI and Fig. 6).

TABLE XI

Group	Treatment	Tumour weights (g.) after 28 days..... Individual	Mean
I (9 rats)	2 x 25 mg. 3:4-benz-pyrene in oil	5.8 4.1 2.6 2.1 1.7 1.1 0.7 0.6 0.2	2.1
II (13 rats)	2 x 5 c.c. sesame oil	59.2 48.1 46.1 37.4 35.0 33.1 31.3 29.5 27.6 26.4 20.0 6.4 3.2	31.0

Note: Originally termed 1:2-benzpyrene. See Cook et al., (1936, p. 2).



A B

10 cm.

Fig. 6: Experiment 11; Walker carcinoma.

3:4-benzpyrene 50 mg.

Tumours after 28 days.

A: Controls...mean weight 31.0 g.

B: Treated.... " " 2.1 g.

Experiment 12: Walker carcinoma.

3:4:5:6-dibenzacridine.

This compound was chosen as being related to the polycyclic hydrocarbons and possessing moderate carcinogenicity the effects of which are delayed as compared with say 1:2:5:6-dibenzanthracene (Barry et al., 1935).

Twenty rats were grafted in two groups of fifteen and five. These were injected with 50 mg. 3:4:5:6-dibenzacridine in oil and oil alone respectively. The mean weights after 32 days (Table XII) were examined statistically and the value for P found to be less than 0.01. The inhibition is thus of considerable significance although much less than in the case of the substances used in previous experiments.

TABLE XII

Group	Treatment	Tumour weights (g.) after 32 days..... Individual			Mean
I (13 rats)	2 x 25 mg. 3:4:5:6-dibenzacridine	45.5 32.2 27.5 12.0 7.5	36.7 29.2 25.5 11.5	36.4 29.1 25.2 10.7	25.3
II (5 rats)	2 x 5 c.c. sesame oil	79.5 41.4	66.2 29.0	46.2	52.5

B. Chrysene and certain compounds of benzanthracene type.

The following experiments were carried out with synthetic chrysene and certain compounds of benzanthracene type, the carcinogenicity of which is weak, doubtful, or, in some cases, (e.g., 3-methyl and 7-methyl-1:2-benzanthracene), apparently nil.

Experiment 13: Jensen sarcoma.

Chrysene.

Fifteen rats were grafted. One group received colloidal chrysene daily in 0.75 per cent. gelatin and the other gelatin solution alone. At the end of the experiment the mean weight of treated tumours was less than one third that of the controls (Table XIII).

TABLE XIII

Group	Treatment	Tumour weights (g.) after 17 days..... Individual	Mean
I (8 rats)	Colloidal chrysene (0.03% in 0.75% gelatin daily) total 30 mg.	5.0 4.1 4.0 3.6 1.2 1.2 0.7 0.5	2.54
II (7 rats)	0.75% gelatin daily	18.0 14.3 11.6 4.6 3.6 3.1 2.3	8.20

Experiment 14: Walker carcinoma.

Chrysene.

Thirty rats after grafting were divided into two groups which received chrysene in oil and oil alone respectively, in two doses on successive days. Again chrysene produced considerable inhibition of the tumour growth-rate (Table XIV).

TABLE XIV

Group	Treatment	Tumour weights (g.) after 19 days..... Individual			Mean
I (12 rats)	2 x 30 mg. chrysene in oil	6.0 3.6 1.2 0.7	5.6 2.0 1.0 0.6	4.6 1.4 0.9 0.5	2.34
II (13 rats)	2 x 5 c.c. sesame oil	34.7 27.5 23.8 21.3 4.7	32.2 26.2 23.8 15.2	28.0 26.1 21.4 9.4	22.60

Experiment 15: Jensen sarcoma.

1:2-benzanthracene.

1:2-benzanthracene was tested simultaneously with 5:6-cyclopenteno-1:2-benzanthracene (see Experiment 9). Of thirty rats grafted, twenty received colloidal 1:2-benzanthracene daily --- to a total dosage of 15 mg. --- while ten received the control solution alone. The treatment resulted in a moderate degree of inhibition (Table XV and Fig. 7)

TABLE XV

Group	Treatment	Tumour weights (g.) after 18 days.....			
		Individual			Mean
I (20 rats)	2.5 c.c. 1:2-benzan-:thracene daily (total 15 mg.)	21.2 14.7 9.6 5.3 3.6 3.0 1.1	17.6 11.8 9.2 4.7 3.4 2.1 1.0	17.0 10.6 6.1 4.1 3.3 1.5	7.5
II (10 rats)	2.5 c.c. 0.5% gelatin daily	33.4 24.8 13.2 4.7	29.7 15.9 8.9	29.5 15.9 6.4	18.2

A B C

10 cm.

Fig. 7: Experiment 15; Jensen sarcoma.

1:2-benzanthracene 15 mg.

A: Injected controls...mean weight 18.2 g.
B: Uninjected controls. " " 24.9 g.
C: Treated " " 7.5 g.

Experiment 16: Walker carcinoma.

1:2-benzanthracene.

Twelve of twenty-four rats were given 1:2-benzanthracene in sesame oil and the rest oil alone, in two daily doses immediately after implantation. (Table XVI).

TABLE XVI

Group	Treatment	Tumour weights (g.) after 21 days..... Individual			Mean
I (8 rats)	2 x 30 mg. 1:2-benz-anthracene in oil	5.3 2.2 0.7	5.0 1.6 0.6	4.0 1.4	2.6
II (12 rats)	2 x 5 c.c. sesame oil	25.4 20.4 16.2 13.0	22.5 18.6 15.7 11.1	21.1 17.0 14.6 5.0	16.7

Experiment 17: Walker carcinoma.

3-methyl-1:2-benzanthracene.

3-methyl-1:2-benzanthracene was given to fifteen rats in two doses on the days immediately after implantation. The substance was tested simultaneously with 3:4:5:6-dibenzacridine (Exper-:iment 12), with common controls. Thirteen treated animals bore tumours at the end of the experiment. (Table XVII).

T A B L E XVII

Group	Treatment	Tumour weights (g.) after 32 days..... Individual			Mean
I (13 rats)	2 x 25 mg. 3-methyl-1:2-benz-anthracene in oil	14.5 10.7 6.5 5.1 2.7	14.5 8.8 6.5 4.0	11.1 7.1 5.7 3.0	7.7
II (5 rats)	2 x 5 c.c. sesame oil	79.5 41.4	66.2 29.0	46.2	52.5

Experiment 18: Walker carcinoma.

4-methyl-1:2-benzanthracene;

7-methyl-1:2-benzanthracene.

On the two days following implantation, three groups of fifteen rats were injected with 4-methyl-1:2-benzanthracene, 7-methyl-1:2-benzanthracene and control oil respectively (Table XVIII).

TABLE XVIII

Group	Treatment	Tumour weights (g.) after 15 days..... Individual			Mean
I (10 rats)	2 x 25 mg. 4-methyl-1:2-benz-anthracene in oil	3.3 1.2 1.0 0.7	2.1 1.0 0.9	1.2 1.0 0.9	1.3
II (12 rats)	2 x 25 mg. 7-methyl-1:2-benz-anthracene in oil	3.0 1.3 1.1 0.7	2.8 1.1 0.9 0.7	2.5 1.1 0.9 0.6	1.4
III (14 rats)	2 x 5 c.c. sesame oil	16.2 9.7 8.1 7.0 4.6	12.7 8.6 7.8 5.0	10.5 8.3 7.2 4.6	8.5

Experiment 19: Walker carcinoma.

6-methyl-1:2-benzanthracene.

Two groups of twelve implanted rats were given 6-methyl-1:2-benzanthracene in oil, and oil alone, respectively (Table XIX).

TABLE XIX

Group	Treatment	Tumour weights (g.) after 30 days..... Individual	Mean
I (10 rats)	2 x 25 mg. 6-methyl-1:2-benz-anthracene in oil	5.3 4.5 4.3 4.0 3.4 2.6 2.2 1.6 1.4 0.5	3.0
II (8 rats)	2 x 5 c.c. sesame oil	52.9 43.4 33.4 30.0 28.7 28.7 26.8 19.3	32.9

C. Related oestrogenic compounds.

In a study of the oestrogenic activity of condensed-ring compounds, Cook, Dodds, Hewett and Lawson (1934) described work carried out with oxygen derivatives of phenanthrene and certain diols related to 1:2:5:6-dibenzanthracene. The most active oestrogens in these classes were 1-keto-1:2:3:4-tetrahydrophenanthrene and the d-n-propyl compound respectively. The following experiment was performed to determine the action of these substances on the growth of the Walker tumour.

Experiment 20: Walker carcinoma.

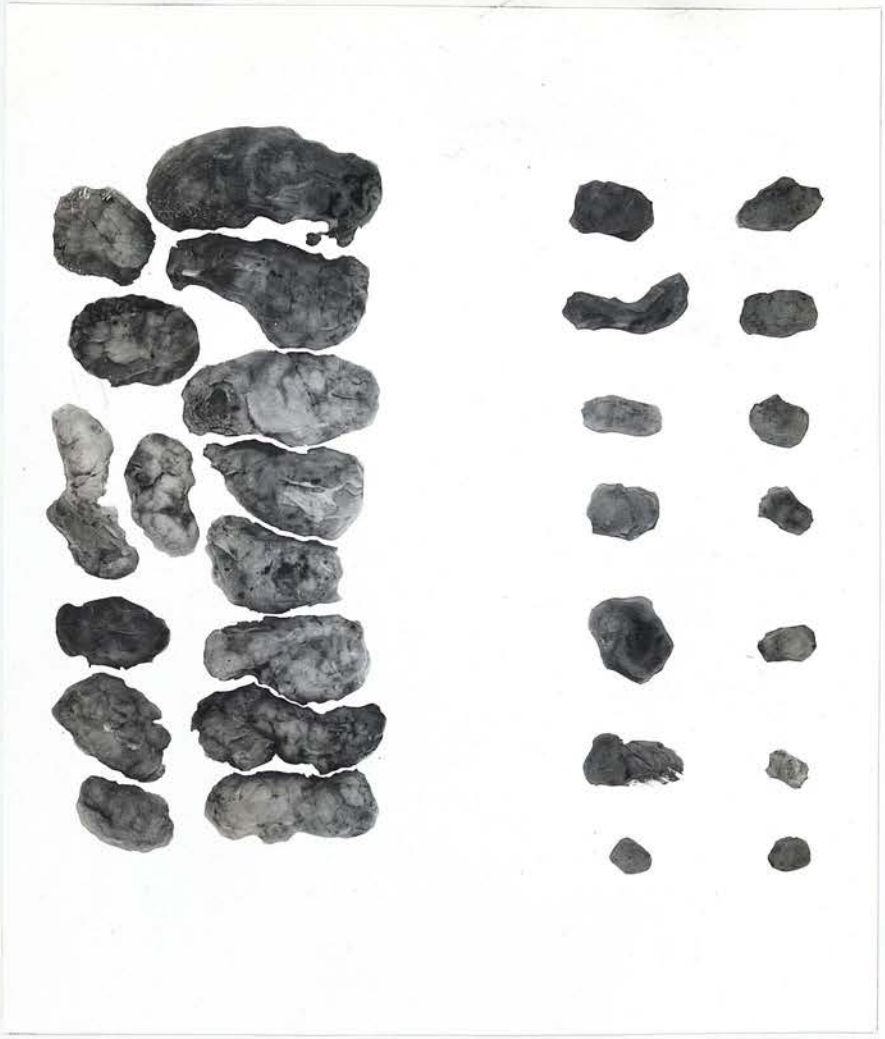
1-keto-1:2:3:4-tetrahydrophenanthrene;

9:10-dihydroxy-9:10-di-n-propyl-9:10-dihydro-1:2:5:6-dibenzanthracene.

Forty-five rats were implanted and used in groups of fifteen to test these substances with a common control. The phenanthrene derivative proved quite inactive as regards growth-inhibiting power while the dibenzanthracene diol showed moderate potency (Table XX and Fig. 8).

TABLE XX

Group	Treatment	Tumour weights (g.) after 28 days..... Individual			Mean
I (15 rats)	2 x 25 mg. 1-keto-1:2:3:4-tetrahydrophenanthrene	45.1 35.0 33.5 29.6 14.8	42.2 34.8 31.8 26.9 13.7	35.5 34.2 30.9 25.4 9.8	29.5
II (13 rats)	2 x 25 mg. 9:10-dihydroxy-9:10-di-n-propyl 9:10-dihydro-1:2:5:6-dibenzanthracene.	9.7 6.4 3.8 2.7 0.9	6.7 6.0 2.7 2.1	6.5 3.9 2.7 0.9	4.2
III (15 rats)	2 x 5 c.c. sesame oil	54.7 28.4 21.6 19.5 13.9	38.4 26.8 21.6 15.1 9.7	37.1 23.9 20.1 14.6 8.8	23.6



A B

10 cm.

Fig. 8: Experiment 20; Walker carcinoma.

9:10-dihydroxy-9:10-di-n-propyl-
9:10-dihydro-1:2:5:6-dibenzanthracene.
50 mg.

A: Controls....mean weight 23.6 g.

B: Treated..... " " 4.2 g.

D. Non-carcinogenic hydrocarbons and related substances.

The following tests were carried out with a number of non-carcinogenic hydrocarbons and related substances, under experimental conditions identical with those already described.

Experiment 21: Jensen sarcoma.

Anthracene.

Of nineteen implanted rats, ten were treated daily with a gelatin-protected colloid of anthracene until each had received a total of 12 mg. in fifteen injections. The rest were given gelatin alone. The treatment had no inhibitory effect on the rate of tumour growth (Table XXI).

TABLE XXI

Group	Treatment	Tumour weights (g.) after 18 days..... Individual			Mean
I (7 rats)	2.5 c.c. colloidal anthracene daily (total 12 mg.)	34 25 8	33 19	29 12	22.8
II (7 rats)	2.5 c.c. 0.5% gelatin daily	28 19 9	25 17	25 15	19.7

Experiment 22: Jensen sarcoma.

Phenanthrene.

Twelve freshly implanted rats were treated with phenanthrene under the same conditions as for anthracene in the last experiment (Table XXII). In this case the colloid proved markedly unstable.

T A B L E XXII

Group	Treatment	Tumour weights (g.) after 15 days..... Individual				Mean
I (11 rats)	2.5 c.c. colloidal phenanthrene daily (total 12 mg.)	21 14 6	18 12 4	16 12 4	14 12	12.0
II (11 rats)	2.5 c.c. 0.5% gelatin daily	19 12 8 6	17 10 7 6	16 9 7		10.6

Experiment 23: Jensen sarcoma.

Phenanthrene.

After implantation, twelve rats were treated with phenanthrene as in Experiment 22 and controlled with a group of six. As before there was no trace of an inhibiting effect (Table XXIII).

TABLE XXIII

Group	Treatment	Tumour weights (g.) after 15 days..... Individual			Mean
I (11 rats)	2.5 c.c. colloidal phenanthrene daily	21 16 9 6	19 12 7 5	16 10 6	11.5
II (6 rats)	2.5 c.c. 0.5% gelatin daily	19 9	11 9	10 9	11.2

Experiment 24: Jensen sarcoma.

1:2-cyclopentenophenanthrene.

This test was made concurrently with Experiment 13, the controls being common to both. The treated animals were eight in number and each received a total of 30 mg. in the form of colloid. Far from there being any inhibition, the mean weight of the treated tumours was greater than that of the controls (Table XXIV). However, this difference proved not to be significant, the value of P lying between 0.2 and 0.3.

TABLE XXIV

Group	Treatment	Tumour weights (g.) after 17 days..... Individual		Mean
I (8 rats)	Colloidal 1:2-cyclo-penteno-phenanthrene 0.03% in 0.75% gelatin daily (total 30 mg.)	24.6 16.6 11.5 5.3	21.2 12.2 6.3 2.2	12.5
II (7 rats)	0.75% gelatin daily	18.0 11.6 3.6 2.3	14.3 4.6 3.1	8.2

Experiment 25: Walker carcinoma,
1:2-cyclopentenophenanthrene;
dehydronorcholene.

Forty-five rats were grafted and divided into three equal batches. Group I received 1:2-cyclopentenophenanthrene in two injections in oil, Group II the same quantity of dehydronorcholene, while Group III were given the equivalent of sesame oil (Table XXV). Although dehydronorcholene proved completely inactive, the mean weight of the tumours treated with 1:2-cyclopentenophenanthrene was considerably greater than that of the control group, as also occurred in Experiment 24. In this case the t method gave a value for P between 0.1 and 0.05.

TABLE XXV

Group	Treatment	Tumour weights (g.) after 34 days..... Individual			Mean
I (15 rats)	2 x 25 mg. 1:2-cyclopentenophenanthrene in oil	73.0	72.1	69.7	49.0
		61.4	58.5	52.2	
		50.6	48.5	48.2	
		45.5	44.2	43.2	
		40.5	16.2	11.6	
II (12 rats)	2 x 25 mg. dehydronorcholene in oil	72.0	61.7	46.4	35.8
		44.5	40.0	38.7	
		37.4	29.4	26.0	
		15.0	10.5	8.7	

.....continued over.....

.....TABLE XXV continued.....

66

Group	Treatment	Tumour weights (g.) after 34 days..... Individual			Mean
III (13 rats)	2 x 5 c.c. sesame oil	72.4	68.2	51.2	
		48.0	47.0	44.5	36.4
		26.5	23.7	22.0	
		21.4	20.5	14.0	
		13.6			

Experiment 26: Walker carcinoma.

Pyrene; fluoranthene.

Forty-five rats were used to test pyrene and fluoranthene, which were administered in colloidal form by daily injection. The compounds had no influence of any kind on the rate of growth (Table XXVI).

T A B L E XXVI

Group	Treatment	Tumour weights (g.) after 21 days..... Individual				Mean
I (14 rats)	Colloidal pyrene (total 40 mg.)	29.1	24.5	21.1	20.1	
		17.6	17.2	16.8	15.3	16.2
		15.3	11.0	10.3	9.9	
		9.7	9.5			
II (15 rats)	Colloidal fluoranthene (total 40 mg.)	29.4	27.0	23.9	23.8	
		18.7	16.9	16.7	16.4	17.2
		16.1	15.2	15.0	13.5	
		10.5	9.1	5.4		
III (15 rats)	Control gelatin	30.1	23.7	22.4	19.1	
		18.0	17.8	17.1	16.5	15.8
		15.7	13.2	12.0	9.9	
		8.5	8.0	4.7		

Experiment 27: Walker carcinoma.

Pyrene.

This test was carried out simultaneously with Experiment 7, a group of fifteen rats serving as common controls to two experimental series. The rats were implanted on the same occasion from a single donor, and the time of injection was the same for all. In this part of the experiment two doses of 25 mg. pyrene in oil were given on successive days. This produced no interference with the normal rate of tumour growth, and the final result (Table XXVII and Fig. 9) presents a striking contrast to that of Experiment 7 (Table VII and Fig. 4).

T A B L E XXVII

Group	Treatment	Tumour weights (g.) after 21 days..... Individual			Mean
I (14 rats)	2 x 25 mg. pyrene in oil	48.9 40.6 33.4 23.5 13.5	46.2 37.4 27.4 20.3 37.0	40.9 36.3 25.2 17.9	32.0
II (15 rats)	2 x 5 c.c. sesame oil	49.6 37.5 29.7 29.0 20.5	46.6 35.7 29.7 28.5 17.4	38.0 33.2 29.2 22.9 15.7	30.9

A B

10 cm.

Fig. 9: Experiment 27; Walker carcinoma.

Pyrene 50 mg.

A: Controls....mean weight 30.9 g.

B: Treated..... " " 32.0 g.

Experiment 28: Rous fowl sarcoma.

Pyrene.

Concurrently with Experiment 8 twelve fowls implanted with the Rous sarcoma were each given 90 mg. pyrene in oil. There was undue mortality in both experimental and control groups, but pyrene had no inhibitory effect (Table XXVIII). The mean weight of the test tumours was actually considerably greater than that of the controls, but the difference was without significance (P 0.2-0.3).

T A B L E XXVIII

Group	Treatment	Weight of tumour tissue (g.) after 18 days..... Individual			Mean
I (7 fowls)	3 x 30 mg. pyrene in oil	76 46 8	62 32	60 32	45.1
II (9 fowls)	sesame oil alone	53 42 22	46 35 14	46 28 13	33.2

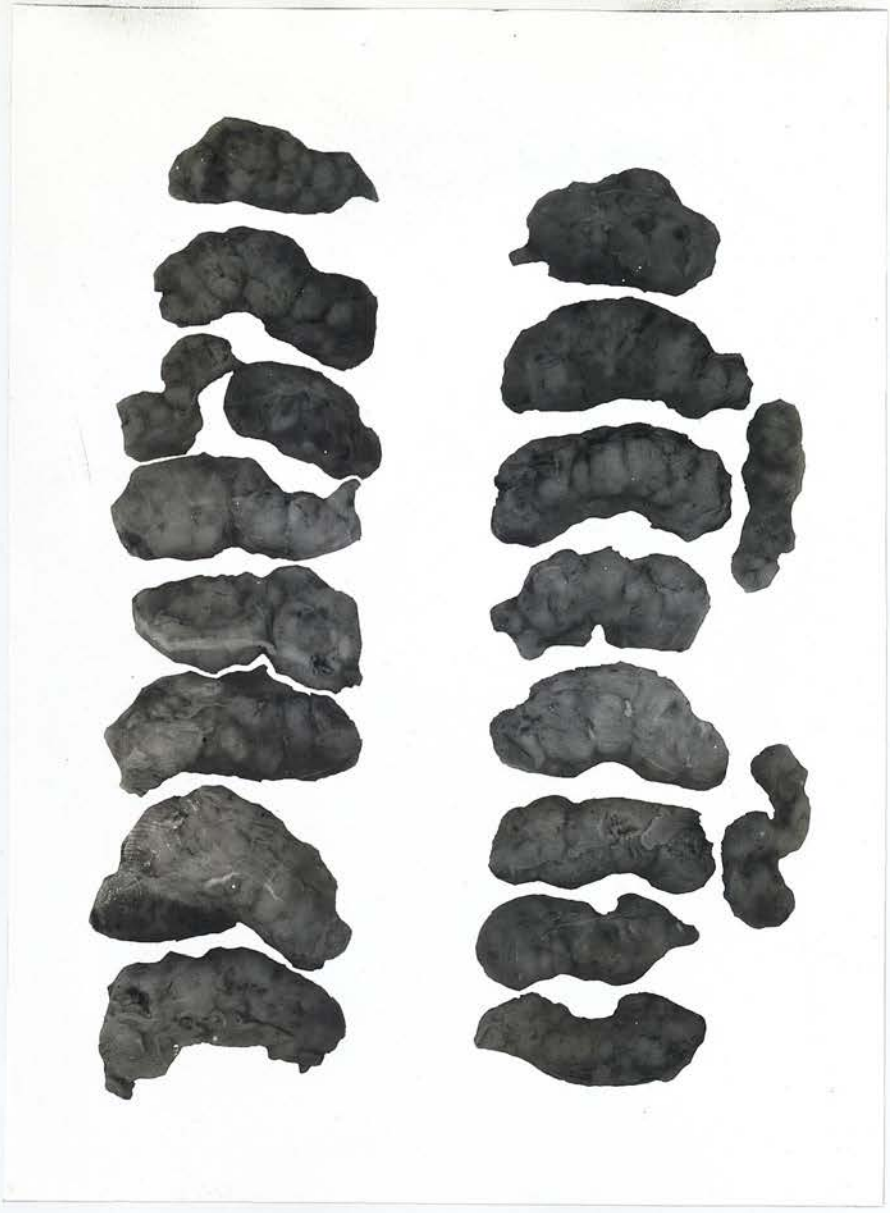
Experiment 29: Walker carcinoma.

Triphenylene.

Twenty-four rats were used in two groups. The mean weight of the treated tumours at the 26th day was less than that of the controls (Table XXIX and Fig. 10) but the difference was without significance (P between 0.5 and 0.4).

T A B L E XXIX

Group	Treatment	Tumour weights (g.) after 26 days..... Individual		Mean
I (10 rats)	2 x 25 mg. triphenylene in oil	47.2 38.9 31.9 25.5 13.0	43.5 38.4 26.0 21.9 9.6	29.6
II (9 rats)	2 x 5 c.c. sesame oil	71.1 43.1 34.2 22.4 10.0	46.2 38.6 30.5 20.4	35.2



A B

10 cm.

Fig. 10: Experiment 29; Walker carcinoma.

Triphenylene 50 mg.

Tumours after 26 days.

A: Controls....mean weight 35.2 g.

B: Treated..... " " 29.6 g.

Experiment 30: Walker carcinoma.

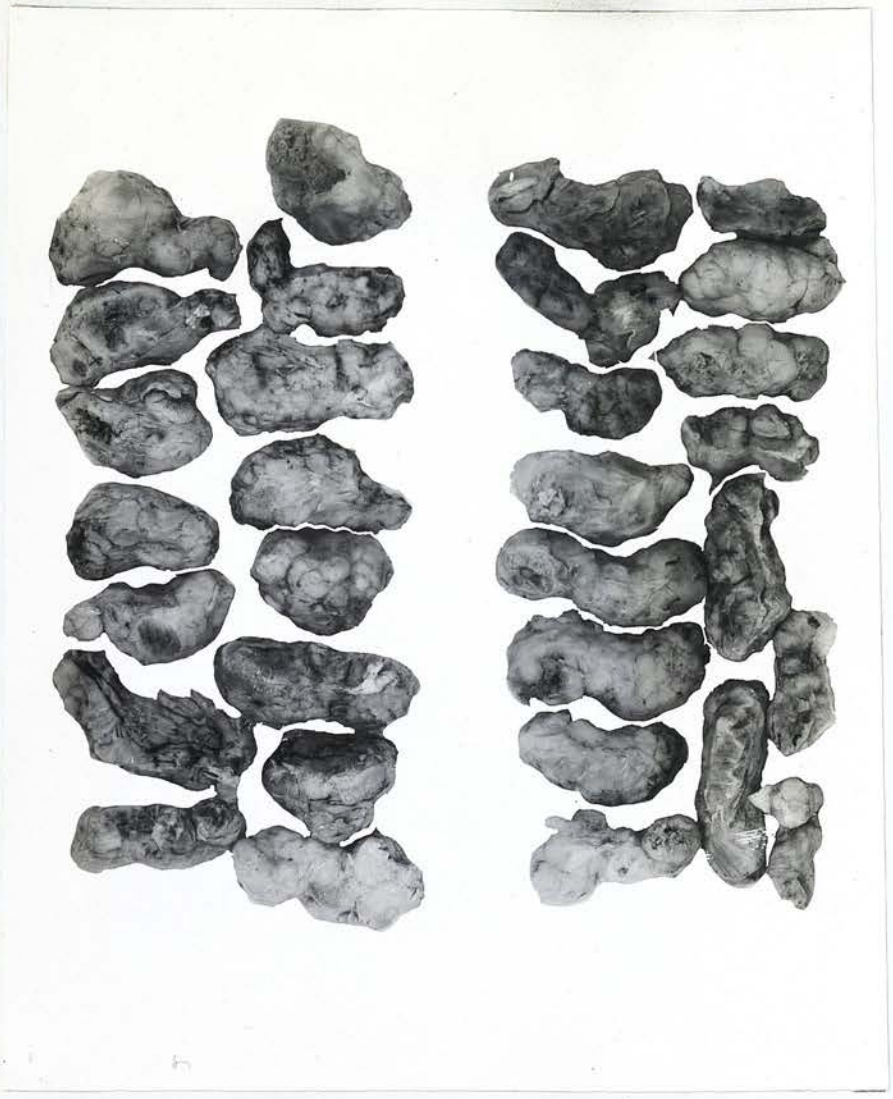
Dodecahydro-1:2-benzanthracene;
(Cook and Hewett 1934)

Perylene.

Forty-six implanted rats were used to test the influence of these substances in doses of 50 mg. There was no significant difference between the mean weights of the tumours treated with dodecahydro-1:2-benzanthracene and their controls (Table XXX) and although the mean weight of the perylene-treated tumours (Fig. 11) was lower than that of the controls by several grams, the calculated value for P was 0.1

T A B L E XXX

Group	Treatment	Tumour weights (g.) after 32 days..... Individual				Mean
I (15 rats)	2 x 25 mg. dodecahydro-1:2-benz-anthracene	63.5	58.7	54.0	52.0	
		51.9	44.1	41.5	39.8	41.6
		38.5	38.0	36.8	27.5	
		27.5	26.9	24.2		
II (16 rats)	2 x 25 mg. perylene in oil	56.9	52.1	50.1	47.5	
		41.5	37.4	35.9	35.2	33.7
		32.3	31.9	30.9	21.7	
		20.5	18.9	14.8	12.2	
III (15 rats)	2 x 5 c.c. sesame oil	47.9	47.9	43.7	42.0	
		41.7	41.5	40.5	40.0	40.0
		39.8	39.6	38.6	38.1	
		36.5	34.3	29.2		



A B

10 cm.

Fig. 11: Experiment 30; Walker carcinoma.

Perylene 50 mg.

Tumours after 32 days.

A: Controls....mean weight 40.0 g.

B: Treated..... " " 33.7 g.

Experiment 31: Walker carcinoma.

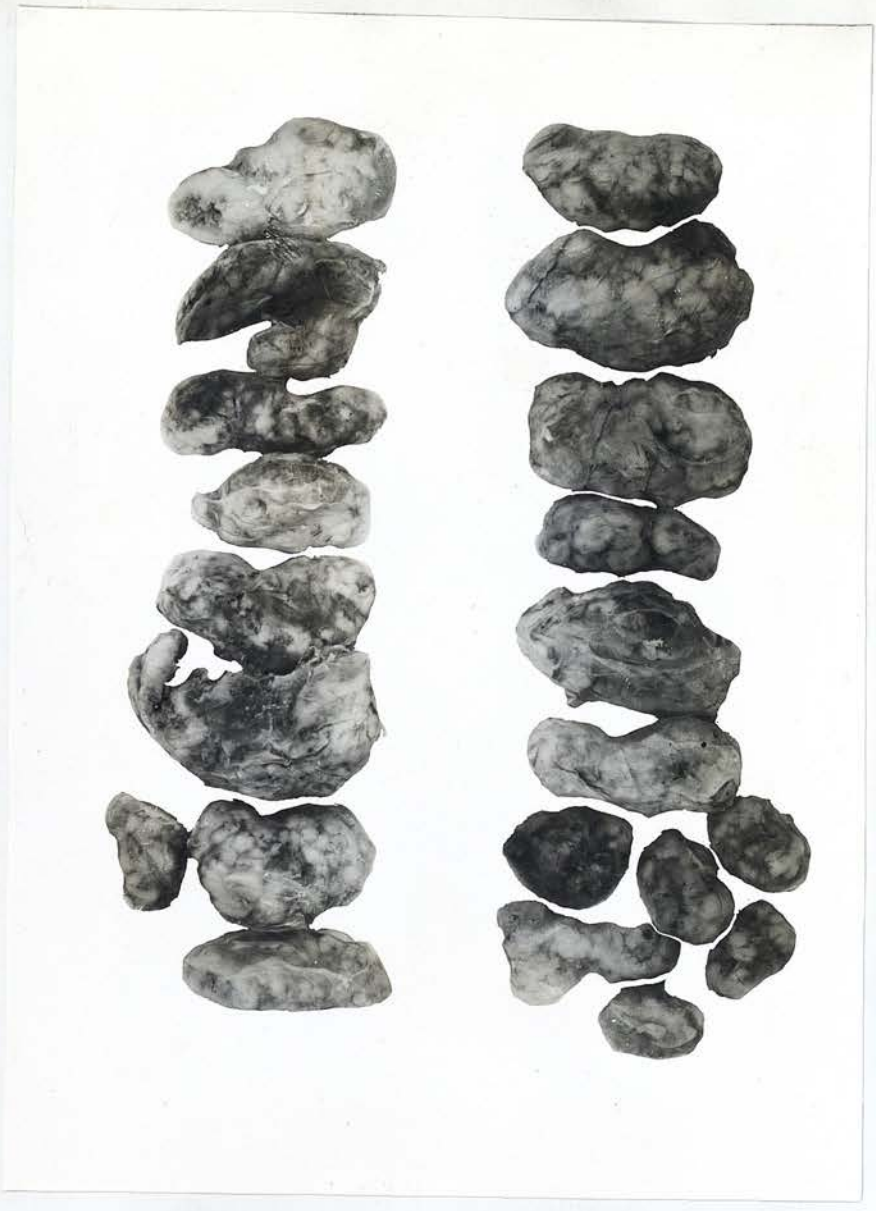
1:9-benzanthrone;

diphenylene oxide.

Thirty-six rats were used in three equal groups. Although the mean weight of the treated sets was lower than that of the controls, (Table XXXI and Fig. 12), no significance could be attached to the differences. The range of weights covered by the individual data was the same in all these groups, and the value for P in each half of the experiment was as high as 0.2

TABLE XXXI

Group	Treatment	Tumour weights (g.) after 30 days..... Individual			Mean
I (12 rats)	2 x 25 mg. 1:9-benz-anthrone	68.8 36.4 17.0 10.6	50.6 34.5 15.0 10.6	43.8 21.0 10.7 8.5	27.3
II (11 rats)	2 x 25 mg. di-:phenylene oxide	78.3 33.3 16.1 8.9	37.6 29.2 16.1 6.5	36.2 24.4 13.8	27.3
III (9 rats)	2 x 5 c.c. sesame oil	69.9 43.4 29.4	46.2 41.3 29.2	45.2 30.6 8.9	38.2



A B

10 cm.

Fig. 12: Experiment 31; Walker carcinoma.

1:9-benzanthrone 50 mg.

A: Controls....mean weight 38.2 g.

B: Treated..... " " 27.3 g.

E. Experiments with tumours of known causation.

The following experiments were carried out with two transplantable rat sarcomata of known causation. The first, produced in Dr Hain's laboratory by the subcutaneous injection of 3:4-benzpyrene, appeared some five months after the injections and proved to be easily propagable by grafting. A number of experiments were carried out within the first few generations. The second tumour was a sarcoma 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 three years: it was used in the present work in its 87th. generation.

Experiment 32: The influence of 3:4-benzpyrene on the 4th generation transplants of a tumour induced by the same compound.

Thirty-two rats were implanted from a healthy tumour of the 3rd generation. Half of them were at once given 60 mg. 3:4-benzpyrene in two doses in oil on successive days while the remainder received the control oil alone. The mean weight of the control tumours was just over twice that of the treated group (Table XXXII). This difference was significant since the value for P lay between 0.02 and 0.01.

TABLE XXXII

Group	Treatment	Tumour weights (g.) after 24 days..... Individual			Mean
I (14 rats)	2 x 30 mg. 3:4-benz-pyrene in oil	64.7	21.6	18.7	15.3
		15.8	15.3	14.3	
		13.3	12.7	12.1	
		10.3	8.9	3.5	
		1.9	1.4		
II (14 rats)	Control oil	68.2	57.3	56.0	33.2
		52.9	40.2	39.9	
		34.5	28.9	26.5	
		21.5	19.0	14.8	
		2.7	1.8		

Experiment 33: The influence of 1:2:5:6-dibenz-anthracene on the 6th generation transplants of a tumour induced by 3:4-benzpyrene.

Thirty animals were implanted from a fifth generation sarcoma and half of them at once given 50 mg. 1:2:5:6-dibenzanthracene (Table XXXIII).

TABLE XXXIII

Group	Treatment	Tumour weights (g.) after 24 days..... Individual			Mean
I (11 rats)	2 x 25 mg. 1:2:5:6-dibenz-anthracene in oil	18.9 6.3 4.7 2.2	12.5 6.0 3.1 1.7	6.5 6.0 3.0	6.4
II (12 rats)	2 x 5 c.c. sesame oil	56.1 30.7 22.0 11.0	33.4 28.7 18.2 6.9	30.9 26.6 14.1 4.4	23.6

Experiment 34: The influence of 3-methyl-1:2-benzanthracene on the 5th generation transplants of a tumour induced by 3:4-benzpyrene.

Of twenty rats implanted from a healthy tumour of the 4th generation half were given 50 mg. 3-methyl-1:2-benzanthracene in oil and the remainder oil alone (Table XXXIV).

T A B L E XXXIV

Group	Treatment	Tumour weights (g.) after 26 days..... Individual			Mean
I (9 rats)	2 x 25 mg. 3-methyl-1:2-benz-anthracene	26.4 10.7 4.5	13.5 8.4 2.1	11.6 8.1 2.0	9.7
II (8 rats)	2 x 5 c.c. sesame oil	81.8 44.7 34.4	63.0 40.7 23.2	62.2 37.1	48.4

Experiment 35: The influence of 1:2:5:6-dibenz-anthracene on the 87th generation transplants of a tumour induced by the same compound.

Thirty rats were implanted from a healthy tumour of the 86th generation of the LR-10 sarcoma. Fifteen of these were then given daily injections of colloidal 1:2:5:6-dibenzanthracene for 17 days during which time each received about 30 mg. of the hydrocarbon. The remaining animals were given the control material (0.5 per cent. gelatin) alone. The result was a moderate degree of inhibition (Table XXXV).

T A B L E XXXV

Group	Treatment	Tumour weights (g.) after 17 days..... Individual			Mean
I (14 rats)	Colloidal 1:2:5:6-dibenz-anthracene daily (total 30 mg.)	3.5 2.5 2.1 1.6 0.6	3.2 2.5 1.9 0.9 0.5	2.8 2.5 1.6 0.7	1.9
II (15 rats)	Control gelatin	12.3 8.8 6.2 4.7 2.4	11.6 7.7 6.0 4.1 1.8	9.9 7.0 4.8 2.6 1.5	6.1

T A B L E X X X V I

Expt. No.	Tumour	Compound	Dose (mg.)	Duration of expt. (days)	Mean wt. treated tumours/mean wt. control tumours	No. of animals	P
2	Jensen sarcoma	1:2:5:6-dibenz-anthracene	8	21	0.20	16	< 0.01
4	"	"	16	21	0.27	22	"
5	"	"	30	18	0.13	13	"
6	"	"	12	14	0.29	19	"
7	Walker carcinoma	"	50	21	0.03	30	"

T A B L E XXXVI continued.....

Expt. No.	Tumour	Compound	Dose (mg.)	Duration of expt. (days)	Mean wt. treated tumours/mean wt. control tumours	No. of animals	P
8	Rous sarcoma	1:2:5:6-dibenz-anthracene	90	18	0.20	16	< 0.01
9	Jensen sarcoma	5:6-cyclo-penteno-1:2-benz-anthracene	15	18	0.19	27	"
10	"	sodium-1:2:5:6-dibenz-anthracene-endo- -succinate	100	18	0.27	24	"
11	Walker carcinoma	3:4-benz-pyrene	50	28	0.07	22	"

T A B L E XXXVI continued.....

Expt. No.	Tumour	Compound	Dose (mg.)	Duration of expt. (days)	Mean wt. treated tumours/mean wt. control tumours	No. of animals	P
12	Walker carcinoma	3:4:5:6-dibenz-acridine	50	32	0.48	18	< 0.01
13	Jensen sarcoma	chrysene	30	17	0.30	15	0.05-0.02
14	Walker carcinoma	"	60	19	0.10	25	< 0.01
15	Jensen sarcoma	1:2-benz-anthracene	15	18	0.41	30	"
16	Walker carcinoma	"	60	21	0.15	20	"
17	"	3-methyl-1:2-benz-anthracene	50	32	0.14	18	"

T A B L E XXXVI continued.....

Expt. No.	Tumour	Compound	Dose (mg.)	Duration of expt. (days)	Mean wt. treated tumours/mean wt. control tumours	No. of animals	P
18	Walker carcinoma	4-methyl-1:2-benz-anthracene	50	15	0.15	24	< 0.01
18	"	7-methyl-1:2-benz-anthracene	50	15	0.16	26	"
19	"	6-methyl-1:2-benz-anthracene	50	30	0.09	18	"

T A B L E XXXVI continued.....

Expt. No.	Tumour	Compound	Dose (mg.)	Duration of expt. (days)	Mean wt. treated tumours/mean wt. control tumours	No. of animals	P
20	Walker carcinoma	1-keto-1:2:3:4-tetra-hydro-phenanthrene	50	28	1.25	30	c. 0.2
20	"	9:10-di-hydroxy-9:10-di-n-propyl-9:10-dihydro-1:2:5:6-dibenz-anthracene	50	28	0.18	28	$<$ 0.01
21	Jensen sarcoma	anthracene	12	18	1.16	14	c. 0.5
22	"	phenanthrene	12	15	1.13	22	0.6-0.5

T A B L E XXXVI continued.....

Expt. No.	Tumour	Compound	Dose (mg.)	Duration of expt. (days)	Mean wt. treated tumours/mean wt. control tumours	No. of animals	P
23	Jensen sarcoma	phenanthrene	12	15	1.02	17	0.9
24	"	1:2-cyclo-penteno-phenanthrene	30	17	1.52	15	0.3-0.2
25	Walker carcinoma	"	50	34	1.34	28	0.1-0.05
25	"	dehydronor-cholene	50	34	0.98	25	> 0.9
26	"	pyrene	40	21	1.02	29	0.9-0.8
26	"	fluoranthene	40	21	1.08	30	0.6-0.5

T A B L E XXXVI continued.....

Expt. No.	Tumour	Compound	Dose (mg.)	Duration of expt. (days)	Mean wt. treated tumours/mean wt. control tumours	No. of animals	P
27	Walker carcinoma	pyrene	50	21	1.03	29	0.8-0.7
28	Rous sarcoma	"	90	18	1.36	16	0.3-0.2
29	Walker carcinoma	triphenyl-:ene	50	26	0.84	19	0.5-0.4
30	"	dodeca-hydro-1:2-benz-anthracene	50	32	1.04	30	0.7-0.6
30	"	perylene	50	32	0.84	31	0.1
31	"	1:9-benz-anthrone	50	30	0.71	20	0.2

T A B L E XXXVI concluded.....

Expt. No.	Tumour	Compound	Dose (mg.)	Duration of expt. (days)	Mean wt. treated tumours/mean wt. control tumours	No. of animals	P
31	Walker carcinoma	diphenyl-:ene oxide	50	30	0.71	20	0.2
32	Benzpyrene-induced sarcoma (4th generation)	3:4-benz-pyrene	60	24	0.46	28	0.02-0.01
33	" (6th generation)	1:2:5:6-dibenz-anthracene	50	24	0.27	23	< 0.01
34	" (5th generation)	3-methyl-1:2-benz-anthracene	50	26	0.20	17	"
35	Dibenzanthr-:acene-induced tumour LR-10 87th generatn.	1:2:5:6-dibenz-anthracene	30	17	0.31	29	"

III. D I S C U S S I O N.

A. Results.

Table XXXVI summarises the experiments already described. The ratio "mean weight of tumours from treated animals: mean weight of control tumours" is an index of the inhibiting effect, if any, produced. Throughout the work the usual convention $P < 0.05$ was taken as expressing the limit of significance.

It is obvious that compounds with acknowledged carcinogenic activity produced inhibition of the growth of the tumours used. Confirmatory results have recently been published by Morelli and Guastalla (1936) who obtained inhibition of the Jensen and Walker tumours with 3:4-benzpyrene.

Secondly, inhibitory power was shown, usually to a somewhat less extent, by chrysene, 1:2-benzanthracene, 4-methyl-1:2-benzanthracene, 6-methyl-1:2-benzanthracene, 3-methyl-1:2-benzanthracene, and 7-methyl-1:2-benzanthracene, substances whose carcinogenicity is either exceedingly weak (as in the case of the first four named) or nil, as in the last two. Chrysene was stated by Twort and Fulton (1930) and Bottomley and Twort (1934) to possess a low order of carcinogenic power, while Barry and Cook (1934)

and Barry et al. (1935) found only slight activity in experiments with highly purified chrysene derived from coal tar. Later work was carried out with synthetic chrysene not open to contamination with constituents of tar (Cook et al. 1936). "The synthetic compound was applied to the skin of 20 mice of which 5 lived for more than 440 days. The last mouse died on the 853rd day bearing a large epithelial tumour, which had appeared first on the 711th day; this showed some downgrowth, but did not reach the superficial layer of voluntary muscle. No other mouse showed any other tumour. Hence the carcinogenic power of chrysene is of an extremely low order." In addition, the subcutaneous injection in ten rats of solutions of chrysene in lard gave spindle-celled tumours in two cases. The same authors state that while 1:2-benzanthracene itself showed little if any carcinogenicity the introduction of alkyl groups into positions 5 and 6 invariably led to activity.

Lastly, the remaining non-carcinogenic compounds proved quite inactive as regards growth-inhibitimg power: the mean weight of the treated tumours was less than that of the controls in five out of fifteen experiments, and in only one of these (Experiment 30: perylene) was the value for P as low as 0.1. As has been mentioned, the case of

1:2-cyclopentenophenanthrene was of additional interest since the data suggested that this compound might actually possess a stimulating action; the differences however were not judged significant. According to Cook et al. (1936) 1:2-cyclopentenophenanthrene applied to the skin of mice was non-carcinogenic, although the subcutaneous injection of a solution in lard gave a spindle-celled tumour --- which however did not grow even in autograft --- in one of ten rats.

These results show a correlation between carcinogenicity and growth-inhibitory power, but this relationship is not quantitatively simple, since 1:2:5:6-dibenzanthracene proved to be more inhibitory than 3:4-benzpyrene, although their order of carcinogenicity is the reverse. If it be assumed that the two properties are connected etiologically, this finding suggests various possibilities such as the following: (a) that carcinogenicity is dependent on a certain optimal, and not a maximal, inhibiting power, or (b), that the relation between them is quantitatively simple but is disturbed by other factors such as the "toxicity" of individual compounds. Again, while 6-methyl-1:2-benzanthracene is the most actively inhibitory (Experiment 19) as well as the most carcinogenic among those methyl

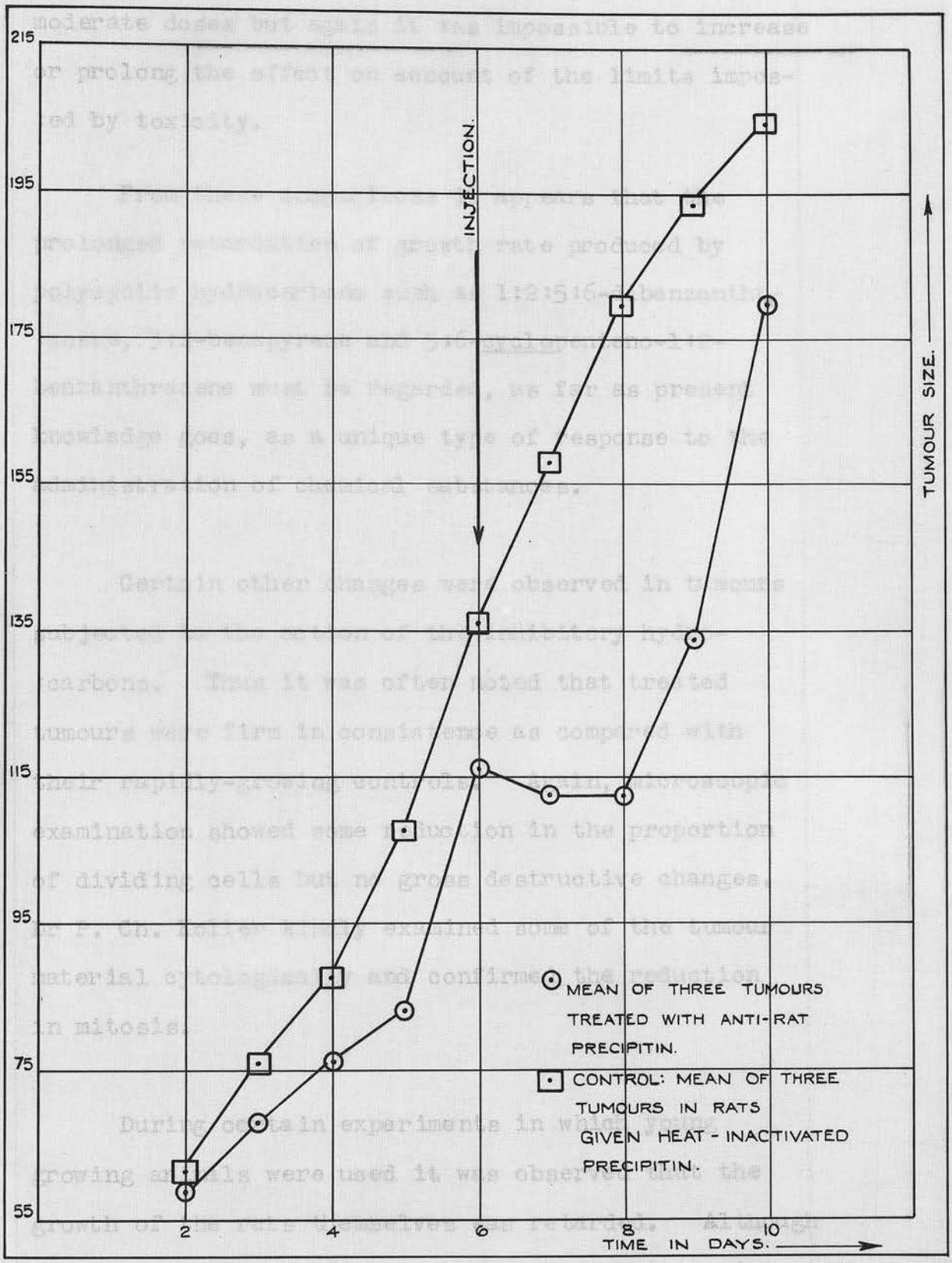
derivatives of 1:2-benzanthracene which were tested, the 3-methyl and 7-methyl compounds also showed inhibitory properties (Experiments 17, 18, 34) although they are classed with the non-carcinogenic derivatives of 1:2-benzanthracene (Barry et al., 1935). In any comparison between these biological properties it should also be borne in mind that while carcinogenicity has been determined mainly in mice, these experiments on growth-inhibition have been carried out almost exclusively with rats. Thus the statements made here about the carcinogenic, or non-carcinogenic, character of various compounds are derived chiefly from experiments upon the skin of the mouse. The accessibility of this particular test-object is in a sense accidental, and its response, which is that of a single tissue in a single species, may not give a complete picture of the property in question.

Figs. 1-3 show that the effect of 1:2:5:6-dibenzanthracene was to produce an immediate and prolonged retardation in the growth rate even when the substance was administered in only two injections. In addition it can be seen that the retardation was relatively constant in any one experiment. It may also be noted that the control data are in agreement

with the conclusions reached by Mayneord (1932) in the case of the Jensen sarcoma and Schrek (1935) for the Walker and Flexner-Jobling carcinomata, that the unimpeded growth of these tumours is a linear function of the time over a considerable period.

The contrast between the growth-inhibitory action of substances such as 1:2:5:6-dibenzanthracene and the inactivity of most of the non-carcinogenic hydrocarbons is sufficiently striking, but it is important to enquire if the action is characteristic. Prior to this work a number of experiments had been carried out to determine the effect on rat tumours of various toxic agencies such as anti-rat precipitin and colchicine, and these experiments provided a useful standard by which to judge the significance of the inhibition produced by the carcinogenic hydrocarbons. Interference with growth in such experiments was obviously due to a serious poisoning effect on the whole animal, and it was found that no lasting inhibition could be produced by amounts approaching the lethal dose. Fig. 13 illustrates the influence on the growth of the Jensen sarcoma of a suitable amount of anti-rat precipitin: temporary retardation was followed by rapid recovery. This action could not be intensified since larger doses proved highly toxic. In the case of colchicine some irregularity in growth rate was produced by

FIGURE 13


215
195
175
155
135
115
95
75
55
INJECTION.
TUMOUR SIZE.
2
4
6
8
10
TIME IN DAYS.
MEAN OF THREE TUMOURS TREATED WITH ANTI-RAT PRECIPITIN.
CONTROL: MEAN OF THREE TUMOURS IN RATS GIVEN HEAT - INACTIVATED PRECIPITIN.

moderate doses but again it was impossible to increase or prolong the effect on account of the limits imposed by toxicity.

From these comparisons it appears that the prolonged retardation of growth rate produced by polycyclic hydrocarbons such as 1:2:5:6-dibenzanthracene, 3:4-benzpyrene and 5:6-cyclopenteno-1:2-benzanthracene must be regarded, as far as present knowledge goes, as a unique type of response to the administration of chemical substances.

Certain other changes were observed in tumours subjected to the action of the inhibitory hydrocarbons. Thus it was often noted that treated tumours were firm in consistence as compared with their rapidly-growing controls. Again, microscopic examination showed some reduction in the proportion of dividing cells but no gross destructive changes. Dr P. Ch. Koller kindly examined some of the tumour material cytologically and confirmed the reduction in mitosis.

During certain experiments in which young growing animals were used it was observed that the growth of the rats themselves was retarded. Although the degree of inhibition was much more striking in the case of the tumours, it was concluded that the effect was in no sense specific for tumour tissue

and that the latter simply shared in a retardation of growth affecting the body as a whole. This impression was fully confirmed in another series of experiments on the influence of carcinogenic hydrocarbons on body-growth (see also Haddow, Scott and Scott 1937). This investigation also demonstrated the value of the young growing rat as a test-object in work of this kind, since the material permits greater uniformity than can be attained with transplantable tumours even under the best conditions.

Apart from their action on body-growth and tumour-growth the carcinogenic hydrocarbons produced other effects which are no doubt correlated. In some experiments it was noted that the food-intake of the treated rats appeared to be less than that of the controls, although their general health and activity remained good. The fertility of the treated animals was very definitely lowered, and in many cases the ovary showed suppression of ovulation and the testis a variable reduction of spermatogenesis (Figs. 14-15). In one case the administration of 50 mg. 1:2:5:6-dibenzanthracene at the first appearance of the placental sign was followed by prolongation of the gestation period and death of the foetuses <u>in utero</u>.

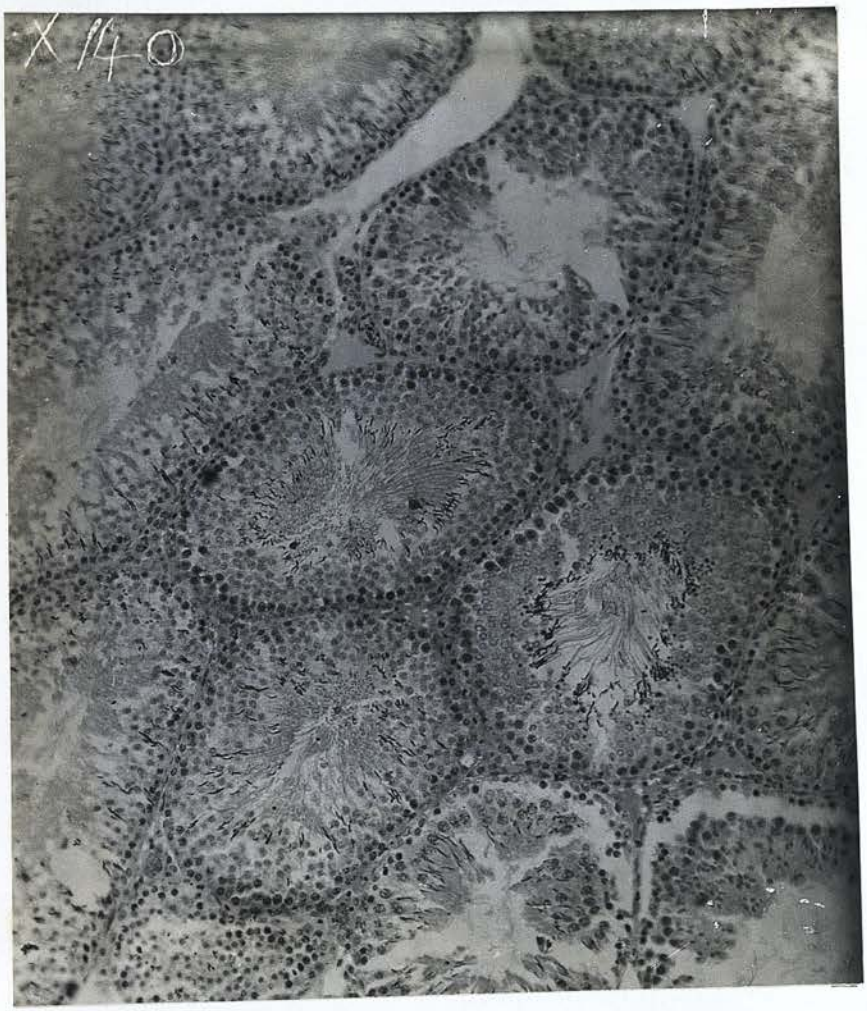


Fig. 14a.

Testis from rat treated with 50 mg. pyrene 21 days previously.

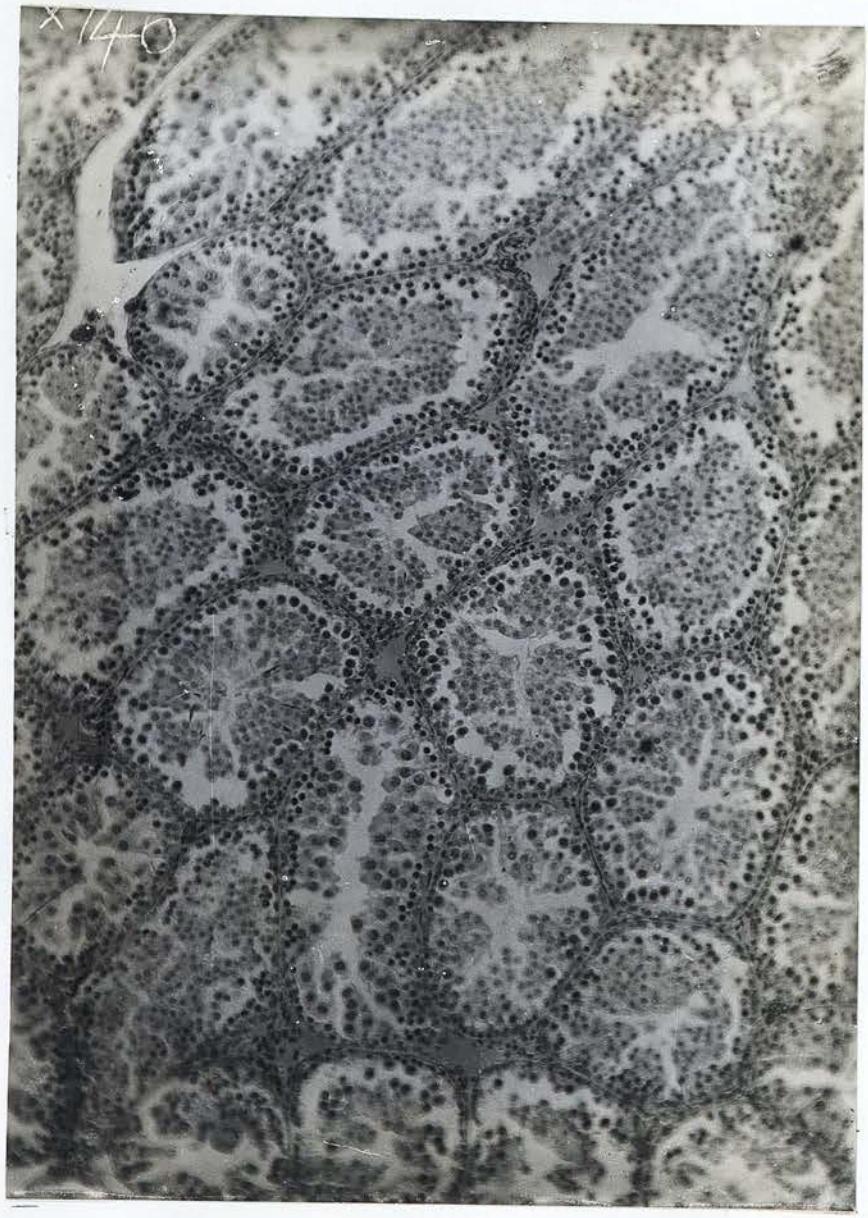


Fig. 14b.

Testis from rat treated with 50 mg. 1:2:5:6-dibenzanthracene 21 days previously.

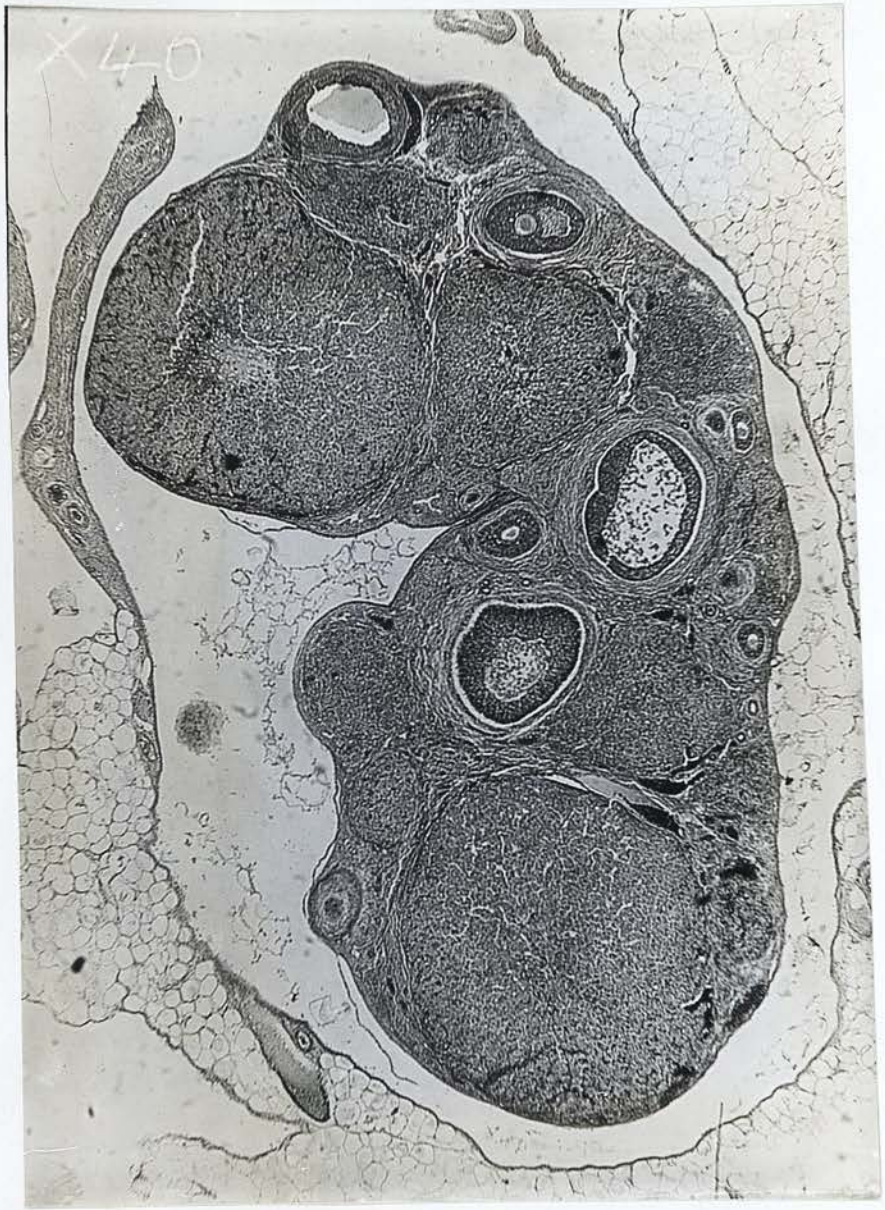


Fig. 15a.

Ovary from rat given control oil 21 days previously.

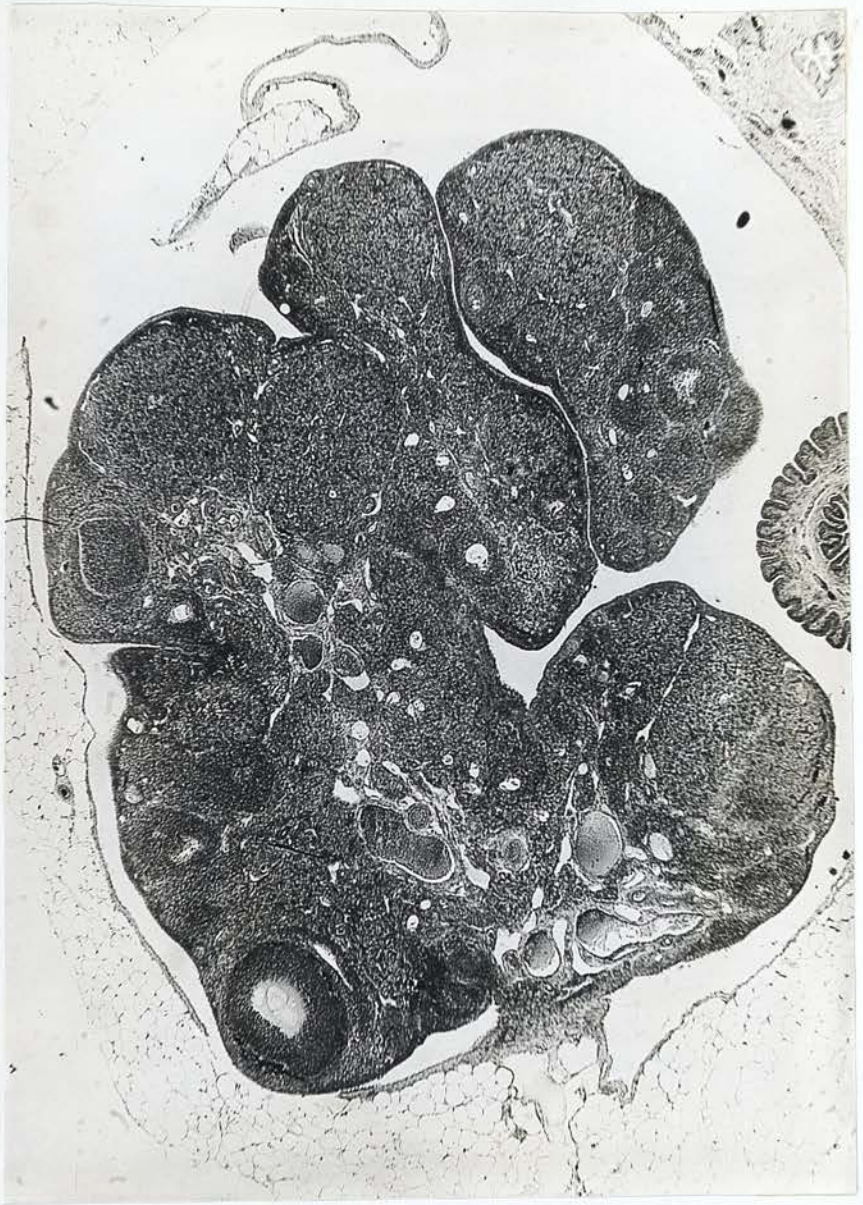


Fig. 15b.

Ovary from rat given 50 mg. 6-methyl-1:2-benzanthracene 21 days previously.

Routine histological examination revealed no undoubtedly significant changes in the liver, kidney, spleen, suprarenal, thyroid or bone-marrow. The influence of the active substances is thus specially evident in the case of growing tissues, and is unique in so far as doses which produce little sign of general toxicity or tissue damage may bring about a profound and continued alteration in the rate of growth. The phenomenon is clearly independent of toxic action in the non-specific sense, especially since recognised poisons such as colchicine evoke a different type of response. On the other hand it is equally true that the carcinogenic hydrocarbons tested have all shown some degree of toxicity. This manifested itself not by any immediate ill-effect but rather by an increased susceptibility to infection which should perhaps be regarded as a secondary effect of the retardation of growth itself. Picard and Laduron (1934 a, b) studied the toxicity of 3:4-benzpyrene in mice and found that doses of the order of 35 mg. produced atrophic changes in the spleen, thymus gland and.lymph nodes. More recently Polson (1936) has described experimental liver necrosis in the rabbit produced by the intraperitoneal injection of suspensions of 1:2:5:6-dibenzanthracene: the results showed considerable variation and were not duplicated in the case of 3:4-benzpyrene.

According to Zondek (1936 a, b) prolonged administration of large doses of oestrin produces considerable inhibition of body-growth; the subcutaneous injection of 5,000 m.u. dimenformon twice weekly in rats 4-6 weeks old retarded growth to the extent of nearly 45 per cent. after about 4 months. The treated animals ate less food, and Zondek referred to the possible interpretation that the animals eat less in consequence of the long treatment and are therefore retarded in their growth. But in Zondek's opinion the intake isreduced because the animals have ceased growing and so require less food. The growth-inhibiting effect of oestrin was produced by percutaneous as well as subcutaneous administration and in birds as well as in rodents.

Zondek also brought forward evidence that (a) prolonged administration of oestrin interferes with the anterior lobe of the pituitary; (b) the process does not affect all the anterior pituitary functions equally and simultaneously, since atrophy of the genitals is the first effect in the rat; (c) elimination of the gonadotropic hormone is followed by failure of the growth hormone. He regarded the stunting of growth produced by oestrin as a pituitary dwarfism since it could be abolished by giving an extract rich in growth hormone.

In the present experiments the synthetic oestrogens 1-keto-1:2:3:4-tetrahydrophenanthrene and 9:10-dihydroxy-9:10-di-n-propyl-9:10-dihydro-1:2:5:6-dibenzanthracene behaved differently in their action on tumour growth, the latter proving moderately inhibitory and the former inactive. The former substance is non-carcinogenic (Barry et al., 1935) and the latter has given no tumours as yet, although the experiments are incomplete (Burrows, unpublished). It should also be noted that the oestrogenic activity of the keto compound is of an altogether lower order than that of the di-n-propyl diol (Cook, Dodds, Hewett and Lawson 1934). Wolfe (1936) studied the action of this dibenzanthracene diol on the pituitary and found that it prevented the changes which occur after castration and also induced extreme degranulation of the basophiles and moderate degranulation of the eosinophiles. In this comparison between the effects of carcinogenic and oestrogenic substances it may be noted that Cramer and Horning (1936) described arrest of cell division in the mouse testis after prolonged treatment with oestrin, while Tuchmann (1936; also Tuchmann and Demay 1936) obtained similar regressive changes following the administration of either folliculin or 3:4-benzpyrene in guinea-pigs.

It is clear that at least a part of the general inhibition of growth produced by the carcino-:genic hydrocarbons may be explicable on the basis suggested by Zondek for oestrin, i.e., as an interference with pituitary function. Nevertheless it also seems probable on account of the insolubility of the cancer-producing compounds that in certain circumstances their action must be restricted to the cells in the immediate vicinity. Tumour-induction by means of the carcinogenic hydrocarbons is essent-:ially a local process, and this in itself indicates that such substances may act directly on exposed cells.

B. Interpretation and significance.

These results show that the carcinogenic hydrocarbons possess unusual growth-inhibiting properties and are not direct growth-stimulating substances as has been assumed for instance by Needham (1936). We have thus a paradox in that the continued exposure of normal cells to a strongly inhibitory agent may result in the appearance of a rapidly growing malignant tumour. The following hypothesis is put forward to explain this apparent contradiction.

The available evidence points rather to the origin of cancer as a result of a change in cells at one time normal, than to any selective process. The cancer cell must therefore be regarded as a variant of its normal prototype, from which it differs in metabolism and in the character of growth-rate. Moreover, the study of its behaviour during prolonged transplantation shows that the type of variant it represents is discontinuous, permanent and irreversible. On these grounds it seemed that the origin of cancer could be resolved into one of the origin of variations in general and of discontinuous irreversible variations in growth-rate in particular.

In a study of variation in bacteria it was found that certain principles seemed to be applicable to the phenomena of variation in other types of cell. Firstly, the sources of variation were found to be mainly if not entirely environmental in origin. Secondly, for the purpose of inducing variation in respect of a given character there appeared to be two main requirements: (a) a cell inherently capable of variation in respect of that character; (b) a source of environmental interference with the character in question which is freely compatible with cell viability and so with the power to vary. In particular it was found that variants characterised by permanently increased growth-rate were produced not by any process of direct growth stimulation but rather as a sequel to a long continued period of growth inhibition. It was concluded that when the growth of a potentially variable organism is inhibited by a process which allows the majority of the affected cells to survive, a relatively small number may undergo an irreversible change in their metabolism such that they can then multiply even in an environment which makes this difficult or impossible for their parent cell. The problem arose whether this might apply to the new cell-race which constitutes a malignant tumour, and whether the production of cancer might also be due to a

sustained inhibition of the affected normal cells leading to the emergence of a new and advantageous variant with a permanently increased fission rate.

It is of interest that x- and gamma-rays, which in certain circumstances are associated with the induction of tumours (e.g., Ludin 1934, Ross 1936), have growth-inhibiting powers to which they owe their therapeutic application. This is specially significant since recent evidence (e.g., Medical Uses of Radium, 1934: M.R.C. Special Report No. 204, pp. 10-12) tends to show that the effect of such agencies is inhibitory under all conditions of their action, low dosages and short non-lethal exposures producing a retardation of growth which is followed by recovery. The association of cancer with these radiations is therefore unlikely to be due to direct stimulation.

If the emergence of a chemically produced tumour is a response of normal cells to a long-continued inhibition, it is of the greatest theoretical interest to know the reaction of the new race of tumour cells to the hydrocarbon which induced their appearance. Experiment 32 shows that a rat sarcoma produced by 3:4-benzpyrene was apparently relatively resistant (at the time of the 4th transplanted generation) to the inhibitory action of the same hydrocarbon. Comparative tests

showed that while the Walker tumour was markedly sensitive to inhibition by 3:4-benzpyrene, the ratio of the mean weight of treated tumours to that of the controls being 1:14 (Experiment 11), the correspond-:ing ratio in the case of the benzpyrene-induced tumour was approximately 1:2.

No undue stress can possibly be laid on this single observation at present, as it merely gives a certain amount of passive support to the idea that such a growth represents a new and relatively resistant cell-race which appears as an emancipation from the inhibitory conditions produced by the hydrocarbon. On the other hand, if confirmation of this view is obtained in other experiments with tumours induced by similar means, two further problems will arise regarding (a) the specificity of drug-resistance in the cells of such tumours; and (b) the stability of this resistance during the course of transplantation. With regard to the first question it can be seen from Experiments 33 and 34 that the benzpyrene-induced tumour was inhibited by 1:2:5:6-dibenzanthracene and 3-methyl-1:2-benz-anthracene, although to a less degree by the former substance than was the Walker carcinoma. As to the second question, the only information at present available is given by Experiment 35, which shows that

a tumour originally induced by 1:2:5:6-dibenz-anthracene proved moderately sensitive to inhibition by the same compound when tested in the 87th generation, after about three years of propagation.

IV. S U M M A R Y.

1. A number of carcinogenic compounds, including 1:2:5:6-dibenzanthracene, 5:6-cyclopenteno-1:2-benzanthracene and 3:4-benzpyrene, produced a considerable inhibition in the rate of growth of the Jensen and Walker tumours.

2. This activity was also shown to a variable extent by chrysene and certain compounds of benzanthracene type, the carcinogenicity of which is either feeble or nil.

3. A series of related non-carcinogenic compounds showed no inhibitory influence when tested under the same conditions.

4. Of the synthetic oestrogens 1-keto-1:2:3:4-tetrahydrophenanthrene and 9:10-dihydroxy-9:10-di-n-propyl-9:10-dihydro-1:2:5:6-dibenzanthracene the latter proved to be inhibitory and the former quite inactive.

5. These and other effects are discussed in relat-:ion to the mode of action of tumour-producing substances in general.

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VI. APPENDIX

Data for Experiments

1 and 3.

E X P E R I M E N T 1

Jensen sarcoma. Individual tumour measure-:ments (length x breadth x thickness in cm.) Injections from 10th day after implantation.

Days after implantation	Dibenzanthracene colloid daily			
	Rat 1	Rat 2	Rat 3	Rat 4
10	4.7	4.2	3.9	2.8
11	4.4	5.6	5.1	3.8
12	6.9	8.2	7.3	4.6
13	8.2	11.0	9.3	5.6
14	11.9	14.7	10.6	7.3
15	12.6	20.0	12.7	7.3
16	17.2	23.0	13.2	9.5
17	21.2	20.5	13.4	10.9
18	19.9	22.3	13.0	9.4
19	20.2	26.3	15.6	10.6
20	23.2	25.0	17.2	11.2
21	23.2	31.9	16.3	12.5
22	26.4	29.5	17.5	13.5
23	21.2	33.0	19.7	12.9
24	20.5	27.9	19.5	15.0

E X P E R I M E N T 1

Jensen sarcoma. Individual tumour measurements (length x breadth x thickness in cm.).

Days after implantation	Control			
	Rat 5	Rat 6	Rat 7	Rat 8
10	7.3	3.5	3.7	1.8
11	7.6	5.6	5.2	2.2
12	14.2	8.2	8.0	3.2
13	21.0	13.3	12.7	4.8
14	27.5	17.6	16.7	6.5
15	31.4	22.8	21.2	8.6
16	27.0	30.0	25.0	10.4
17	38.7	36.9	28.7	12.8
18	46.0	35.2	31.8	15.1
19	46.5	45.0	42.2	16.6
20	47.8	42.5	40.0	20.8
21	57.1	52.0	died	26.6
22	90.3	55.5	-	33.2
23	75.0	62.2	-	38.6
24	80.5	60.2	-	43.3

E X P E R I M E N T 3

Individual tumour dimensions (length x breadth in cm.)

Days after implantation	Group 1 --- Control untreated			
	Rat 1	Rat 2	Rat 3	Rat 4
5	2.5	1.1	4.0	3.0
6	4.0	2.1	4.9	4.2
7	4.6	2.7	7.9	5.4
8	5.4	5.4	9.9	5.6
9	5.0	8.1	11.0	8.4
10	5.4	7.3	10.8	7.6
11	6.8	8.1	12.6	9.4
12	7.2	7.8	13.8	10.1
13	7.6	7.8	13.0	11.0
14	8.0	8.6	15.6	11.2
15	8.7	9.6	16.0	14.6
16	9.7	11.1	16.6	12.2
17	10.4	11.4	19.0	13.9
18	11.2	12.5	21.2	18.1
21	16.2	17.0	24.6	24.1
23	21.6	21.6	23.2	29.2
25	26.2	22.8	26.6	32.0
29	35.0	33.2	30.6	30.8
31	39.5	32.8	27.2	35.6
35	43.0	33.6	34.2	died
44	died	36.0	died	-

E X P E R I M E N T 3

Individual tumour dimensions (length x breadth in cm.)

Days after implantation	Group 2 --- Control --- 5 c.c. oil					
	Rat 5	Rat 6	Rat 7	Rat 8	Rat 9	Rat 10
5	2.0	3.8	2.6	3.8	4.3	4.3
6	2.6	4.9	2.6	4.0	5.3	5.6
7	3.9	6.6	3.4	4.2	6.9	8.1
8	5.0	6.6	4.4	4.8	8.7	8.1
9	5.9	8.5	4.9	6.5	10.8	13.0
10	6.6	9.6	5.9	7.3	13.3	12.4
11	7.3	10.4	5.9	8.0	12.7	13.9
12	8.2	9.0	6.3	9.6	12.5	10.4
13	8.6	12.7	6.8	7.7	15.2	13.0
14	10.3	12.3	8.7	8.4	16.5	16.6
15	10.6	12.0	10.5	7.9	19.2	18.0
16	11.4	14.7	11.3	9.0	17.8	16.3
17	13.2	17.0	11.2	9.2	19.9	18.6
18	15.7	18.3	13.9	10.2	21.4	19.5
21	19.2	19.0	17.3	14.3	24.3	26.2
23	26.3	26.9	19.0	14.9	26.6	32.0
25	died	28.5	19.7	16.6	30.0	30.1
29	-	26.9	28.1	19.6	died	died
31	-	32.8	30.0	17.7	-	-
35	-	31.1	29.6	17.0	-	-
44	-	26.2	died	died	-	-

E X P E R I M E N T 3

Individual tumour dimensions (length x breadth in cm.)

Days after implantation	Group 3 - 25 mg. dibenzanthracene. Rat11	Rat12	Rat13	Rat14	Rat15	Rat16
5	2.0	3.1	2.3	2.0	3.9	3.2
6	2.9	6.4	3.3	2.8	5.7	4.2
7	3.5	8.0	4.0	3.3	7.0	6.9
8	2.8	9.9	4.4	5.5	8.4	6.0
9	3.9	11.1	5.9	6.3	10.5	7.0
10	4.2	12.7	5.4	5.8	10.6	7.6
11	4.2	12.7	5.1	7.0	10.7	7.4
12	3.7	12.4	5.4	6.9	10.5	7.3
13	3.8	11.3	4.9	5.4	11.0	6.6
14	3.8	10.4	5.4	5.5	9.6	7.1
15	3.9	9.2	5.6	5.6	8.1	6.4
16	4.0	9.2	5.6	5.6	9.4	5.6
17	4.0	10.2	5.6	5.7	9.7	5.9
18	5.0	9.7	6.0	4.9	9.7	6.6
21	5.1	8.6	7.4	6.5	9.0	6.4
23	5.2	9.8	7.7	5.7	8.6	8.0
25	3.8	9.5	6.7	5.1	8.8	7.2
31	3.6	9.0	6.6	7.4	9.5	8.4
35	2.9	9.5	8.8	8.1	10.6	7.8
44	3.2	8.4 died	8.2	10.6	9.0	10.1

The influence of certain carcinogenic and other hydrocarbons on body-growth in the rat

I. INTRODUCTORY.

In the course of this work on the mode of action of tumour-producing agents it was found, as already described, that certain of the carcinogenic hydrocarbons studied at the Research Institute of the Royal Cancer Hospital (Cook et al., 1932; Cook 1932; Barry et al., 1935) possessed growth-inhibiting properties (Haddow 1935; Haddow and Robinson 1937). This was demonstrated by recording the effect of these substances, administered by intraperitoneal injection, on the behaviour of transplantable tumours growing subcutaneously in the rat. During these experiments it was observed, as already noted, that treated animals appeared to grow less rapidly as compared with their controls. Because of this it was felt that the reduction in tumour growth-rate was probably not a tumour-specific effect but rather the result of an inhibition of growth affecting the body as a whole. Hence further experiments were carried out to determine the action of these and other substances on the body-growth of rats.

II. EXPERIMENTAL.

(1) Animals.

The animals used were from an inbred colony of hooded rats of the Lister strain, maintained under standard conditions of housing and feeding. The diet was one of bread and milk (alternating with biscuit and milk) with additions of cod-liver oil and marmite; fresh greens, whole oats and water were also supplied daily. The room-temperature was kept at 65-70 degs. F.

(2) General design of experiments.

Rats to be used for a given experiment were obtained from several litters, (usually four but occasionally five) born within a space of 48-72 hours; when about 55 days of age they were ear-marked and weighed individually each day for at least a week. This procedure indicated the rate of increase and its variation, and also enabled the animals to adjust themselves to handling. At the end of this prelim-:inary control period they were arranged in groups, special care being taken to ensure that each of these contained, as far as possible, an equal share from every litter and the same proportion of males and females. The following data show that the groups agreed closely not only in composition and sex ratio but also in their initial mean weight and rate of

growth. The majority of the experiments contained three such balanced groups so that two substances might be tested simultaneously with a common control.

The rats in each set were then injected with the appropriate compound, usually in a single dose but occasionally in two, while the controls received an equal volume of the solvent alone. The individual litters were reconstituted immediately after injection and kept in separate cages to the end of the experiment. Individual daily weighings were then continued for as long a period as was thought to be necessary.

(3) Technique of administration.

All the hydrocarbons and related substances were dissolved in sesame oil, in the hot air oven, in concentrations of 0.5 or 1.0 per cent. The resultant sterile solutions were injected intra-:peritoneally. Other substances (vide infra) were dissolved in suitable concentration in sterile water or saline and administered by the same route.

(4) Recording of data.

A complete record was kept of the individual data from day to day, and graphs were also constructed to indicate the rate of increase of each group as a whole. For the latter purpose it was found necessary to apply corrections in certain cases in respect of (a) the death of animals, and (b) the occurrence of pregnancy. These adjustments were in no case serious and they are indicated where required in the tables which follow. It should be pointed out that the animals used were of such an age as to enable conclusive results to be obtained before the first pregnancies occurred. In some experiments, however, the observations were continued well into the period of sexual maturity, and in these cases the data for males alone were employed.

The mean figures at certain selected periods of different experiments are presented in tabular form, while the individual data on which these are based are given in the Appendix. The whole course of each experiment is also shown in graphs (Figs. 16-26).

A. Hydrocarbons and related substances.

Experiment 36. 1:2:5:6-dibenzanthracene; pyrene.

The first experiment was planned to determine the action on normal growth of a carcinogenic hydrocarbon (1:2:5:6-dibenzanthracene) and a non-carcinogenic compound (pyrene). These were selected as representative of substances which had already been studied in the work described above, when 1:2:5:6-dibenzanthracene proved to be inhibitory (as tested on the growth of the Jensen and Walker tumours) and pyrene to be quite inactive in this respect.

Forty rats (46-48 days old) were used from four litters of 12, 11, 10 and 8. After a preliminary control period they were divided into three groups as follows. Groups I and II were composed of four animals from each of two litters and three from each of the remaining two, while Group III contained two animals from one litter, four from another, and three from each of the remaining two. The groups were also adjusted so as to contain comparable numbers of males and females.

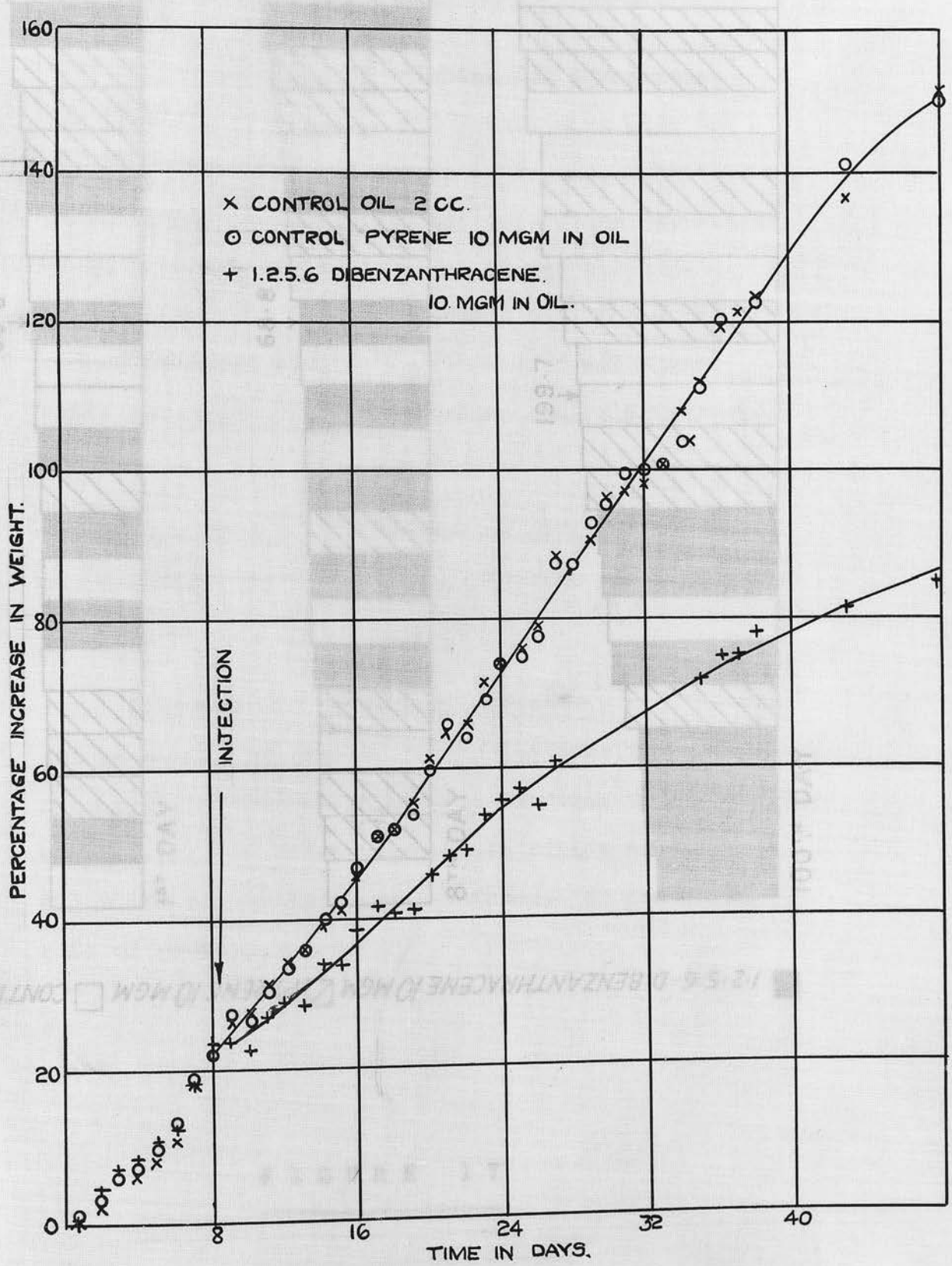
On the 8th day the controls each received 2 c.c. sterile sesame oil; those in the second group 10 mg. 1:2:5:6-dibenzanthracene, and those in the remaining group 10 mg. pyrene, in 2 c.c. oil.

Table XXXVII summarises the position at the 43rd day, Fig. 16 indicates the respective growth-rates in the three groups to the 48th day, and Fig. 17 shows the relative distribution of individual weights at the 1st., 8th. and 100th. days in the male animals surviving thus far.

Pyrene under these conditions was totally without influence on the body-growth, while the same dose of 1:2:5:6-dibenzanthracene produced an immediate and prolonged retardation in the growth-rate amounting to 50 per cent. of the normal and continuing, with no indication of recovery, at least for fifteen weeks.

In view of this result it was decided to study the influence of varying doses of 1:2:5:6-dibenz-:anthracene.

FIGURE 16


× CONTROL OIL 2 CC.
⊙ CONTROL PYRENE 10 MGM IN OIL
+ 1.2.5.6 DIBENZANTHRACENE. 10 MGM IN OIL.
INJECTION
PERCENTAGE INCREASE IN WEIGHT.
TIME IN DAYS.
0
20
40
60
80
100
120
140
160
8
16
24
32
40

55·5
68·8
199·7
1ST DAY
8TH DAY
100TH DAY.
1:2:5:6-DIBENZANTHRACENE 10 MGM. PYRENE 10 MGM. CONTROL

FIGURE 17

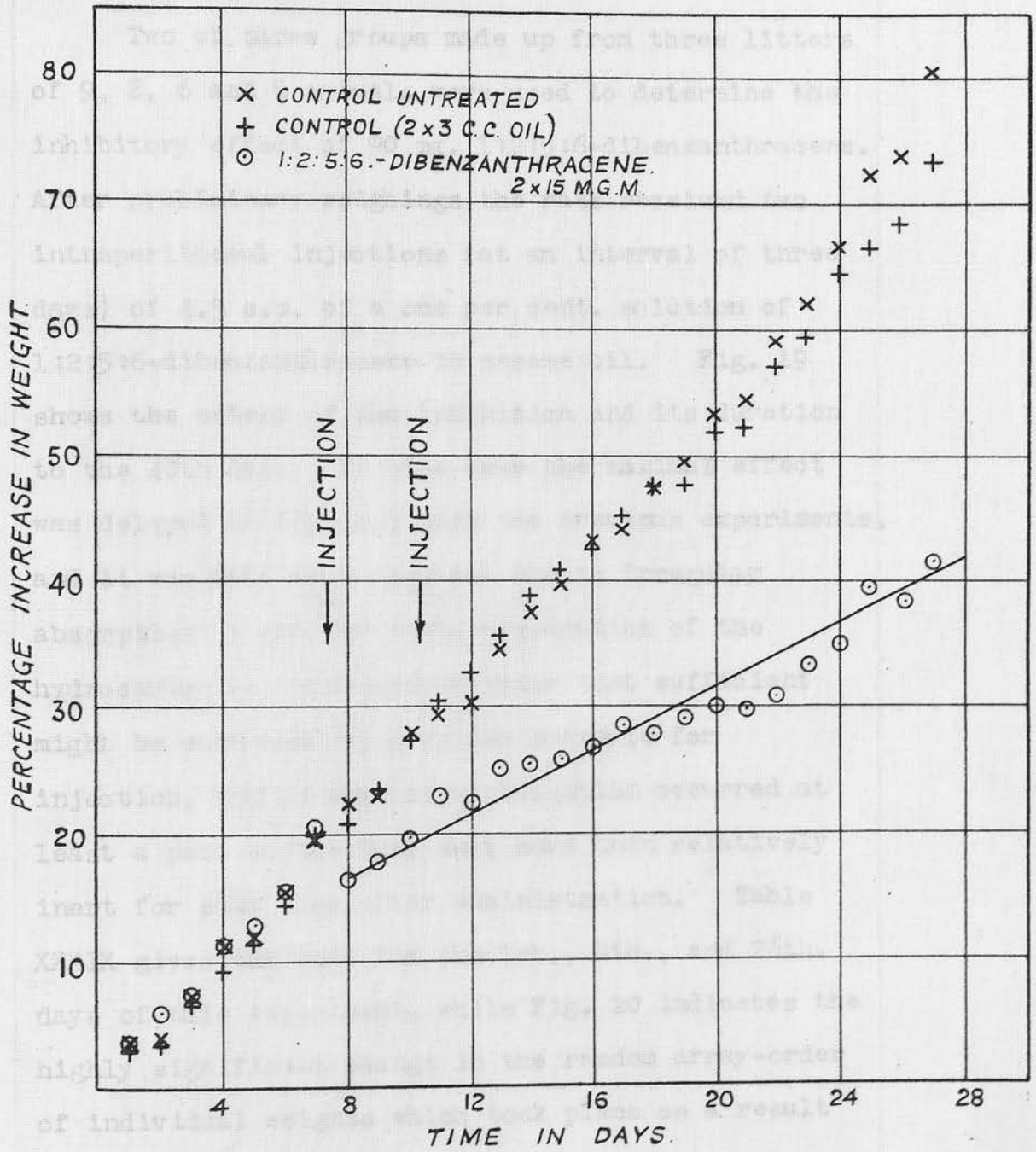
Experiment 37. 1:2:5:6-dibenzanthracene.

After preliminary weighing for seven days, four litters of 12, 9, 7 and 7 rats were used to provide three balanced groups as before. Animals in one group then received two injections (separated by an interval of two days) of 15 mg. 1:2:5:6-dibenzanthracene in 3 c.c. sesame oil; those in the second received similar injections of oil alone; while the third group remained an entirely untreated control.

Fig. 18 shows the course of events to the 26th day and Table XXXVIII the data for the male animals to the 38th day.

Although the inhibitory effect was not increas-:ed in simple proportion to the increase in dose of hydrocarbon, the result clearly confirms the first experiment, and shows that the injection of sesame oil alone was quite without influence on the normal rate of growth.

FIGURE 18

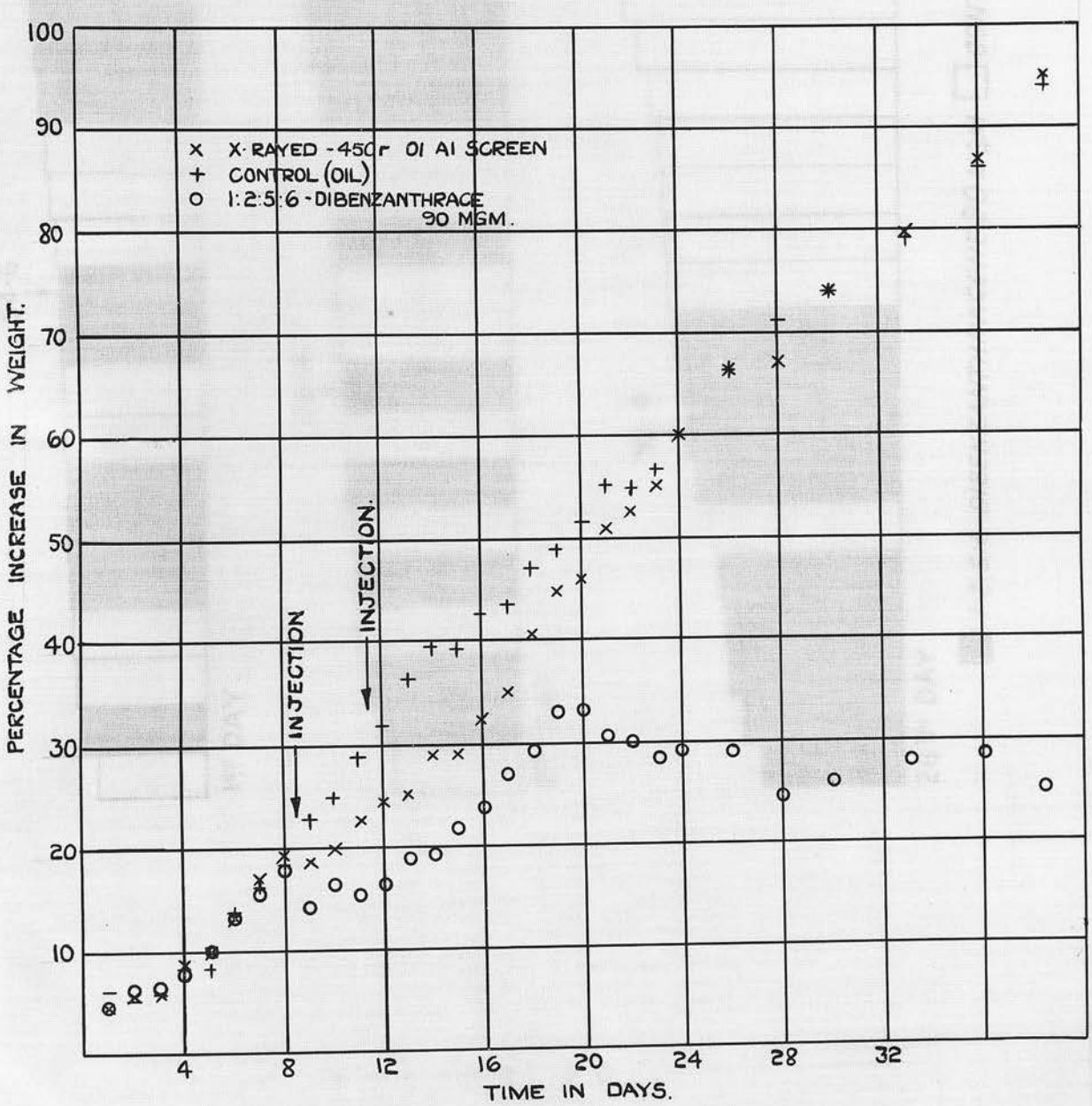

× CONTROL UNTREATED
+ CONTROL (2×3 C.C. OIL)
⊙ 1:2:5:6.-DIBENZANTHRACENE. 2×15 M.G.M.
INJECTION
INJECTION
PERCENTAGE INCREASE IN WEIGHT.
TIME IN DAYS.
80
70
60
50
40
30
20
10
4
8
12
16
20
24
28

Experiment 38 (part i).

1:2:5:6-dibenzanthracene.

Two of three groups made up from three litters of 9, 8, 6 and 5 animals were used to determine the inhibitory effect of 90 mg. 1:2:5:6-dibenzanthracene. After preliminary weighings the rats received two intraperitoneal injections (at an interval of three days) of 4.5 c.c. of a one per cent. solution of 1:2:5:6-dibenzanthracene in sesame oil. Fig. 19 shows the extent of the inhibition and its duration to the 40th day. In this case the maximal effect was delayed as compared with the previous experiments, and it was felt that this was due to irregular absorption: a one per cent. preparation of the hydrocarbon was employed in order that sufficient might be contained in a volume suitable for injection, and as some crystallisation occurred at least a part of the dose must have been relatively inert for some time after administration. Table XXXIX gives the data for the 1st., 8th., and 26th. days of this experiment, while Fig. 20 indicates the highly significant change in the random array-order of individual weights which took place as a result of the treatment.

FIGURE 19


X X-RAYED -450r 01 Al SCREEN
+ CONTROL (OIL)
O 1:2:5:6-DIBENZANTHRACE 90 MGM.
INJECTION
INJECTION.
PERCENTAGE INCREASE IN WEIGHT.
TIME IN DAYS.
100
90
80
70
60
50
40
30
20
10
4
8
12
16
20
24
28
32

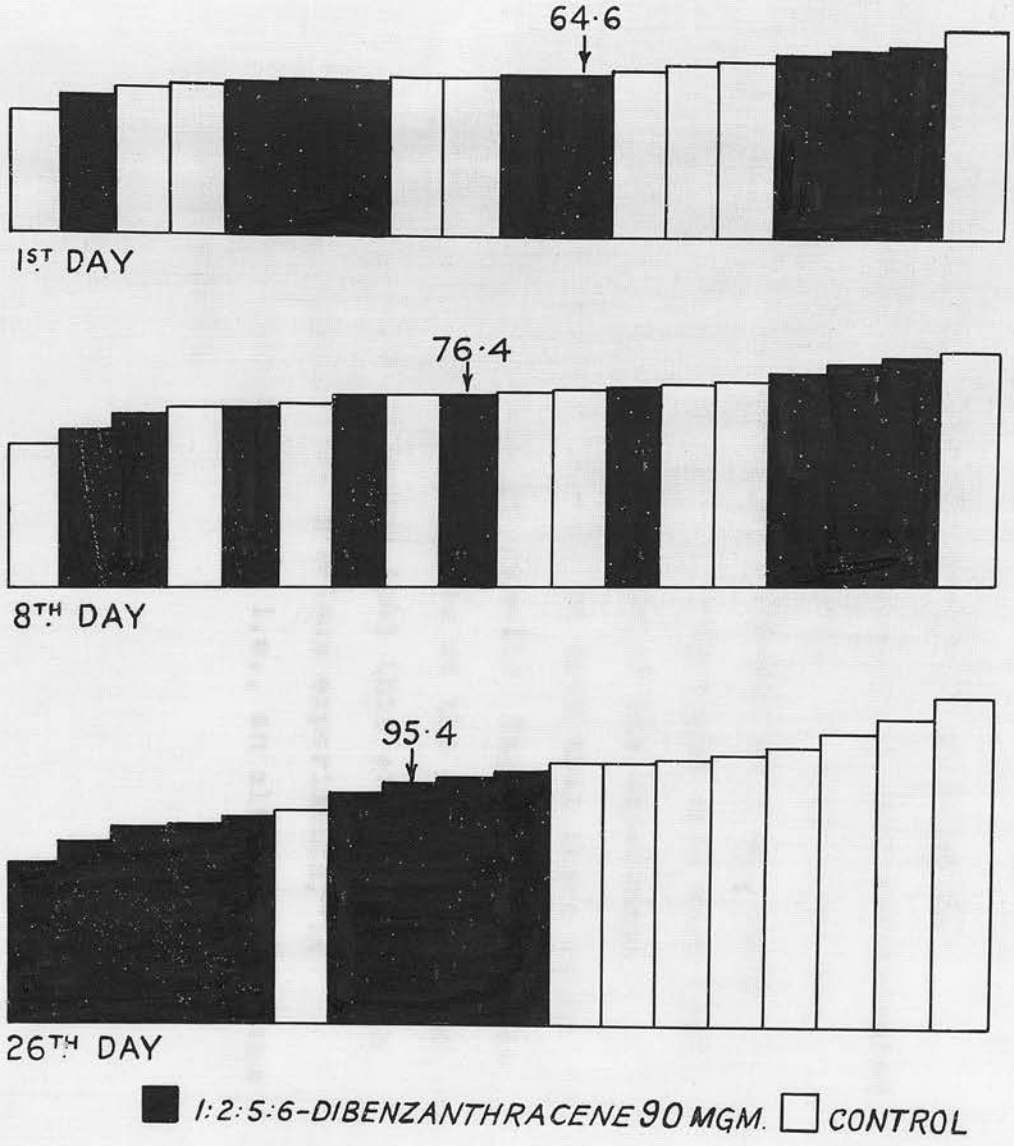
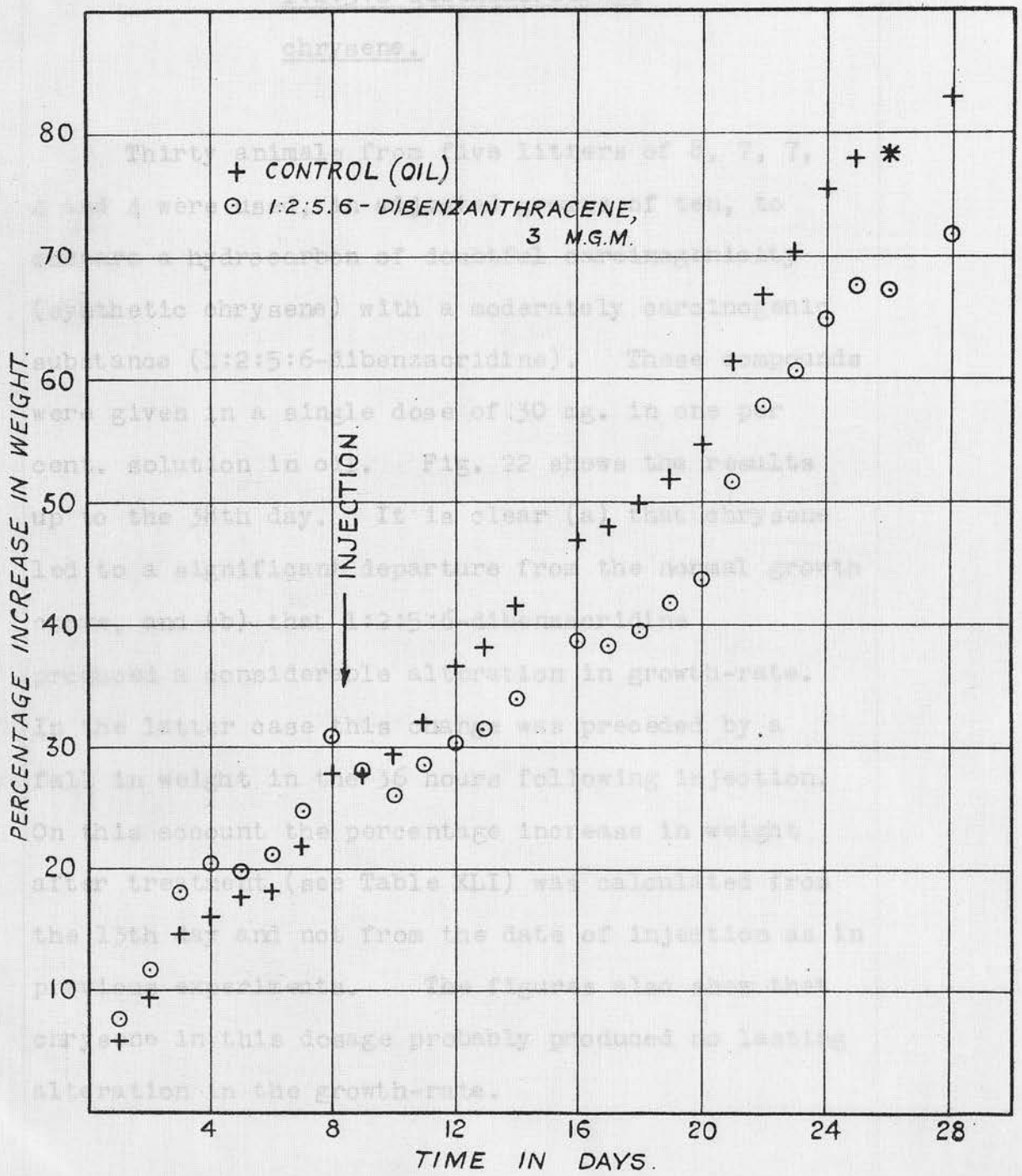

64·6
1ST DAY
76·4
8TH DAY
95·4
26TH DAY
1:2:5:6-DIBENZANTHRACENE 90 MGM.
CONTROL

FIGURE 20

Experiment 39. 1:2:5:6-dibenzanthracene.

When the inhibiting effect of 1:2:5:6-dibenzanthracene on body growth had been demonstrated for doses of 10, 30 and 90 mg., an experiment was carried out to test the influence of 3 mg. under the same conditions. Twenty-eight rats were used and Fig. 21 shows the course of the experiment during a month. It will be seen that there is an undoubtedly significant result: Table XL gives the details for the male animals at the 1st., 8th., and 34th. days, and it seems that this effect, although much less than in the previous experiments, is probably of the same type, i.e., an alteration in the rate of growth.

FIGURE 21


+ CONTROL (OIL)
⊙ 1:2:5.6 - DIBENZANTHRACENE, 3 M.G.M.
INJECTION
PERCENTAGE INCREASE IN WEIGHT.
TIME IN DAYS.
10
20
30
40
50
60
70
80
4
8
12
16
20
24
28

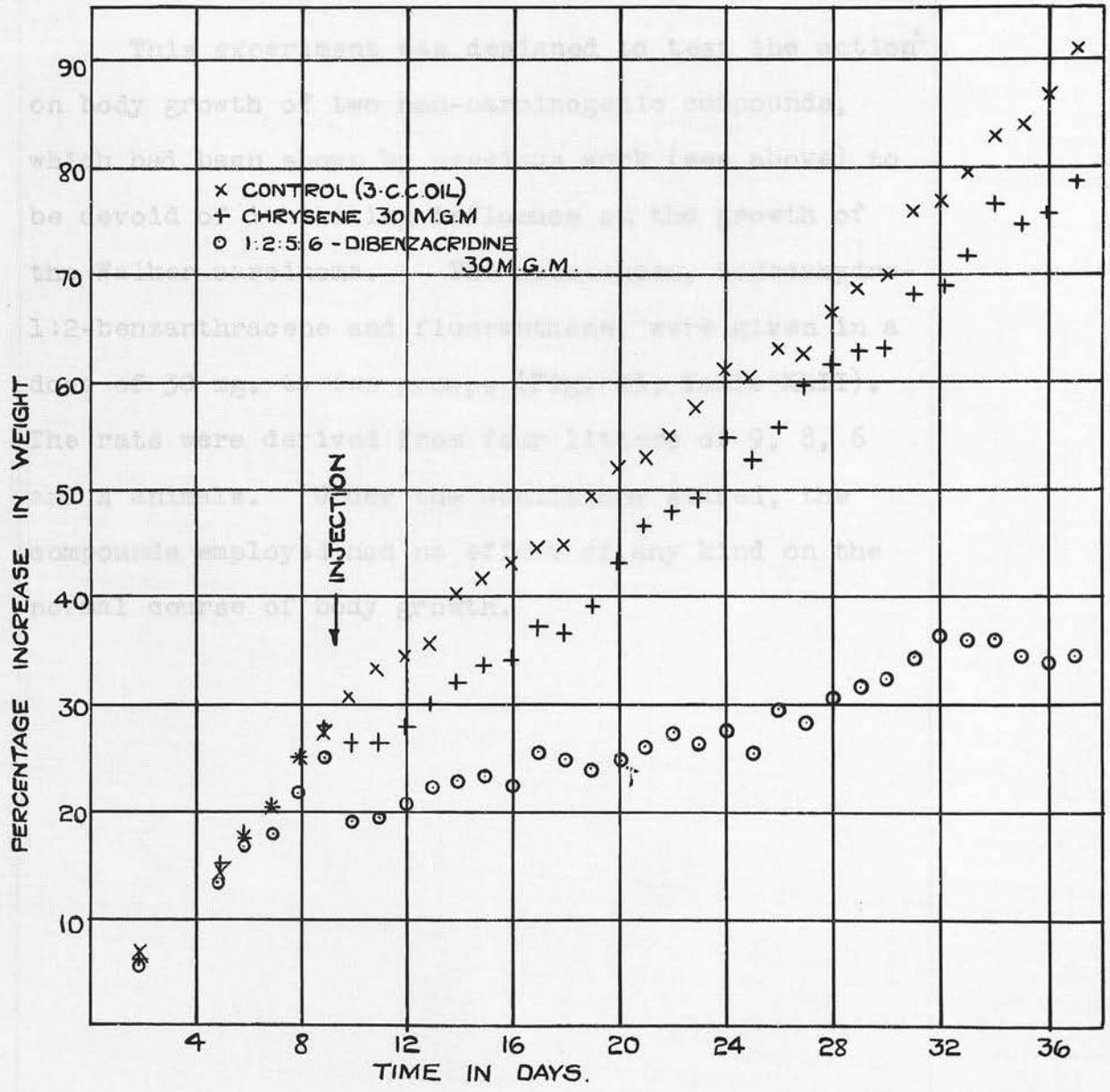
Experiment 40.

1:2:5:6-dibenzacridine;

chrysene.

Thirty animals from five litters of 8, 7, 7, 4 and 4 were used, in adjusted groups of ten, to compare a hydrocarbon of doubtful carcinogenicity (synthetic chrysene) with a moderately carcinogenic substance (1:2:5:6-dibenzacridine). These compounds were given in a single dose of 30 mg. in one per cent. solution in oil. Fig. 22 shows the results up to the 38th day. It is clear (a) that chrysene led to a significant departure from the normal growth curve, and (b) that 1:2:5:6-dibenzacridine produced a considerable alteration in growth-rate. In the latter case this change was preceded by a fall in weight in the 36 hours following injection. On this account the percentage increase in weight after treatment (see Table XLI) was calculated from the 13th day and not from the date of injection as in previous experiments. The figures also show that chrysene in this dosage probably produced no lasting alteration in the growth-rate.

FIGURE 22

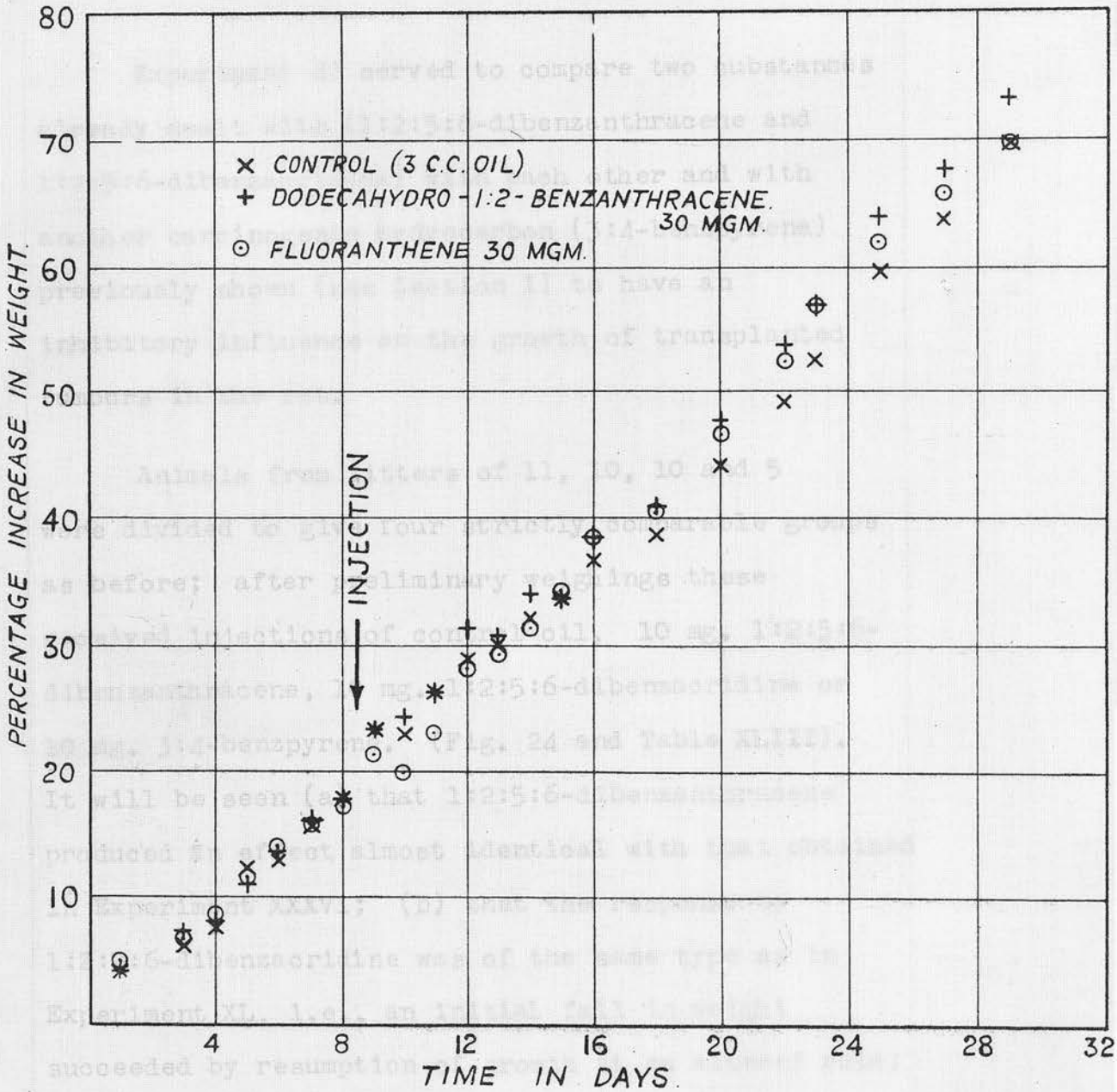

PERCENTAGE INCREASE IN WEIGHT
TIME IN DAYS.
× CONTROL (3·C.C.OIL)
+ CHRYSENE 30 M.G.M
o 1:2:5:6 - DIBENZACRIDINE 30 M.G.M.
INJECTION
10
20
30
40
50
60
70
80
90
4
8
12
16
20
24
28
32
36

Experiment 41.

Dodecahydro-1:2-benzanthracene; fluoranthene.

This experiment was designed to test the action on body growth of two non-carcinogenic compounds, which had been shown by previous work (see above) to be devoid of inhibiting influence on the growth of the Walker carcinoma. The substances, dodecahydro-1:2-benzanthracene and fluoranthene, were given in a dose of 30 mg. to two groups (Fig. 23, Table XLII). The rats were derived from four litters of 9, 8, 6 and 4 animals. Under the conditions stated, the compounds employed had no effect of any kind on the normal course of body growth.

FIGURE 23.

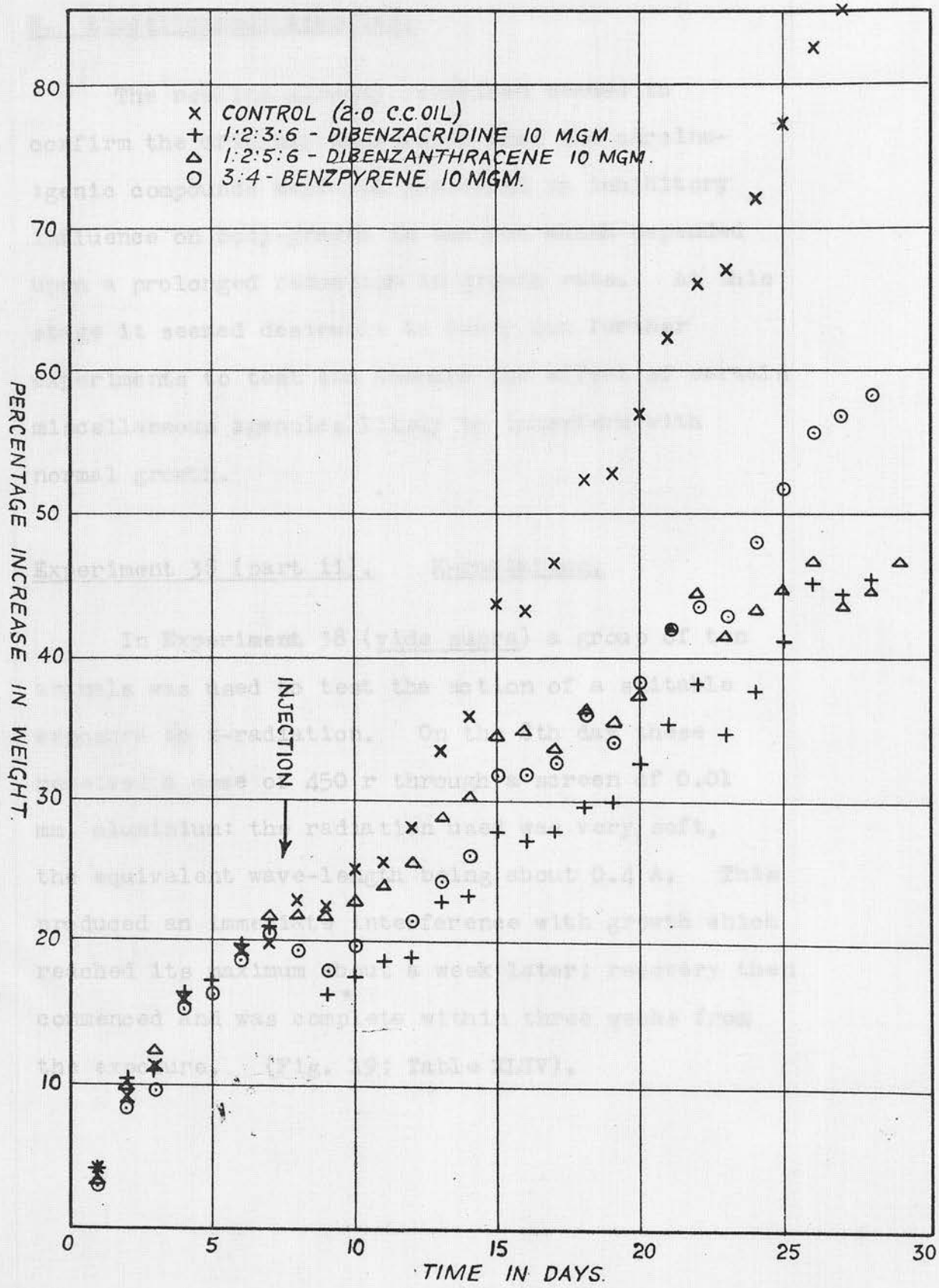

80
70
60
50
40
30
20
10
PERCENTAGE INCREASE IN WEIGHT.
× CONTROL (3 C.C. OIL)
+ DODECAHYDRO-1:2-BENZANTHRACENE. 30 MGM.
⊙ FLUORANTHENE 30 MGM.
INJECTION
4
8
12
16
20
24
28
32
TIME IN DAYS.

Experiment 42. 1:2:5:6-dibenzanthracene;
1:2:5:6-dibenzacridine;
3:4-benzpyrene.

Experiment 42 served to compare two substances already dealt with (1:2:5:6-dibenzanthracene and 1:2:5:6-dibenzacridine) with each other and with another carcinogenic hydrocarbon (3:4-benzpyrene) previously shown (see Section I) to have an inhibitory influence on the growth of transplanted tumours in the rat.

Animals from litters of 11, 10, 10 and 5 were divided to give four strictly comparable groups as before; after preliminary weighings these received injections of control oil, 10 mg. 1:2:5:6-dibenzanthracene, 10 mg. 1:2:5:6-dibenzacridine or 10 mg. 3:4-benzpyrene. (Fig. 24 and Table XLIII). It will be seen (a) that 1:2:5:6-dibenzanthracene produced an effect almost identical with that obtained in Experiment XXXVI; (b) that the response to 1:2:5:6-dibenzacridine was of the same type as in Experiment XL, i.e., an initial fall in weight succeeded by resumption of growth at an altered rate; and (c) that while 3:4-benzpyrene produced the same initial effect as 1:2:5:6-dibenzacridine, the subsequent inhibition of growth was less than in the case of 1:2:5:6-dibenzacridine or 1:2:5:6-dibenz-:anthracene.

FIGURE 24.


× CONTROL (2·0 C.C. OIL)
+ 1:2:3:6 - DIBENZACRIDINE 10 M.GM.
△ 1:2:5:6 - DIBENZANTHRACENE 10 MGM.
○ 3:4 - BENZPYRENE 10 MGM.
INJECTION
PERCENTAGE INCREASE IN WEIGHT.
TIME IN DAYS.
0
5
10
15
20
25
30
10
20
30
40
50
60
70
80

B. Miscellaneous agencies.

The results already described seemed to confirm the original impression that the carcino-:genic compounds employed possessed an inhibitory influence on body-growth in the rat which depended upon a prolonged reduction in growth rate. At this stage it seemed desirable to carry out further experiments to test and compare the effect of certain miscellaneous agencies likely to interfere with normal growth.

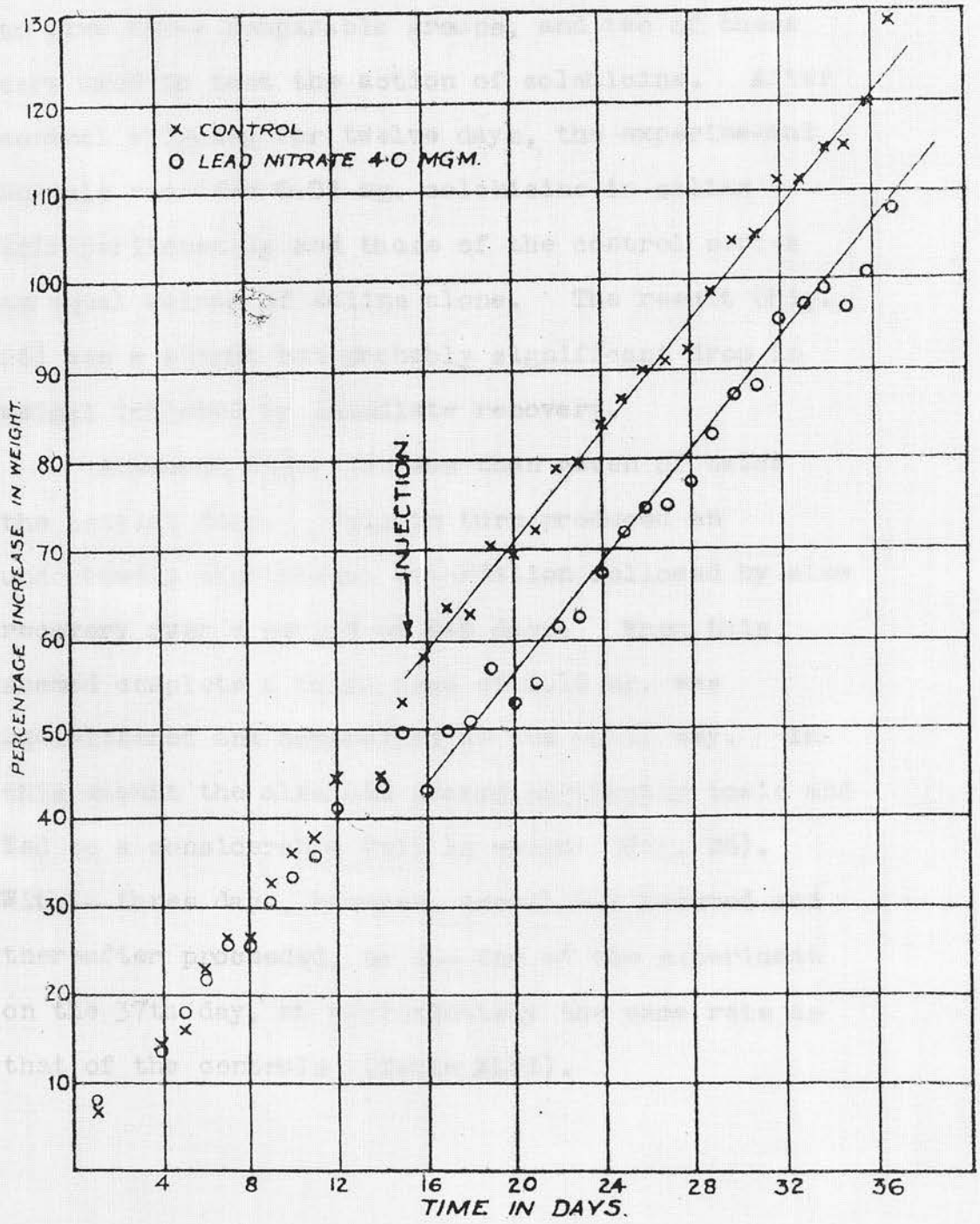
Experiment 38 (part ii). X-radiation.

In Experiment 38 (vide supra) a group of ten animals was used to test the action of a suitable exposure to x-radiation. On the 8th day these received a dose of 450 r through a screen of 0.01 mm. aluminium: the radiation used was very soft, the equivalent wave-length being about 0.4 A. This produced an immediate interference with growth which reached its maximum about a week later; recovery then commenced and was complete within three weeks from the exposure. (Fig. 19; Table XLIV).

Experiment 43. Lead nitrate.

Twenty-six animals from four litters were arranged in two groups. In this case the preliminary weighings were continued to the 15th. day. Each rat then received an intraperitoneal injection of 4 mg. lead nitrate in water while the others received a corresponding volume of the solvent alone (Fig. 25). The administration of lead produced an immediate retardation which was soon followed by resumed growth at a rate identical with that of the controls (Table XLV).

FIGURE 25.


X CONTROL
O LEAD NITRATE 4·0 MGM.
INJECTION.
PERCENTAGE INCREASE IN WEIGHT.
TIME IN DAYS.
130
120
110
100
90
80
70
60
50
40
30
20
10
4
8
12
16
20
24
28
32
36

Experiment 44 (part i). Colchicine.

Four litters of 10, 9, 8 and 6 animals served to give three comparable groups, and two of these were used to test the action of colchicine. After control weighing for twelve days, the experimental animals received 0.03 mg. colchicine in saline intraperitoneally and those of the control series an equal volume of saline alone. The result (Fig. 26) was a slight but probably significant drop in weight followed by immediate recovery.

A second injection was then given of twice the initial dose. This in turn produced an undoubtedly significant retardation followed by slow recovery over a period of 5-6 days. When this seemed complete a third dose of 0.12 mg. was administered and controlled in the usual way. In this amount the alkaloid proved manifestly toxic and led to a considerable fall in weight (Fig. 26). Within three days, however, growth was resumed and thereafter proceeded, to the end of the experiment on the 37th day, at approximately the same rate as that of the controls (Table XLVI).

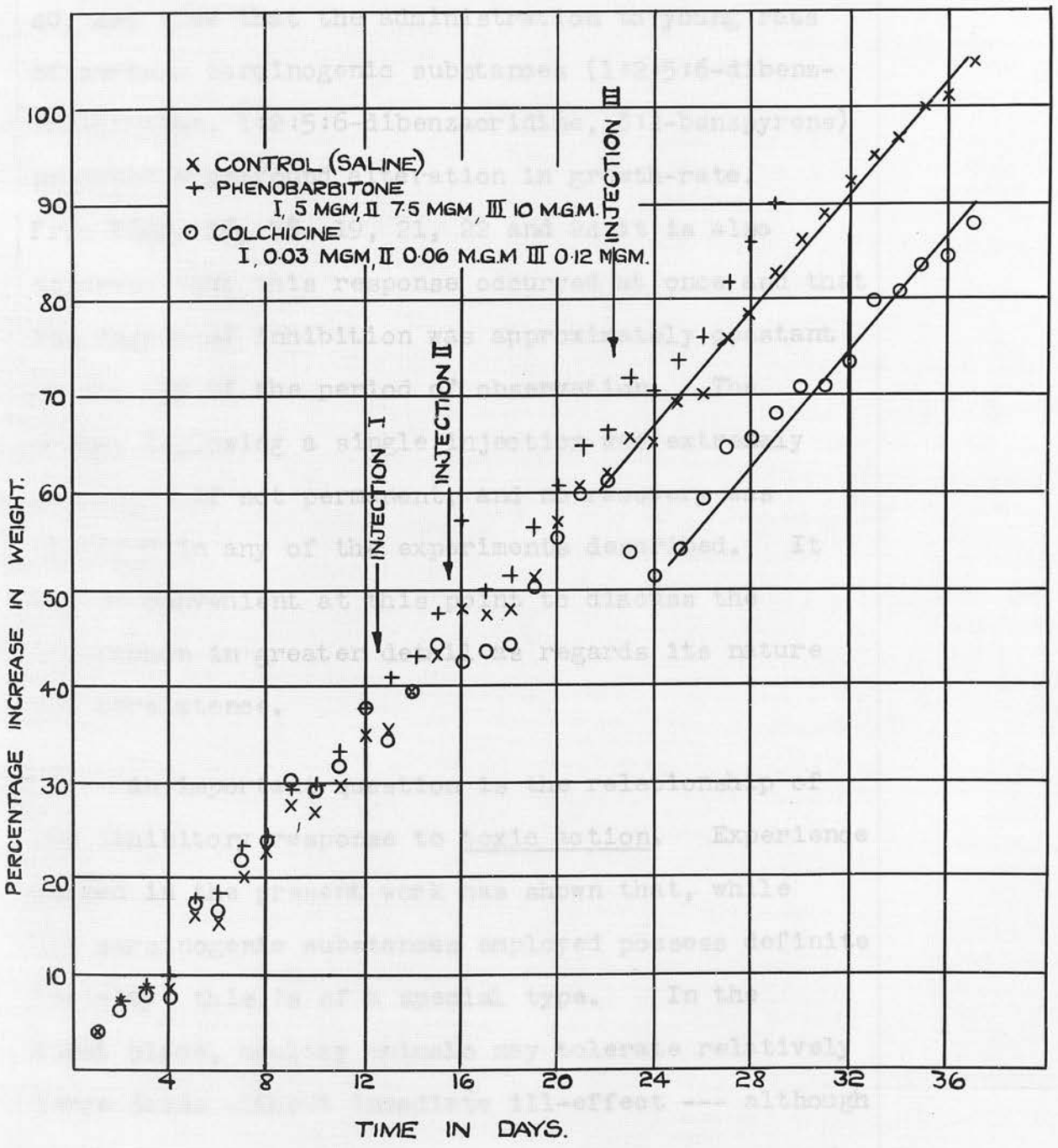
Experiment 44. (part ii).

Phenobarbitone.

A third group was included in Experiment 44 in order to determine the influence on growth of phenobarbitone, using the same controls. Three successive injections were given of 5.0, 7.5 and 10.0 mg., dissolved in saline, on the same dates as the injections of colchicine in part i of the experiment (above).

Although these doses were sufficient to produce incoordination and anaesthesia, it is clear from Fig. 26 and Table XLVII that they had at least no inhibitory influence on growth.

FIGURE 26.


× CONTROL (SALINE)
+ PHENOBARBITONE
I, 5 MGM, II 7·5 MGM, III 10 M.G.M.
⊙ COLCHICINE
I, 0·03 MGM, II 0·06 M.G.M III 0·12 MGM.
INJECTION I
INJECTION II
INJECTION III
PERCENTAGE INCREASE IN WEIGHT.
TIME IN DAYS.
10
20
30
40
50
60
70
80
90
100
4
8
12
16
20
24
28
32
36

III. D I S C U S S I O N.

A. The carcinogenic compounds.

The above results (Experiments 36, 37, 38, 39, 40, 42) show that the administration to young rats of certain carcinogenic substances (1:2:5:6-dibenz-:anthracene, 1:2:5:6-dibenzacridine, 3:4-benzpyrene) produced a profound alteration in growth-rate. From Figs. 16, 18, 19, 21, 22 and 24 it is also apparent that this response occurred at once and that the degree of inhibition was approximately constant to the end of the period of observation. The change following a single injection was extremely prolonged if not permanent, and no recovery was observed in any of the experiments described. It may be convenient at this point to discuss the phenomenon in greater detail as regards its nature and persistence.

An important question is the relationship of the inhibitory response to toxic action. Experience gained in the present work has shown that, while the carcinogenic substances employed possess definite toxicity, this is of a special type. In the first place, healthy animals may tolerate relatively large doses without immediate ill-effect --- although

an initial drop in weight may occur in certain cases --- and the organs of such animals need show no gross or microscopic evidence of damage. On the other hand, spermatogenesis and ovulation may be diminished (Haddow and Robinson 1937, and above, Section I), and clear evidence was obtained in the present work of a considerable lowering of both male and female fertility after administration of 1:2:5:6-dibenz-:anthracene. Again, the impression has been formed that these carcinogenic substances may prove indirectly harmful by lowering the bodily resistance to any existing source of infection; it is of interest that a pulmonary infection was the apparent cause of death in every animal --- control or treated --- which died in the course of these experiments. An indication of the number of deaths can be gained from Table XLIX in the Appendix: mortality was considerable higher in animals receiving carcino-:genic substances, (particularly 1:2:5:6-dibenz-:acridine), but in many cases death occurred only after a fair period following injection.

Since their direct toxic action appears slight in relation to dose it seems that the growth-inhibitory properties of the carcinogenic hydrocarbons, while not necessarily specific, are clearly not dependent on mere toxicity alone. This conclusion

is further supported by the fact that acknowledged poisons such as lead and colchicine produce an entirely different type of effect.

Considerable interest also attaches to the means by which the alteration in growth rate is prolonged, say for several months, following a single injection. The simplest possibilities are (a) that such substances acting on dividing cells produce a change of growth rate which persists after the agent itself has been removed: or (b) that the effect is due to the continuous action of the substance itself or of a derivative. The data available can be readily summarised, although at present they are insufficient to yield an answer to this important question.

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-dibenz-:anthracene were eliminated from chick embryo within a few days of the intramuscular injection in fatty

solution of fræ tions 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 three weeks, and the loss was more complete when the substance was dissolved in lard than when it was given as a colloidal solution in water.

The mode of action of these substances is certainly complex: Peacock (1936) stated that, during the elimination of 3:4-benzpyrene from the blood of fowls, rabbits and guinea-pigs, the bile showed fluorescence due not to benzpyrene itself but to a derivative or derivatives. Again, the urine of rabbits which had received colloidal benzpyrene, dibenzanthracene or anthracene intravenously was found to contain an ether-soluble fluorescent substance not normally present: this indicated that some water-soluble product might be re-absorbed from the gut and excreted by the kidney.

In the course of the present work Dr I. Hieger kindly examined several animals from Experiments 36, 37 and 39. It was found that rats which had received 30 mg. of 1:2:5:6-dibenzanthracene in oil retained some of the hydrocarbon after 40 days. On the other hand, animals which had been injected with doses of 10 mg. and 3 mg. showed no evidence of the presence of 1:2:5:6-dibenzanthracene after 105 and 37 days respectively, i.e., at a time when the inhibitory effect in these experiments was still maintained. The sensitivity of the method of assaying of the hydrocarbon is at present under investigation. Such results are suggestive although further work is obviously required.

B. Comparisons with other substances and agencies.

(1) Related non-carcinogenic compounds.

In contrast to the above-mentioned carcinogenic substances it is clear that the related non-carcino-:genic compounds studied were quite inactive. When taken together these results indicate a certain parallelism, in substances of this class, between carcinogenicity and the power to bring about a long-continued alteration in the rate of growth. In this respect the case of synthetic chrysene is of special interest as that of a substance in which carcinogen-:icity is doubtful or low and inhibiting potency correspondingly weak: it can be seen from Experiment 40 that 30 mg. chrysene gave a response rather less than that produced by only 3 mg. 1:2:5:6-dibenz-:anthracene.

On the other hand, although the carcinogenic activity of 3:4-benzpyrene is much greater than that of 1:2:5:6-dibenzanthracene, which in turn is more potent than 1:2:5:6-dibenzacridine (Barry et al., 1935), Experiment 42 shows that the inhibiting power of these substances is in the reverse order. This would suggest, if carcinogenicity and inhibiting power are intimately connected, that the former is

dependent on an optimal degree of inhibition of growth rather than a maximal, other possible factors being equal. It should also be borne in mind, however, that the carcinogenicity of such substances has mostly been studied in mice, the inhibitory influence exclusively in rats.

The growing rat, adequately controlled, appears to be an excellent test-object for the estimation of inhibitory power. The degree of accuracy attainable is shown by comparing two identical experiments carried out at an interval of about six months: whereas in Experiment 36 (after administration of 10 mg. 1:2:5:6-dibenzanthracene) the ratio "growth-rate of treated animals: growth-rate of controls" was as 458:934, i.e., 0.49, the same ratio for the control and dibenzanthracene animals in Experiment 42 was 208:475, i.e., 0.44. In this section it is not proposed to discuss the etiological connection which may possibly exist between carcinogenicity on one hand and inhibitory power on the other (see Sections I and III).

(2) Toxic agencies.

Experiment 38 (part ii) was included in order to study the retardation of growth produced by a single measured exposure to x-radiation. The growth-rate was temporarily lowered but soon recovered after passing through two periods during which it was successively normal and then greater than normal. No experiments have yet been carried out in this work to depress the growth-rate continuously by suitably spaced exposures.

Lead nitrate was investigated as an example of a heavy metal having toxic properties which often manifest themselves by producing interference with growth. The substance was of additional interest as one which is excreted relatively slowly. The dose given (approximately 50 mg./kilo.) was selected as likely to produce a detectable response without serious poisoning, and corresponded to about one-fifth of the amount found by Buck and Kumro (1930) to produce death after intraperitoneal administration. It has already been noted that the treatment merely resulted in a temporary setback and was entirely devoid of any lasting influence on the rate of growth, which became normal within a few days.

It was thought desirable to investigate the influence of the alkaloid colchicine as a substance possessing not only general toxicity but also special activity as a mitotic poison. The work of Lits (1934) Dustin (1934), Brues and Cohen (1936) and Ludford (1936) has shown beyond all doubt that colchicine produces an arrest of cell division in metaphase which persists for several hours after administration. This phenomenon --- the basis of the so-called caryoclastic crisis --- occurs both in vivo and in vitro, and is most obvious in tissues in which the mitosis rate is normally high, such as the glands of Lieberkuhn (Dustin 1934), the regenerating rat liver (Brues 1936) and various transplantable animal tumours.

The smallest amount employed in the present study (Experiment 44, part i) corresponded closely to the "minimum effective dose" (0.02 mg./100 g.) described by Brues and Cohen (1936) as the smallest dose which gave obvious abnormalities of mitosis in regenerating liver. As has already been noted above, the interference with growth produced by this amount was barely significant. When the dose was doubled a few days later, however, retardation was immediate and pronounced. This in turn was succeeded by recovery both to the original rate of

growth and the expected level of weight. The quantity of colchicine given in the third injection was identical with the "optimal dose" (0.1 mg./100 g.) of Brues and Cohen --- defined by these workers as the average amount giving a maximal number of abnormal mitoses --- and equal to twenty per cent. of the "average lethal dose". The result was a cess-:ation of growth lasting only for a few days and foll-:owed by resumption at the normal rate. It is important to note that this amount of colchicine caused death in three of eleven animals within 72 hours of injection.

The second part of Experiment 44 showed that the doses of phenobarbitone employed had certainly no inhibitory effect on growth. Such negative evidence is of obvious interest in relation to the types of growth-disturbance already described.

3. Retardation of growth produced by deprivation of vitamins and protein.

Osborne and Mendel (1914), in experiments in which prevention of growth was attained by a variety of dietary methods, showed that the capacity to grow remained unaffected. Later (1915) they quoted several remarkable instances of this phenomenon, some of which are collected in Table XLIX.

T A B L E XLIX

....(after Osborne and Mendel, 1915).

Capacity of albino rats to grow at a very late age after suppression of growth by dietary means.

Rat	Growth resumed at...		Final maximum body-wt.(g.)
	Age (days)	Body weight (g.)	
1	552	170	204
2	537	108	187
3	512	58	222
4	479	167	228
5	401	104	259

In this paper Osborne and Mendel wrote: "It should be noted in connection with the fore-going individuals that their curve of growth after the period of suppression was as a rule comparable with that of a growing rat of the same size and sex. The usual rate of body increment was not diminished, but, if anything, was sometimes somewhat accelerated during the resumption of the growth function." In a later paper (1916) they clearly recognised that growth following this type of repression often proceeded at an enormously exaggerated rate. Thus a female rat, on re-feeding after maintenance for some time without growth at a weight of about 100 g., gained 112 g. in 26 days. Again, another showed a gain of 150 g. in 36 days at a size which normally required more than 200 days for the same growth accomplishment.

More recently Clemmesen (1933) retarded the growth of young rats by feeding them on a diet deficient in vitamin-B. The treatment began at four weeks of age and extended for various periods up to 78 weeks. There was considerable mortality, but the survivors had apparently preserved their capacity for normal growth upon adequate re-feeding, so that ultimately the body-weight of the controls was equalled.

Guastalla and Rigoletti (1935) similarly retarded the growth of rats by underfeeding them from 45 to 110 days of age. Again there was high mortality, but the survivors upon subsequent re-feeding grew rapidly and overtook the controls after eight or nine months.

Jackson (1936) carried out extensive experiments on recovery in rats upon re-feeding after prolonged suppression of growth by dietary deficiency of protein. After being weaned at 3 weeks of age (when they weighed about 50 g.) albino rats were maintained at nearly constant body-weight for 15 weeks on a protein-deficient diet. On being re-fed with the normal stock diet the surviving animals grew at first even more rapidly than the standard for corresponding body-weight: at about 9 months of age the female test rats had overtaken the normal controls, although the males still tended to lag somewhat behind.

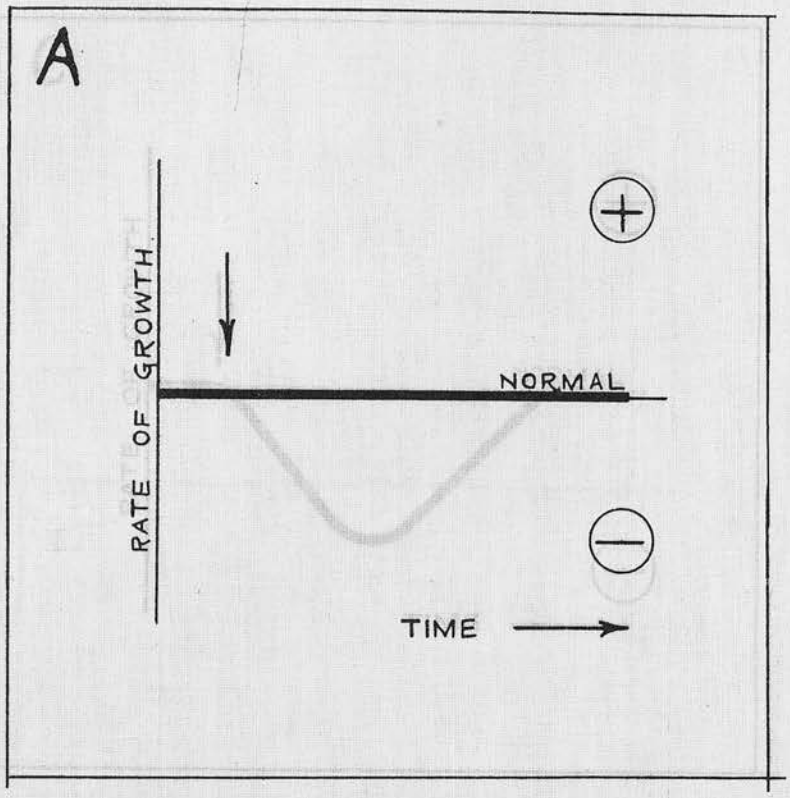
C. General principles.

The above experiments exemplify various fundamental responses which it is advantageous to describe and to classify in terms of rate of growth. Fig. 27, which is confined to reactions described or discussed in the present thesis, is a partly schematic representation along these lines. It would appear that increasing doses of certain substances, of which colchicine is a good example, produce in turn (a) no apparent response (after suitably small dosage); (b) a triphasic response (after medium doses) in which the growth rate successively falls below the normal level, compensates by rising above the normal, and finally subsides to bring about total recovery; and (c) a diphasic response (after larger doses) in which the growth rate is first depressed and then returns to normal without a period of increase above that level.

The outstanding feature in the experiments with x-radiation, lead or colchicine is the uniform tendency to recovery to the original growth rate after a single application or exposure, even in cases where the latter resulted in signs or symptoms of general toxicity. On the other hand, the response to a single injection of the carcinogenic hydrocarbons employed presents a striking difference.

In the case of 1:2:5:6-dibenzanthracene the reaction is strictly monophasic, taking the form of an abrupt fall in growth rate to a level which remains constant for long periods at least. The signific-:ance of this result is of course further increased by contrast with the inactivity of the non-carcino-:genic compounds tested under the same conditions. 1:2:5:6-dibenzacridine is of additional interest as appearing to possess greater inherent toxicity, (as compared with 1:2:5:6-dibenzanthracene), so that the change in growth rate immediately after administration is complicated by a superimposed fall from which recovery soon takes place to the new subnormal level.

For comparative purposes Fig. 27 also indicates (a) the fall in growth rate produced by continued deprivation of protein or vitamin, and (b) the rapid recovery --- with or without compensation --- which follows the complete restoration of these substances to the diet.



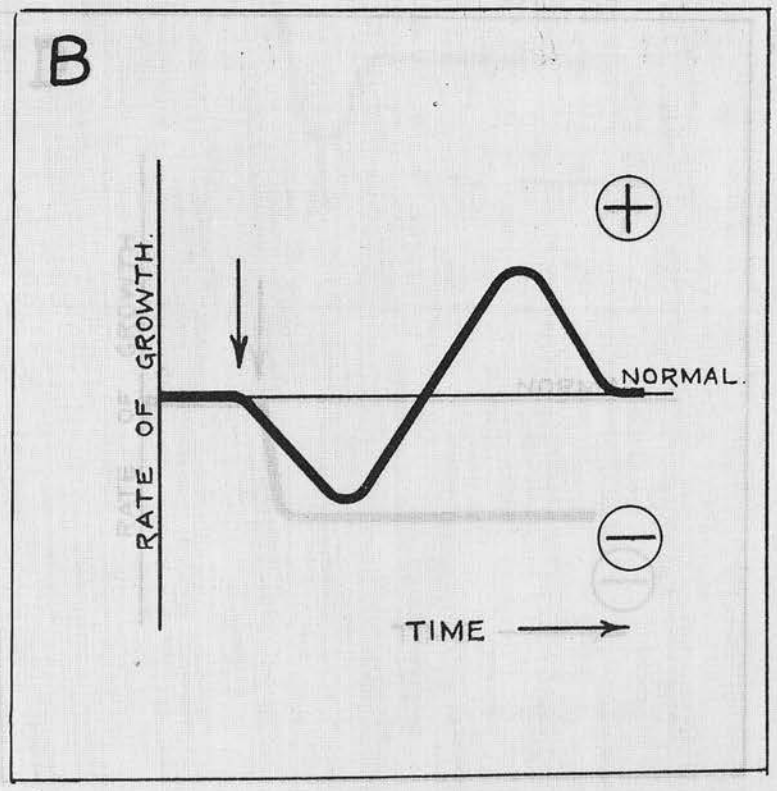
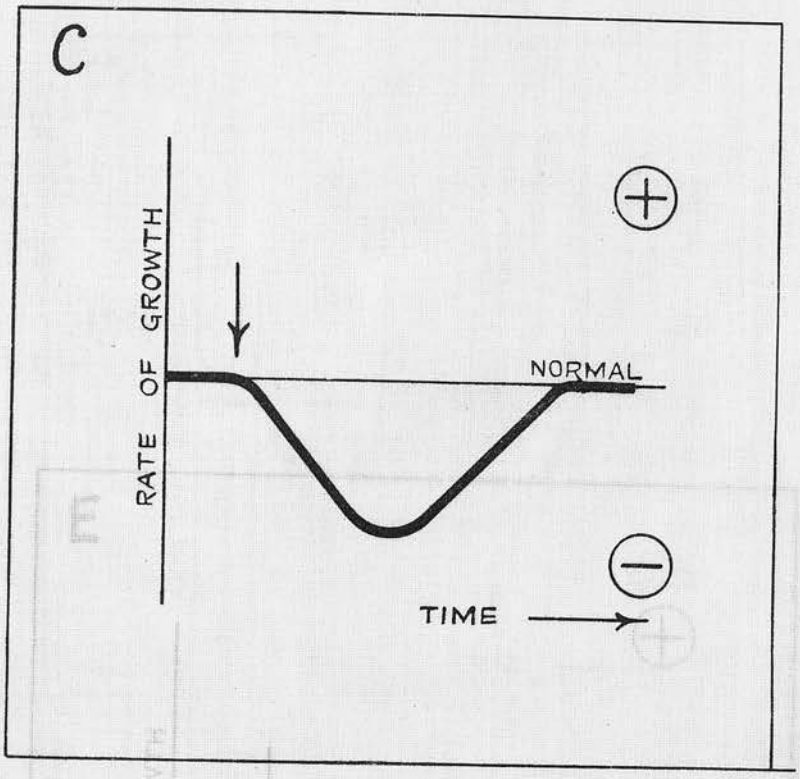


Fig. 27.....continued over.



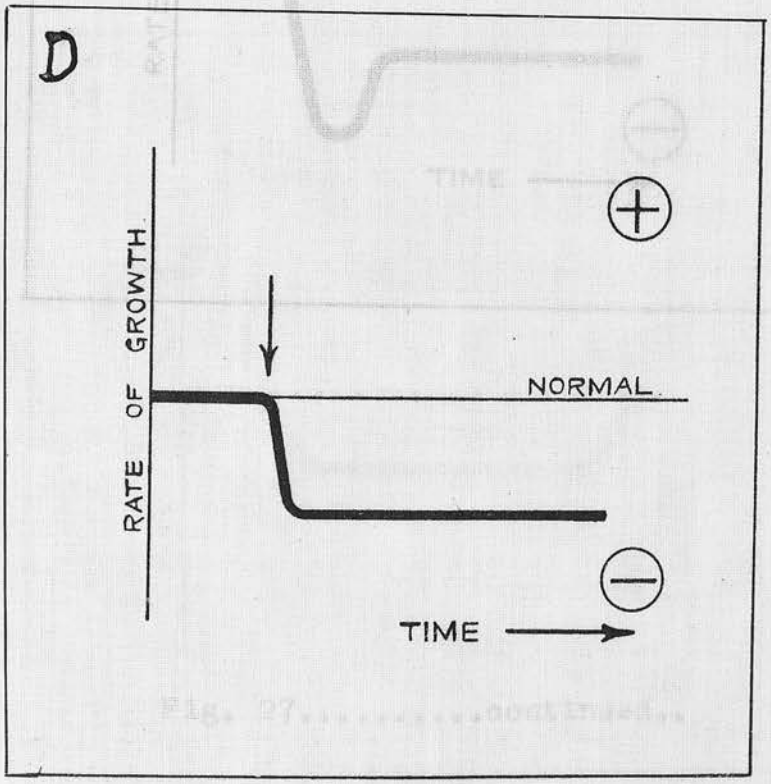


Fig. 27.....continued.....

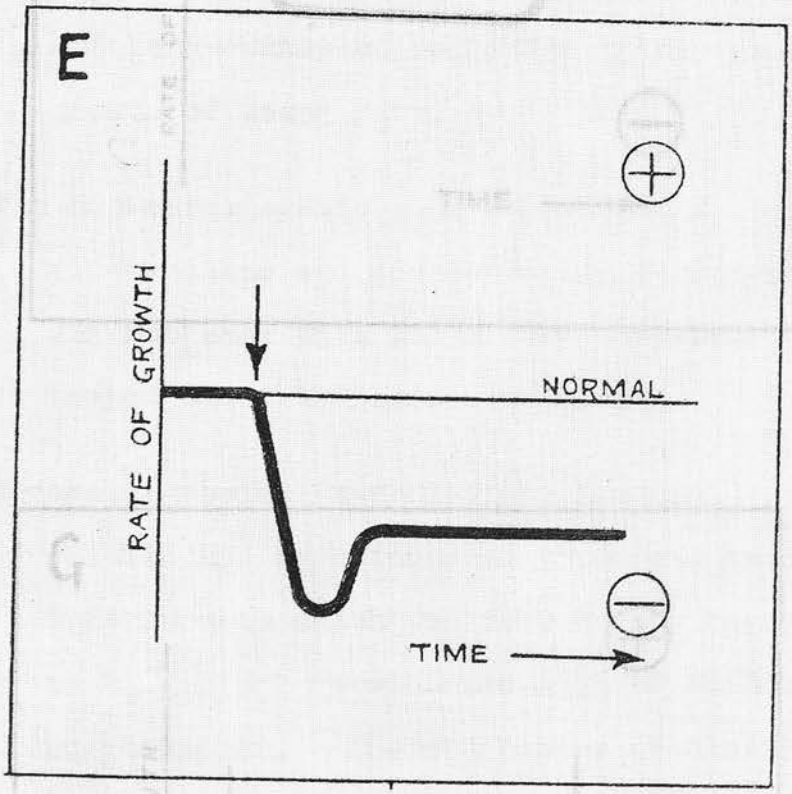
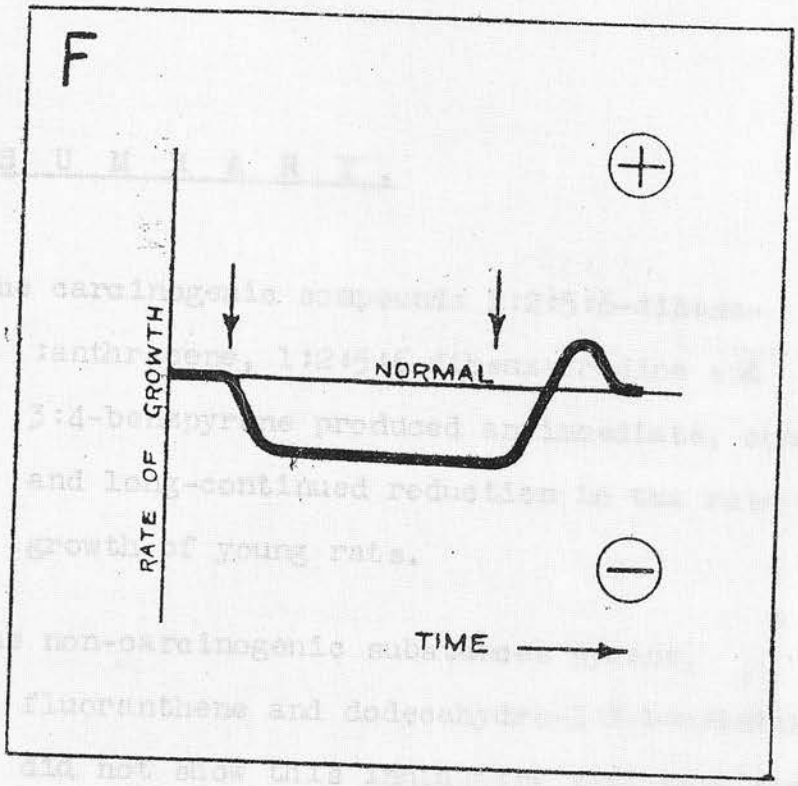


Fig. 27.....continued..



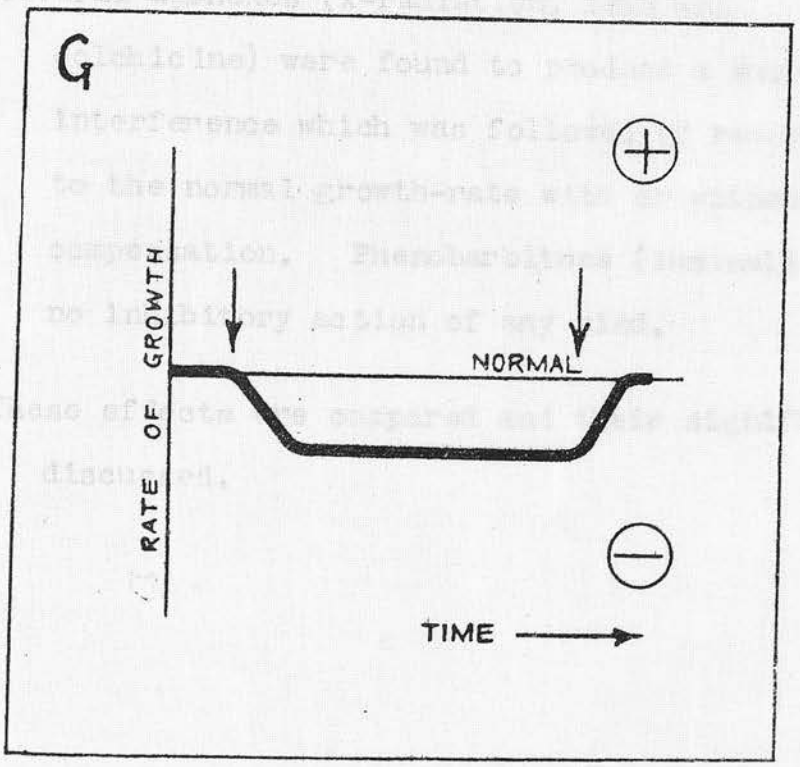


Fig. 27.....continued.

IV. S U M M A R Y.

1. The carcinogenic compounds 1:2:5:6-dibenz-:anthracene, 1:2:5:6-dibenzacridine and 3:4-benzpyrene produced an immediate, constant, and long-continued reduction in the rate of growth of young rats.

2. The non-carcinogenic substances pyrene, fluoranthene and dodecahydro-1:2-benzanthracene did not show this inhibitory influence when tested under the same conditions.

3. Several agencies (x-radiation, lead and colchicine) were found to produce a temporary interference which was followed by recovery to the normal growth-rate with or without compensation. Phenobarbitone (luminal) had no inhibitory action of any kind.

4. These effects are compared and their significance discussed.

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T A B L E X X X V I I

GROUP	TREATMENT ON 8TH DAY	MEAN BODY WEIGHT (GM.) 1ST DAY	8TH DAY	43RD DAY	PERCENTAGE INCREASE IN WT. 1ST-8TH DAY	8TH-43RD DAY
I 9 5	2 c.c. sesame oil i.p.	56.4	69.0	133.5	22.3	93.4
II 9 5	10 mg. 1:2:5:6-dibenzanthracene in oil i.p.	53.8	66.5	97.0	23.6	45.8
III 8 4	10 mg. pyrene in oil i.p.	55.0	67.5	132.7	22.7	96.5

T A B L E XXXVIII

GROUP	TREATMENT ON 8TH AND 11TH DAYS.	MEAN BODY WEIGHT (GM.) 1ST DAY	8TH DAY	38TH DAY	PERCENTAGE INCREASE IN WT. 1ST-8TH DAY	8TH-38TH DAY
I 5	Control untreated	60.4	72.8	121.8	20.5	68.0
II 6	Control oil 2 x 3 c.c. i.p.	68.8	83.1	137.1	20.8	64.9
III 5	1:2:5:6-dibenzanthracene 2 x 15 mg. i.p.	65.6	78.8	108.6	20.1	37.8

T A B L E XXXIX

GROUP	TREATMENT ON 8TH AND 11TH DAYS.	MEAN BODY WEIGHT (GM.) 1ST DAY	8TH DAY	26TH DAY	PERCENTAGE INCREASE WT. 1ST- 8TH DAY	8TH-26TH DAY
I 2 7	Control oil 2 x 4.5 c.c. i.p.	64.0	76.0	107.1	18.7	40.9
II 3 6	1:2:5:6- dibenzanthracene 90 mg. i.p.	65.3	77.0	83.8	17.9	8.8

T A B L E XL

GROUP	TREATMENT ON 8TH DAY	MEAN BODY WEIGHT (GM.) 1ST DAY	8TH DAY	34TH DAY	PERCENTAGE INCREASE IN WT. 1ST-8TH DAY	8TH-34THDAY
I 7	Control oil	58.3	74.3	116.5	27.5	57.0
II 7	1:2:5:6-dibenzanthracene 3 mg. in oil	59.8	77.3	113.0	29.1	46.2

T A B L E XLI

GROUP	TREATMENT ON 8TH DAY	MEAN BODY WEIGHT (GM.) 1ST DAY	8TH DAY	13TH DAY	30TH DAY	PERCENTAGE INCREASE 1ST-8TH DAY	13TH-30TH D
I 4 6	Control oil 3 c.c.	76.2	90.7	100.1	126.3	19.03	26.17
II 4 6	30 mg. chrysene in 3 c.c.	76.3	92.0	95.2	121.9	20.05	28.04
III 4 6	30 mg. 1:2:5:6-dibenzacridine in 3 c.c.	76.3	88.7	86.3	92.7 (corrected to allow for 2 deaths)	16.25	7.41

T A B L E XLII

GROUP	TREATMENT ON 8TH DAY	MEAN BODY WEIGHT (GM.) 1ST DAY	8TH DAY	24TH DAY	PERCENTAGE INCREASE IN WT. 1ST-8TH DAY	8TH-24TH DAY
I 3 6	3 c.c. oil	68.1	80.1	108.5	17.6	35.5
II 4 5	30 mg. dodecahydro-1:2-benzanthracene (one per cent.)	70.7	84.4	115.7	19.2	37.1
III 4 5	30 mg. fluoranthene	70.1	82.1	113.4	17.1	38.1

T A B L E XLIII

GROUP	TREATMENT ON 8TH DAY	MEAN BODY WEIGHT (GM.) 1ST DAY	8TH DAY	10TH DAY	30TH DAY	PERCENTAGE INCREASE IN WT 1ST-8TH DAY	10TH-30TH D.
I 4 5	2 c.c. sesame oil intraperitoneally	66.7	80.1	81.8	120.7 (one animal omitted on account of pregnancy)	19.9	47.5
II 4 5	10 mg. 1:2:5:6-dibenzacridine in oil	64.6	78.1	75.2	97.4 (one sick animal omitted)	20.9	29.5

....continued over.....

T A B L E XLIII continued....

GROUP	TREATMENT ON 8TH DAY	MEAN BODY WEIGHT (GM.) 1ST DAY	8TH DAY	10TH DAY	30TH DAY	PERCENTAGE INCREASE IN WT 1ST-8TH DAY	10TH-30TH D.
III 4 5	10 mg. 1:2:5:6-dibenzanthracene in oil	69.4	84.4	84.4	102.0 (1 sick animal omitted; 1 death)	21.5	20.8
IV 4 6	10 mg. 3:4-benzpyrene in oil	66.9	80.7	78.1	105.3	20.6	34.8

T A B L E XLIV

GROUP	TREATMENT ON 8TH DAY	MEAN BODY WEIGHT (GM.) 1ST DAY	8TH DAY	14TH DAY	PERCENTAGE INCREASE IN WT. 1ST-8TH DAY	8TH-14TH DAY
I 2 7	control	64.0	76.0	89.4	18.7	17.6
II 3 7	x-irradiation (450 r)	63.7	76.1	82.1	19.5	7.9

T A B L E XLV

GROUP	TREATMENT ON 15TH DAY	MEAN BODY WEIGHT (GM.) 1ST DAY	15TH DAY	18TH DAY	34TH DAY	PERCENTAGE INCREASE WT. 1ST-15TH DAY	18TH-34TH D
I 7 6	4 mg. lead nitrate	56.7	84.5	85.3	112.5	48.9	31.8
II 6 7	control	56.7	86.7	92.3	121.8	52.7	32.0

T A B L E XLV I

GROUP	TREATMENT ON 22ND DAY	MEAN BODY WEIGHT (GM.) 1ST DAY	22ND DAY	25TH DAY	36TH DAY	PERCENTAGE INCREASE WT. 1ST-22ND DAY	25TH-36TH D.
I 6 5	0.5 c.c. saline i.p.	65.7	106.4	111.1	130.6 (one animal omitted on account of pregnancy)	61.9	17.5
II 5 6	0.12 mg. colchicine i.p.	64.3	103.3	101.6	121.7	60.7	19.8
				- 3 animals dead -			

T A B L E XLVII

GROUP	TREATMENT	MEAN BODY WEIGHT (GM.) 1ST DAY	MEAN BODY WEIGHT (GM.) 36TH DAY	PERCENTAGE INCREASE IN WT. 1ST-36TH DAY
I 6 5	saline control	65.7	130.6 (one animal omitted on account of pregnancy)	98.7
II 6 5	phenobarbitone (see text)	64.8	136.7 (two animals omitted on account of pregnancy)	110.9

T A B L E XLVIII

Treatment	Dose mg.	No. of animals	Deaths within 20 days of injection	Deaths within 40 days of injection	Deaths within 60 days of injection
1:2:5:6-dibenzanthr-:acene.	3	13	0	-	-
	10 (Expt. 36)	13	0	0	3
	10 (Expt. 42)	9	1	2	-
	30	12	0	0	-
	90	9	0	4	7

...continued over.....

T A B L E XLVIII continued.....

Treatment	Dose mg.	No of animals	Deaths ..... within 20 days of injection	within 40 days of injection	within 60 days of injection
1:2:5:6-dibenzacrid-:ine.	10	9	0	1	-
	30	10	1	5	-
3:4-benz-pyrene.	10	10	0	0	-
chrysene	30	10	0	1	-
Control	0	115	0	3	4

VI. A P P E N D I X .

The following tables show the mean and individual weights (in grams) of animals at various periods of the fore-going experiments, including always the first day (start of preliminary weighings), the day on which the injection or other treatment was given, and another day later in the experiment.

Experiment 36. (Male animals only).

Rat No.	1st day	8th day	100th day
------I. control oil on 8th day.----------			
1	57	66	220
2	56	73	210
3	54	64	200
4	50	62	255
5	78	91	265
6	46	54	170
7	54	71	220
8	50	58	198
9	71	93	260
Mean..	57.3	70.2	222.0
------II. 10 mg. 1:2:5:6-dibenzanthracene on 8th day.			
10	57	73	136
11	55	66	120
12	46	63	138
13	52	64	133
14	50	62	166
15	50	60	178
16	65	84	180
Mean..	53.5	67.4	150.1
------III. 10 mg. pyrene on 8th day.----------			
17	58	72	208
18	48	56	155
19	48	58	260
20	66	84	255
21	64	78	260
22	51	62	195
23	46	58	192
24	60	80	220
Mean..	55.1	68.5	218.1

Experiment 37 (Male animals only).

Rat No.	1st day	7th day	38th day
------I. Control untreated.----------			
1	43	54	97
2	56	68	110
3	54	64	110
4	77	92	155
5	72	86	137
Mean..	60.4	72.8	121.8
------II. Control oil.----------			
6	79	97	150
7	50	62	106
8	81	101	156
9	71	85	141
10	60	75	142
11	72	79	128
Mean..	68.8	83.1	137.1
------III. 30 mg. 1:2:5:6-dibenzanthracene on 7th day.			
12	47	61	108
13	73	93	118
14	55	61	78
15	80	93	123
16	73	86	116
Mean..	65.6	78.8	108.6

Experiment 38

Rat No.	1st day	8th day	14th day	26th day
------I. Control oil on 8th day.----------				
1	58	71	86	102
2	68	81	95	119
3	59	72	83	103
4	81	91	108	127
5	70	80	95	114
6	63	77	87	105
7	65	78	91	108
8	49	57	71	84
9	63	76	89	102
Mean..	64.0	75.9	89.4	107.1
------II. 90 mg. 1:2:5:6-dibenzanthracene on 8th day.				
10	61	69		81
11	55	63		63
12	73	87		99
13	75	89		95
14	62	76		79
15	64	76		78
16	64	79		91
17	72	83		97
18	62	71		71
Mean..	65.3	77.0		83.8
------III. X-irradiation 450 r on 8th day.----------				
19	55	65	73	
20	60	70	81	
21	80	106	114	
22	52	59	62	
23	82	94	99	
24	67	84	90	
25	71	88	92	
26	53	58	64	
27	73	85	92	
28	44	52	54	
Mean..	63.7	76.1	82.1	

Experiment39. (Male animals only).

Rat No.	1st day	8th day	34th day
------I. Control oil.----------			
1	66	82	133
2	40	48	80
3	75	94	130
4	54	74	119
5	65	89	154
6	66	84	123
7	42	49	77
Mean..	58.3	74.3	116.5
------II. 3 mg. 1:2:5:6-dibenzanthracene on 8th day.-			
8	56	67	95
9	73	97	145
10	69	97	126
11	57	74	115
12	54	67	103
13	62	83	122
14	48	56	85
Mean..	59.8	77.3	113.0

Experiment 40

Rat No.	1st day	8th day	13th day	30th day
------I. Control oil on 8th day.----------				
1	90	109	125	166
2	87	106	115	141
3	113	126	131	144
4	93	104	113	133
5	65	80	87	97
6	59	71	81	104
7	53	66	75	102
8	71	82	95	138
9	62	76	83	106
10	69	87	96	132
Mean..	76.2	90.7	100.1	126.3
------II. 30 mg. chrysene on 8th day.----------				
11	80	96	97	128
12	73	84	87	103
13	94	117	124	167
14	73	83	83	102
15	95	113	117	138
16	60	71	71	77
17	78	98	104	144
18	62	78	83	104
19	90	107	110	149
20	58	73	76	107
Mean..	76.3	92.0	95.2	121.9
------III. 30 mg. 1:2:5:6-dibenzacridine on 8th day.-				
21	67	72	65	57
22	98	112	112	131
23	80	86	69	died
24	104	120	124	died
25	81	99	100	109
26	70	82	81	77
27	60	74	73	99
28	65	77	77	86
29	67	79	77	99
30	71	86	85	84
Mean..	76.3	88.7	86.3	92.7

Experiment 41

Rat No.	1st day	8th day	24th day
------I. Control oil on 8th day.----------			
1	71	85	111
2	39	42	53
3	56	72	98
4	62	72	100
5	68	79	107
6	81	91	died
7	60	65	91
8	82	95	131
9	88	110	140
10	87	101	146
Mean..	69.4	81.2	108.5
------II. 30 mg. dodecahydro-1:2-benzanthracene.----			
11	78	93	126
12	73	86	121
13	55	72	96
14	74	89	123
15	67	82	118
16	78	82	104
17	75	91	128
18	66	80	110
Mean..	70.7	84.4	115.7
------III. 30 mg. fluoranthene on 8th day.----------			
19	68	73	105
20	76	94	125
21	70	81	113
22	79	93	134
23	57	66	89
24	59	65	105
25	76	97	120
26	72	90	124
27	74	80	106
Mean..	70.1	82.1	113.4

Experiment 42

Rat No.	1st day	8th day	10th day	30th day
------I. Control oil on 8th day.----------				
1	74	93	99	141
2	80	95	101	pregnant
3	66	85	92	147
4	65	67	61	96
5	75	86	90	149
6	60	73	75	112
7	61	76	71	107
8	53	62	63	94
9	67	84	84	120
Mean..	66.7	80.1	81.8	120.7
------II. 10 mg. 1:2:5:6-dibenzacridine on 8th day.--				
10	83	110	106	153
11	68	85	83	103
12	64	76	72	65
13	64	75	69	93
14	56	61	59	sick
15	60	72	66	83
16	68	79	81	113
17	50	60	59	66
18	68	85	82	103
Mean..	64.5	78.1	75.2	97.4
------III. 10 mg. 1:2:5:6-dibenzanthracene on 8th day.				
19	70	75	77	92
20	81	113	114	149
21	63	78	75	89
22	55	67	68	sick
23	68	73	69	87
24	64	78	78	73
25	50	60	59	died
26	85	107	111	122
Mean..	67.0	81.3	81.3	102.0
------IV. 10 mg. 3:4-benzpyrene on 8th day.----------				
27	77	100	99	130
28	82	102	99	138
29	74	96	94	129
30	63	76	73	87
31	65	74	73	91
32	66	76	73	103
33	61	73	69	101
34	57	65	63	88
35	59	68	62	84
36	65	77	76	102
Mean..	66.9	80.7	78.1	105.3

Experiment 43

Rat No.	1st day	15th day	18th day	34th day
------Control treatment on 15th day.----------				
1	67	87	90	110
2	63	88	92	122
3	45	55	55	72
4	48	66	69	89
5	55	80	82	116
6	47	70	74	95
7	55	82	88	117
8	62	95	101	124
9	59	91	95	125
10	55	90	98	143
11	59	102	112	147
12	61	114	127	166
13	62	107	117	158
Mean..	56.7	86.7	92.3	121.8
------4 mg. lead nitrate on 15th day.----------				
14	41	55	57	69
15	59	81	80	102
16	78	105	106	134
17	48	65	64	85
18	59	80	78	105
19	43	59	62	84
20	44	64	66	90
21	60	90	87	103
22	65	93	100	135
23	68	113	111	152
24	58	97	98	128
25	55	94	99	133
26	60	103	101	142
Mean	56.7	84.5	85.3	112.5

Experiment 44

Rat No.	1st day	22nd day	25th day	36th day
------Control treatment on 22nd day.----------				
1	57	93	101	127
2	89	145	152	154
3	74	127	134	pregnant
4	51	80	82	107
5	64	103	110	133
6	47	65	69	81
7	67	108	112	127
8	50	80	84	111
9	67	124	129	162
10	87	145	147	162
11	70	101	102	142
Mean..	65.7	106.4	111.1	130.6
------0.12 mg. colchicine on 22nd day.----------				
12	74	125	125	151
13	79	137	125	119
14	52	74	died	-
15	50	85	69	100
16	65	113	died	-
17	61	91	died	-
18	45	74	72	89
19	70	109	106	130
20	77	121	118	142
21	65	110	118	138
22	69	97	80	105
Mean..	64.3	103.3	101.6	121.7
------10 mg. phenobarbitone on 22nd day.----------				
23	78			162
24	69			pregnant
25	46			114
26	57			122
27	53			129
28	70			pregnant
29	68			143
30	55			115
31	73			149
32	64			137
33	80			159
Mean..	64.8			136.7

S U M M A R I E S

Section I. The influence of various polycyclic hydrocarbons on the growth-rate of transplantable tumours.

Intraperitoneal administration of a number of carcinogenic hydrocarbons, including 1:2:5:6-dibenzanthracene, 5:6-cyclopenteno-1:2-benzanthracene and 3:4-benzpyrene, produced considerable inhibition in the rate of growth of the Jensen and Walker tumours. The inhibition was however not specific for tumour tissue but affected the growth of the body as a whole. Activity was also shown to a variable extent by chrysene and certain compounds of benzanthracene type, the carcinogenicity of which is either very feeble or nil.

On the other hand, a series of related non-carcinogenic compounds (anthracene, phenanthrene, 1:2-cyclopentenophenanthrene, dodecahydro-1:2-benzanthracene, pyrene, fluoranthene, triphenylene, dehydronorcholene, perylene, 1:9-benzanthrone and diphenylene oxide) showed no inhibiting influence when tested under the same conditions. Of the synthetic oestrogens 1-keto-1:2:3:4-tetrahydrophenanthrene and

9:10-dihydroxy-9:10-di-n-propyl-9:10-dihydro-1:2:5:6-dibenzanthracene, the latter proved to be moderately inhibitory and the former quite inactive.

Although the carcinogenic hydrocarbons must be regarded as toxic substances, their growth-inhibiting effect is not dependent on mere toxicity in any non-specific sense, since manifest poisons evoke an entirely different response. Reference is also made to other effects, particularly on the reproductive tissues.

There is thus a correlation in compounds of this type between carcinogenicity and growth-inhibitory power. It is suggested that the mode of action of these substances in tumour production is indirect, and that they operate by producing a prolonged retardation of the growth of normal cells, which eventually react by a process of discontinuous variation to give a new cell race with a greatly increased fission rate.

Section II. The influence of certain carcinogenic and other hydrocarbons on body-growth in the rat.

In continuation of the work described in Section I, it was found that the carcinogenic compounds 1:2:5:6-dibenzanthracene, 1:2:5:6-dibenzacridine and 3:4-benzpyrene produced an immediate, constant and long-continued reduction in the rate of growth of young rats. The non-carcino-:genic substances pyrene, fluoranthene and dodeca-:hydro-1:2-benzanthracene did not show this inhibitory influence when tested under the same conditions. In comparative experiments, x-radiation, lead and colchicine were found to produce a temporary interference which was followed by recovery to the normal growth-rate with or without compensation. Thus the effect of the carcinogenic hydrocarbons cannot be attributed to toxicity per se.

The inhibition produced by the carcinogenic compounds was extremely prolonged even after a single injection. This feature is discussed in relation to the rate of excretion of such substances.

Reference is also made to other effects, particularly a diminution of both male and female fertility after administration of 1:2:5:6-dibenzanthr-acene.

C E L L U L A R I N H I B I T I O N

A N D T H E O R I G I N O F C A N C E R

CELLULAR INHIBITION AND THE ORIGIN OF CANCER.

Multiplicity of experimental causes.

i. the carcinogenic hydrocarbons.

ii. other chemical substances.

iii. x-radiation, radium and other radio-active elements.

iv. ultraviolet radiation.

v. freezing, heat.

vi. virus action.

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The basis of non-specificity.

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R E F E R E N C E S.

CELLULAR INHIBITION AND THE ORIGIN OF CANCER.

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One of the most striking features of cancer, both in experimental and in human pathology, is the multiplicity of its apparent causes, and it is clear that a variety of chemical and physical agents are capable of producing malignancy in tissues exposed to their action. Among chemical substances with pronounced activity in this respect are the carcinogenic hydrocarbons and related compounds studied at the Research Institute of the Royal Cancer Hospital (Cook, Hieger, Kennaway and Mayneord 1932; Cook 1932; Barry, Cook, Haslewood, Hewett, Hieger and Kennaway 1935): for the most part these are related in their molecular structure to 1:2-benzanthracene (see also Fieser, Fieser, Hershberg, Newman,

Seligman and Shear 1937), and one of them (3:4-benzpyrene) has been shown to be present in coal tar (Cook, Hewett and Hieger 1933). In a continuation of these studies, Boyland and Brues (1937, in press) have recently described the carcinogenic activity of 1:2:5:6- and 3:4:5:6-dibenzcarbazole, and draw attention to the interest of the latter compound on account of its possible derivation from -naphthylamine, one of the bases believed to be responsible for the occurrence of papilloma and carcinoma of the bladder in dye workers (see Schüller 1932, Müller 1932, Berenblum 1932, Ferguson and others 1934, Hueper 1934 and Gehrmann 1935, 1936). There is however no satisfactory evidence of the artifical production of bladder papillomata by -naphthylamine or related substances (see Schär 1930 and Perlmann and Staehler 1933), and Berenblum and Bonser (1937) reported a series of negative experiments.

Of the polycyclic hydrocarbons related to phenanthrene but not to 1:2-benzanthracene, only 3:4-benzphenanthrene possessed pronounced

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Formerly referred to as 1:2-benzpyrene. See Cook, Haslewood, Hewett, Hieger, Kennaway and Mayneord 1937.

carcinogenicity, although chrysene and 1:2-cyclo-pentenophenanthrene gave traces of activity of an extremely low order (Barry et al., 1935). But there is other evidence to show that tumour-producing power need not depend on a single type of chemical structure. Thus the occurrence of sarcoma in mice after subcutaneous injection of a styryl quinoline trypanocide has been studied in detail by Browning, Gulbransen and Niven (1936). Again, Sasaki and Yoshida (1935) and Yoshida (1935) described hepatoma and vesical tumours in rats fed with o-amino-azotoluene, and Otsuka (1935), the occurrence of papilloma of the stomach in mice receiving diazo-aminobenzene. In the former experiments it is interesting to note that p-amino-azotoluene and p-amino-azobenzene were practically inert (see also Yoshida 1932, 1934; Iikubo 1935; Miura 1935; and Shear 1937). Thirdly, Morton, Branch and Clapp (1936) claimed to have produced malignant tumours in mice with triphenylenebenzene and tetraphenyl-methane, two hydrocarbons not in any way related to phenanthrene. This claim awaits confirmation, but it may be noted that Lipschutz (1930) referred to a rat sarcoma produced by the long-continued subcut-:aneous injection of a triphenylene dye.

Of special interest is the appearance of mammary carcinoma --- in male and female mice of both high-cancer and low-cancer-incidence strains --- as a result of prolonged treatment with oestrone, oestrone benzoate and other oestrogenic substances (e.g., Lacassagne 1935, 1936 a, b; Bonser 1935, 1936; Burrows 1935; Cramer and Horning 1936; Gardner, Smith, Allen and Strong 1936a; and Suntzeff, Burns, Moskof and Loeb 1936); and the development of sarcoma in mice at the site of injection of oestro-:genic hormones (Gardner, Smith, Strong and Allen 1936 a, b).

Inorganic substances have also been associated with tumour induction in special circumstances, examples being the production of teratoma of the testis in a small percentage of adult roosters by injection of zinc chloride (Bagg 1936, confirming Michalowsky 1928, 1929), and the relation between arsenic and human cancer (see Franseen and Taylor 1934).

Among physical agents causing cancer the most widely studied are x-rays and the radiations emitted by radium and other radio-active elements. Thus Lacassagne and Vinzent (1929) and Lacassagne (1933 a, b, c) described the production of cancers

in rabbits by the action of x-rays upon inflammatory lesions produced either by infection with Streptobacillus caviae or by the injection of diatomaceous earth. According to a recent editorial article in the American Journal of Cancer (1933, 18, 624) there are now recorded some eighteen examples of sarcoma following x-irradiation of joint tubercul-:osis in humans (see also Becker 1936). Tumour development in rabbits has also been obtained by Burrows (in unpublished experiments) following injection of silica and x-irradiation of the result-:ant focus. Ludin (1934) produced bone sarcoma in the rabbit by prolonged x-irradiation, while Furth and Furth (1936) and Furth and Butterworth (1936) recently reported an increase in the incidence of neoplastic conditions among mice subjected to general x-irradiation. The work of Daels (1925, 1926), Daels and Baeten (1926 a, b), Daels and Biltris (1927, 1931), Biltris (1933), Schurch and Uehlinger (1931) and Mottram (1935) has shown the feasibility of producing animal tumours by prolonged or repeated exposure to radium, while Sabin, Doan and Forkner (1932) obtained two osteogenic sarcomata following the intravenous injection of radium chloride and mesothorium in rabbits. At the 1933 meeting of the Gesellschaft fur Chirurgie Daels exhibited 28 malignant radium-induced tumours resulting from

experiments on several hundred animals. In these cases the latent periods were 6-10 months for the mouse, 8-22 months for the rat, and 12-30 months for the guinea-pig. Dobrovolskaia-Zavadskaia (1935 a,b) also produced carcinoma and sarcoma by implanting radon in the tissues of mice, and Lacassagne and Nyka (1936) observed the development of an osteo-sarcoma in relation to a radon tube implanted to irradiate the hypophysis in a rabbit. As in the case of tumours produced by tar and polycyclic hydrocarbons, those due to radio-active agents have their counterpart in industrial medicine, particularly in the occurrence of osteogenic sarcomata in dial painters (Martland and Humphries 1929; Martland 1931; Schwartz, Knowles, Britten and Thompson 1933). More recently Schürch and Uehlinger (1934, 1935) repeated the experimental production of bone sarcoma by radium and mesothorium in the rabbit. The carcino-:genic action of thorium dioxide, originally described by Roussy, Oberling and Guérin (1934) has also been confirmed by Selbie (1936) and by Prussia (1936).

Of unique interest is the human case described by Ross (1932) in which a radium needle had remained embedded in the interventricular septum of the heart for three years and had apparently caused the

development of a malignant haemangioma in the lobe of the liver immediately subjacent. According to Ross no record could be found of the development of malignancy through exposure to adequately screened radium, and it was decided to attempt the production of tumours by the prolonged action of gamma rays. Platinum tubes containing 0.1 mg. radium element were implanted into the tissues of nine rabbits, of which six developed new growths (osteogenic, spindle-celled and myxomatous sarcomata, squamous-cell carcinoma) in relation to the tubes.

The influence of sunlight in the production of cancer of the skin has long been recognised (e.g., Paul 1918, Larabi 1932), and Findlay (1928, 1930) succeeded in producing cutaneous papillomata and carcinomata by exposing rats to ultraviolet light. Similar findings were reported by Putschar and Holtz (1930 a, b) and Huldschinsky (1933) in the case of rats and by Herlitz, Jundell and Wahlgren (1931) and Wahlgren (1931) for mice. Roffo (1936) has also devoted much attention to the role of ultraviolet rays in this connection. Further evidence of the production of malignant tumours by means of ultra-violet irradiation was reported by Beard, Boggers and von Haam (1936), and von Haam, Alexander and Beard (1936) described studies in which they employed a rat tumour obtained by this method.

Tumours have also been produced by repeated local freezing of the tissues (Berenblum 1929, confirmed by Mansens 1931), or by heat, of which the Kashmir kangri burn offers a striking illustration (see Neve 1930). An interesting report in the latter connection is that by Tur (1935) of heat-produced neoplasms in the developing avian blastoderm.

Lastly must be mentioned the progression to carcinoma of virus-induced papillomata (Rous and Beard 1935) and the carcinogenic effect of the same virus upon previously tarred skin (Rous and Kidd 1936).

From the above summary it is clear that carcinogenic agents have certainly no chemical or physical feature in common. This lack of specificity may be more apparent than real, and it is conceivable that a deeper knowledge of the chemical changes involved would show that these processes have more in common than appears. The hope of such an under-:standing is seen in the suggestion, originally made by Kennaway and Cook (1932) that cancer-producing substances may be formed in the body by abnormal sterol metabolism.

Biological non-specificity in other fields.

On the other hand it iswell recognised that characteristic biological effects may be induced by equally diverse means. While the most extreme instance is the non-specific ity of lethal agents, there are numerous other illustrations. Thus in the case of the stimulation of normal growth it is probable, according to Willmer (1935), "that cell division and growth are dependent on a series of conditions for their fulfilment and there is no one factor which is responsible for growth by itself, although at any one time one particular condition or substance may determine by its presence or absence whether growth shall or shall not occur." Other examples include (a) the production of artificial parthenogenesis by a variety of methods (Loeb 1913; Tchakotine 1935); (b) the production of caryoclastic effects by substances as different as sodium caco-:dylate and colchicine (Dustin 1934); (c) the similar effects of parasympathetic drugs such as pilocarpine and muscarine, which are structurally unrelated but act selectively at the same sites (see Ing 1937); (d) the production of a non-specific "irritative" reticulocytosis, e.g., by potassium arsenite (Minot and Castle 1935). Again, Dodds and Lawson (1936) described the production of oestrus by synthetic compounds not possessing the phenanthrene

nucleus, the most active of these substances being 1:2-dihydroxy-1:2-di-α-naphthylacenaphthene and the simplest 4:4'-dihydroxydiphenyl. The activity of diphenyl compounds in relation to mammary growth was later discussed by Grant (1937). The question of the specificity of hormone and other reactions has also been reviewed by Dodds (1936). This important problem of biological response in relation to chemical structure has further been dealt with by Bergmann (1935) in quoting another example. "Until quite recently the organic chemist was inclined to conclude from a similarity in physiological behaviour of two unknown substances that if not identical they were at least very closely chemically related. That such conclusions might lead to serious mistakes has been shown by Fritz Koegl in his excellent studies on the growth promoting substances of plants. He found that the original growth hormone, auxine, which is present in seedling tips, is a cyclopentene derivative, while the hormone found in the urine is partly, and in micro-organisms is exclusively, the "hetero-auxine" which is indolacetic acid. These two substances which are chemically absolutely different can replace each other in every known reaction on young oat or corn seedlings. They show only a slight difference in activity." According to a recent report by Snow (1937) benzoyl oxide and

benzoyl peroxide also possess activity, although to a less extent than auxine or hetero-auxine: benzoic acid proved inactive. Crook, Davis and Smith (1937) also described plant growth hormone activity in certain synthetic substances such as thionaphthene-β-acetic acid and naphthalene-α-acetic acid, although these again were considerably less potent than indole-β-acetic acid.

The basis of non-specificity.

From the facts themselves and from these analogies it is clearly possible that the apparent lack of chemical or physical specificity in cancer-producing agents may in part be real. But since the pathological end-result is the same in all these cases it is probable that the cellular processes involved are not entirely dissimilar. In other words, such processes may have a common physiological basis, the same types of interference with function being induced by a wide range of initial causes. The position has been well stated by Findlay (1930). "The forms of stimulation which are now known to cause cancer are.....of a most diverse character, though the properties of the cancer cell are similar in all cases. It therefore follows that whatever the forms of external stimulus, the metabolic changes in the tissues produced by these stimuli must of necessity

be essentially similar." Loeb (1935) gave it as his opinion that although the carcinogenic hydro-:carbons are more efficient than other factors in producing cancer, in principle they probably do not act differently to other conditions which may cause it.

The literature contains several references which indicate that different carcinogenic influences may summate, a finding to be expected if cancer can result not only from a single chain of abnormal events but from several which, although differing in chemical detail, lead by different routes to the same type of interference with function. For example, it was reported by Findlay (1928) that when mice were tarred and exposed to ultra-violet light at the same time, the period necessary for the induction of cancer was shorter than when either tar or ultra-violet light alone was employed. Berenblum (1930) also found that the application of two different carcinogenic agents (tar and repeated freezing) during two consecutive periods produced a much greater incidence of tumours than that of one agent alone, a result which indicates that the tissue changes prior to the appearance of a tumour are not specific but that such agents can be inter-changed during the period of induction. Again, "the increase

in frequency of a naturally occurring form of cancer (e.g., the occurrence of cancer of the lung in 20 per cent. instead of 5 per cent. of mice) in the presence of a known carcinogenic agent suggests that this agent can summate with the unknown, naturally occurring, carcinogenic factor to give an effective stimulus." (see Cook et al., 1937). Additional evidence in favour of the summation hypothesis was described by Hieger (1936). Lastly, Mayneord and Parsons have obtained results (in unpublished experiments) which suggest that the production of sarcoma in mice by the subcutaneous injection of sodium-1:2:5:6-dibenzanthracene-9:10-endo-α/β-succinate is accelerated by exposure to x-rays.

The basis of non-specificity in cases of this kind is probably the extremely limited number of possible physiological responses. As regards division, for instance, a normal cell can respond to interference in only three ways: (a) by continuing growth at the normal or at a temporarily increased or decreased rate; (b) by ceasing growth; or (c) by undergoing malignant change so as to divide at a permanently increased rate.

Tumour-inducing agents and the artificial induction of mutations.

The question therefore arises --- how can we interpret the change from normal to cancerous in terms of the basic physiology of growth? Before proceeding to this question attention must once again be directed to the striking circumstance that a number of the physical agents associated with cancer production represent the only means available for the experimental production of mutations. Among more recent examples may be cited the production of mutations in somatic cells of Drosophila melanogaster by x-rays (Patterson 1929), the induction of hereditary changes in mice by the same means (Snell 1935) and of a gene mutation in Oenothera by radium (Brittingham 1936), and the artificial production of mutations (Macdougall 1929, 1931; Altenburg 1933, 1934) and other genetic effects (Stadler and Sprague 1936 a, b, c; see also Science 1937, 85, 57) by ultra-violet light. Reference may also be made to the acceleration of mutation in resting seeds which is effected by increased temperatures. It was found by Navashin and Shkvarnikov (1933) that the effect of moderate heat between 50 and 60 C. acting for 20 days upon dry seeds of Crepis tectorum L. was comparable with that produced by allowing them to age at room temperature for from six to seven years. In parallel experiments

with x-rays a similar effect was obtained by subjecting dry seeds to a dosage of 15,000 r. Cartledge, Barton and Blakeslee (1936) studied temperature and age as factors in the production of increased mutation rates in Datura seeds and pollen, and stated that high temperatures induce mutations of the same sort but at a lower rate than the harsher radiation treatments or than the more effective natural ageing processes. They concluded that age of the cells, if not itself a cause of mutation, must increase susceptibility to those factors, such as heat, which are effective in producing mutative changes (see also Goldschmidt 1929).

Such parallelism at once suggests that cancer may be regarded simply as a special case in the biology of variation, and the problem resolves itself into one of the origin of variations in general.

Variant characters of the cancer cell.

i. morphology.
ii. growth-rate.
iii. metabolism.

If the cancer cell is to be regarded as a variant of the corresponding somatic cell, greatest attention must be paid to the characters in which it differs from the normal. Differences in morphology and function are inconstant, and according to Ludford (1925) "there exists for the cancer cell no precise diagnostic character of any kind" in the former respect. But such differences as do exist are of importance in that they indicate dedifferent-:iation --- in various degrees --- as a feature of the change from normal to malignant.

The most striking and constant property of the cancer cell is its increased rate of growth, which, as Schrek (1936) has clearly shown, in most cases approaches, although it rarely exceeds, that of the normal embryonic tissues. The feature next in importance is that of metabolic behaviour. Warburg (1931) was the first to show that malignant cells differ from the majority of normal body-cells in having a high aerobic glycolysis. But it soon became evident that, although practically all

tumours showed strong aerobic glycolysis, this property was not necessarily connected with the process of normal growth, still less with malignancy. Beginning with a study of the normal tissues which exhibit a tumour-like aerobic fermentation but do not utilise the resultant energy for uncontrolled growth, Dickens and Weil-Malherbe (1936; see also 13th Ann. Rept. British Empire Cancer Campaign, 1936, pp. 160-162) discovered a possible difference between normal and tumour metabolism through a classification based on respiration rather than glycolysis. According to these authors, "this classification.....proves to be more reliable as a method of distinguishing metabolism of tumour and normal tissues, than the original view of Warburg that aerobic glycolysis was such a criterion. Thus retina and medulla of kidney prove to have respiratory-quotients of close to unity, in conformity with our rule respecting normal, highly-glycolysing tissues. In these normal tissues the ability to oxidise carbohydrate is not injured despite their high aerobic glycolysis. In tumours the defective (i.e., insufficient or incomplete) oxidation of carbohydrate is shown by the lowered respiratory quotient.....The present position may be summed up by the statement that the metabolic peculiarities associated with tumour-growth are a

combination of (a) aerobic glycolysis with (b) a lowered efficiency of carbohydrate oxidation, indicated by a lowered respiratory quotient. Whilst either peculiarity alone may be found in normal tissues, the occurrence of the two together seems at present to be a peculiarity of tumour tissue: there being a general, though not entirely clear-cut, transition from normal tissues, through benign to malignant growths."

Nature of the cellular change.

i. discontinuity; irreversibility.

ii. transformation or selection.

Those characters in which variation has occurred having been defined, the next problem must concern the type of variation involved. From the transplantation experiments of the past thirty years an enormous bulk of evidence has accumulated to show that, although the cancer cell in its turn is not incapable of further variation (e.g., Bittner 1931; Cloudman 1932), no reversion to the normal state occurs. Bittner showed that hereditary genetic changes might occur in the tumour cell during the process of transplantation, and that the cell might thus deviate from the genetic constitution of the individual which gave rise to it. Cloudman 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. But such changes are obviously in the direction of further dedifferent-

:iation or change by loss. Secondly, the study of experimental carcinogenesis indicates that the change to malignancy, while it may be preceded by non-hereditary cellular alterations, is discontinuous in nature.

Notions of this kind are not entirely new. For instance, Lewis (1935) presented the view that malignant cells are permanently altered units, that is, new cellular types or species derived from normal cells which have been altered by environmental influences or agents of one sort or another. He stressed the important fact that after malignant cells are once established they multiply indefinitely in vivo as well as in vitro, independently of the special environment or agents which produced them. Menétrier (1926) had long regarded a malignant tumour as a new cell race, although he erred in attributing undue importance to the role of selection in inducing its appearance. It is practically certain, on the contrary, that the change from normal to malignant involves an adaptive transformation --- or series of transformations --- and that this must be regarded as the dominant process. Again, it is highly improbable that the pathological environ-:ment which leads to tumour formation is such as to favour or select rapidly growing cells: from what is known it is clear that x- and gamma-rays would

certainly tend to act in the opposite direction, on account of the relative sensitivity of rapidly growing cells to these agents. Third, that no highly selective influence is present in the local induction of cancer is shown by the fact that the malignant cell grows as well --- and often even better (e.g. Willis 1932) --- when it escapes to normal tissues where the causative agent does not operate. Lastly, it is self-evident that the cancer cannot be present as such in either normal or pre-cancerous tissues --- as is apparently the substance of a recent statement by Fischer (1936) --- and equally evident, therefore, that at least some measure of transformation is involved. Fischer has stated that it is possible by repeated autogenic transplant-:ation of normal tissues in young mice to produce carcinoma in a short time. But even if this should be substantiated it by no means follows, as Fischer further claims, that the phenomenon is a selective one. The problem finds a complete parallel in the origin of drug-resistant strains of trypanosomes. According to Yorke (1935) 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 impossible. The importance of conversion was stressed by Shaw (1932), who referred to it as a

process "in which a single differentiated cell or cell group becomes altered under some change in environment so that its previous differentiation and specialisation disappear or become modified. In contrast to the processes of repair, this dedifferent-:iation remains established."

It may be objected that a theory of this kind in relation to cancer adds nothing to the known facts but is rather a transcription in other terms. This is largely true, and is, indeed, partly as it should be. The sole justification for such a change lies in the fact that it may indicate a new approach more clearly than before.

General biology of variation: environmental alterations.

If the cancer is to be regarded as a discontinuous and irreversible variant of a normal somatic cell, differing sharply from the latter in possessing an increased rate of division and an altered type of metabolism, further information should be sought through an investigation of the conditions which govern the origin of variations of this kind. It is noteworthy that several workers (e.g., Lacassagne 1936 a) have been impressed by the physiological resemblances between malignant cells and unicellular organisms, and Shaw (1932) emphasised the capacity for independent existence which most metazoan cells retain. "The neoplastic process is founded upon the potentiality for independence of all the cellular entities which, in their aggregation and sufficient co-ordination, give us a composite living body. The cell is primar-:ily an individual and secondarily a citizen. The tissue culture slide, the persistence of cell life for several days after somatic death, the free grafting of tissues, the vitality of the spermato-:zoon, and the self-sufficiency of the ovum --- all these proclaim a capacity for survival of cells in the disjoined state, either when specially prepared for separation as are the reproductive cells or when

artificially separated as in the other instances." In the writer's opinion it is only through the study of the physiology of unicellular organisms that the fundamental principles of variation can be clearly deciphered.

In the course of work along these lines it was found that aspects of bacterial variation presented distinct advantages in this connection. In material of this kind the sources of variation are found to be mainly if not entirely environmental in origin, and it may be said that no variation will occur in an organism placed in an optimal environment to which it is completely adapted. Secondly, for the induction of variation in a given character there appear to be two main requirements: (a) a cell which is inherently capable of variation in respect of that character; (b) a source of environmental interference with the character in question. In the latter case, the interference must be so relatively specific as not to produce serious damage to other characters or functions of the cell; in particular it must obvious-:ly be non-lethal and freely compatible with survival if variation is to occur. Special attention was directed to the environmental conditions which govern the origin of variants with a permanently increased growth-rate, andit has been found that these are produced not by any process of direct growth-stimulation, as might be expected, but appear

as a sequel to a long-continued period of growth repression. In particular it is found that when the growth of a potentially variable organism is inhibited by a process which allows the majority of the affected cells to survive, a relatively small number may undergo an irreversible change in their metabolic properties in virtue of which they are then able to achieve active multiplication in an environment which makes this difficult or even impossible for their parent cell.

Inhibition and stimulation in experimental radiology.

The question arises whether this conception may apply to the origin of the new cell race which constitutes a malignant tumour. That such is at least a possibility is shown by the carcinogenic action of both x- and gamma-radiation. The interesting association between these physical agents and tumour-production has been widely neglected for a variety of reasons. For example, x-ray cancer in man has been studied mainly in relation to the dermatitis and destructive changes on the basis of which it arises. Again, the experimental production of cancer by radium was regarded as due more likely to α- or β- than to γ-radiation. But the more recent experimental work of Ross (1936) has clearly shown that radium, screened so as to filter practically all except gamma rays, is capable of producing cancer in a high proportion of rabbits subjected to chronic local irradiation.

The carcinogenic role of these agents might conceivably depend, as has indeed been assumed, on a stimulative or formative action exerted by low dos-:ages over a protracted period. As has been pointed out by Packard (1933), this suggestion arose under the influence of the Arndt-Schultz principle, according to which small doses might show the reverse

effect of larger and so might actually promote certain physiological activities including cell-division. In view of its importance this subject has been widely investigated, but with results which show that, far from producing any true stimulation, these radiations are in all circumstances injurious to the cells which absorb them. When tissues growing in vitro were first subjected to short exposures to radium, a wave of mitotic activity was frequently observed some time after the irradiat-:ion. Canti (1929) pointed out that this increase was not a real stimulation but rather a compensation following inhibition; and Canti and Spear (1929) showed numerically how cell division altered after such an exposure. In these experiments the dose was just above the minimum necessary to produce any effect at all upon mitosis, and the duration of exposure short compared to the average time a cell takes to divide. Mitosis gradually decreased to a minimum which was reached 80 minutes after exposure; recovery then took place and mitosis was at the normal rate after 2-3 hours; this recovery was then followed by a further rise in the number of cells in division, the maximum count being reached 4 hours after exposure; the count then returned to normal after a further period of two hours. Thus the increase in mitosis compensated almost exactly

for the fall originally observed. Essentially similar results were also obtained by Whitman (1933). As Willmer (1935) has pointed out, this compensation is due to the fact that "all those cells whose division has been temporarily inhibited divide at the same time as those which would, and do, divide in the ordinary course of events." The results of Canti and Spear were substantially confirmed by the work of Robertson (1935) on the behaviour of the protozoon Bodo caudatus on release from irradiation with gamma rays. After exposure there occurred a gradual rise in the rate of multiplication of the irradiated cells, until complete compensation in the total number had been obtained. The general principle has now been shown to apply not only in vitro but also in vivo (Spear 1935; and Medical Uses of Radium, Special Report Series No. 204, 1935).

In a study of the mitotic reaction of tumour tissue to various doses of radiation Dustin (1932) described a hyperkinetic phase following exposure, but he concluded: "Toutefois ces faits n'impliquent pas la conclusion d'un effet excitateur, direct, immediat sur la division cellulaire maligne. Cet effet est secondaire et consecutif à une chute précoce et rapide de l'index cinétique dès la première atteinte par les radiations. Toutes les

radiations, quelle que soit leur intensite, auraient des effets destructeurs ou au moins inhibiteurs."

In view of such observations as those made by Martland and Sabin, and the suggestion that tumours evoked by radio-activity are "due to stimulation causing an activated growth of mesenchyme or speeding up of the somatic cell", Flinn, Victor, Stillman and MacDonald (1934) undertook work to determine whether or not radio-active materials have a direct stimulating action on embryonic tissues in vitro. They used radium chloride in concentrations similar to those in the bones in dial painters' disease, but obtained no evidence of direct stimulation either of growth or oxygen consumption. In a further study of the effects of radio-active deposits in the body (with particular reference to alpha-radiation), Flinn (1934) concluded that the weak doses from such deposits do not stimulate growth but are always destructive, and he supported this view by clinical evidence from a number of cases of industrial radium poisoning, none of which gave any sign of marrow stimulation. He further suggested that malignancy arising in relation to such deposits is caused by an over-response to an essentially destructive action. Martland (1931) also regarded the production of malignancy in radio-active persons as due to the destructive effects of

continuous bombardment with alpha-particles.

In addition to the above there may also be mentioned the evidence from a large number of recent investigations, e.g., the action of x-rays on wound healing (Ritchie 1933) and on the metabolism and growth of transplantable tumours (Bancroft, Beck and Russell 1935); the action of gamma radiation from radium on the developing chick embryo (Wilson 1935), on the rat retina (Glucksmann and Tansley 1936), and on the human leucocytes (Goodfellow 1936); and the effects of x-rays and radium on embryonic development (Butler 1936) and on regeneration (Curtis 1936). X-ray and radium injuries have also been discussed in considerable detail by Colwell and Russ (1934), Reiss (1935) and by Tod (1936) and Hunter (1936). Further, the recent classification of the physiological and pathological effects of irradiation by the Committee on Safety and Standards of the American Roentgen Ray Society (Amer. Jour. Roentgenol., 1936, 35, 266-267) shows that the great majority of such effects are in the nature of (a) arrest of development, (b) atrophy, or (c) destruction.

It therefore seems justifiable to conclude that the ionizing action of gamma- and roentgen-irradiation is essentially damaging in its effects

under all conditions, and, therefore, that the production of cancer by these agents must be related to this destructive action and not to a primary stimulation. In a recent survey of the biological effects of x-radiation Clark (1936) wrote: "Continued acceleration of metabolism cannot be induced by roentgen rays or radium, which always cause degenerative changes or have no effect whatever. Irradiation of certain tissues, such as the skin, repeated over a long period may cause hyperplasia of the epithelium, and this in turn may lead to malignant transformation. This is not stimulation in the sense here employed, but the alteration of a normal to an aberrant function due to chronic irritation."

Growth-inhibitory action of the carcinogenic hydrocarbons.

If the production of cancer by such physical agents is a reaction by which the cell emancipates itself from their inhibitory effects, it becomes of interest to determine the influence of other tumour-producing agents, and particularly the carcinogenic hydrocarbons, on normal growing tissues. As a result of experiments along these lines it has now been shown (Sections I and II) that the intraperit-:oneal injection of a number of carcinogenic compounds, including 1:2:5:6-dibenzanthracene, 5:6-cyclopenteno-1:2-benzanthracene and 3:4-benzpyrene, produces considerable inhibition of the Jensen and Walker tumours and of the Crocker mouse sarcoma (unpublished experiments). This effect was however not specific for tumour tissue but, in young animals, affected the growth of the body as a whole. It is also of interest that the administrat-:ion of 1:2:5:6-dibenzanthracene produced a considerable diminution in both male and female fertility. Activity was also shown to a variable extent by chrysene and certain compounds of benzanthracene type, the carcinogenicity of which is either very feeble or nil. On the other hand, a series of related non-carcinogenic compounds (anthracene, phenanthrene, 1:2-cyclopenteno-

phenanthrene, 1-keto-1:2:3:4-tetrahydrophenanthrene, dodecahydro-1:2-benzanthracene, pyrene, fluoranthene, triphenylene, dehydronorcholene, perylene, 1:9-benzanthrone and diphenylene oxide) showed no inhibitory influence when tested under the same conditions. Confirmatory results have been published by Morelli and Guastalla (1936), who obtained inhibition of the Jensen and Walker tumours with 3:4-benzpyrene.

There is thus a correlation in compounds of this type between carcinogenicity and growth-inhibitory power, and it is therefore a possibility, as in the case of x-rays and radium, that the mode of action of these substances in tumour production is indirect, and that they operate by producing a prolonged retardation of the growth of normal cells, which eventually react by a process of discontinuous variation to give a new cell race with a greatly increased rate of division. In these experiments the compounds were administered intraperitoneally in colloidal suspension or in solution in oil, in order to secure a satisfactory degree of general absorption. It is important to note that a significant inhibition of somatic growth in young rats was produced by as little as 3 mg. 1:2:5:6-dibenzanthracene, i.e., by an amount well within the range of dosage in which these substances are

used for the production of tumours. There is some evidence to show that the systemic effects are greatly diminished or even absent following subcut-:aneous administration in lard --- as employed for instance in the induction of sarcomata --- although the local inhibitory effect of an active substance under these conditions must be considerable.

Probably the only other experimental work suggesting that the carcinogenic hydrocarbons possess growth-inhibitory properties is that of Lewis (1935) who found that when certain of these compounds (1:2:5:6-dibenzanthracene, 3:4-benzpyrene and methylcholanthrene) were added to cultures of chick embryo tissue, the cells developed photosensitivity, the photodynamic action causing definite changes in the state of the cell protoplasm which were often accompanied by inhibited cell division. In cultures that had approximately 0.05 to 0.1 per cent. hydrocarbon in their media, the cells remained undamaged and the process of mitosis continued in a normal manner while they were studied under dull illumination but, on exposure to bright light, the cells became changed and mitosis was inhibited within two to ten minutes. Cells in cultures which had been growing in the presence of one of the hydrocarbons exhibited much greater photosensitivity than those in cultures to which the suspension of

hydrocarbon had just been added.

Although the carcinogenic compounds used all showed some degree of toxicity, this was of a low order in relation to dose. It was further concluded that their inhibitory effects could not be attributed to toxicity per se, since manifest poisons (e.g., lead, colchicine, anti-rat precipitin) produced an entirely different response, namely, a temporary retardation followed by recovery to the normal growth-rate with or without compensation. A feature of the inhibition was its extreme prolongation, even after a single injection, and it appears that this must be regarded, so far as our present knowledge goes, as a unique type of response to the administration of chemical substances.

Relation to tumour-induction by radio-active agents.

It will be seen that the theory of tumour induction under discussion demands just this type of interference with growth, and it is important in relation to their carcinogenic action that x-rays and radium are also capable of producing a similar inhibition under certain circumstances. The necessary conditions are (a) continuous exposure to a relatively weak source of radiation (as in the experiments of Ross (1936) with radium), or (b) prolonged or repeated exposures. In experiments of the former type it is certain that there must exist a gradient or zones of intensity passing from a maximum at the source of radiation to a minimum in more distant tissues; the corresponding biological changes in most cases must therefore be (a) death of the cells nearest the source if this be sufficiently intense; (b) in cells receiving less intense radiation, a continued arrest of growth without impairment of viability or the capacity to vary; (c) in tissues more distant but affected by the radiation, probably changes of a reversible nature permitting fluctuational adjustment and recovery; and (d) in tissues sufficiently remote from the source, no effect. It is therefore in the cells of zone (b) that the changes required for carcinogenesis are realised, and this being so the

emergence of a tumour will depend only on two further conditions: (a) that the environmental alteration is maintained for a sufficiently prolonged period, and (b) that the affected cells are inherently capable --- as is usually the case --- of undergoing the type of variation which, it is suggested, is the biological reaction to such a change.

From recent experimental and clinical observations on the rationale of radiotherapy (Cramer 1936; Mottram and Morton 1936) it is also clear that, although individual small exposures produce merely temporary inhibition followed by recovery, suitable spacing of such radiation may lead, according to Cramer, to a dormant condition "in which the malignant cells, while retaining their viability, fail to grow."

In connection with the inhibitory reactions produced by the carcinogenic hydrocarbons it is of great interest that the prolonged administration of large doses of oestrin has been described by Zondek (1936 a, b; see also the confirmatory statements of Arguello 1937) as causing considerable inhibition of body-growth: the subcutaneous injection of 5,000 m.u. dimenformon twice weekly in rats 4-6 weeks old retarded growth to the extent of nearly 45 per cent. after about 4 months. Zondek also brought forward evidence that the administration of oestrin under

these conditions produces a physiological inhibition of the anterior lobe of the pituitary; in these circumstances it is remarkable fact that similar treatment has resulted in the appearance of pituitary hyperplasia or tumours in mice in a large proportion of cases (Cramer and Horning 1936; McEuen, Selye and Collip 1936; Zondek 1936; see also Burrows 1936).

The inhibition concept in the literature.

Views similar to those put forward above, and concerning the relation of inhibitory processes not only to cancer induction but also to variation in general, have received expression from time to time in other places. Thus the general principle connecting environmental change with biological response was discussed by Noël 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..... provided that the modification is not detrimental to existence under the altered conditions." To take a concrete example, Sartory, Sartory and Meyer (1926), in a study of the action of radium on cultures of Aspergillus, described the appearance of a sexual form which they attributed to the abiotic action of the radiation....."Le radium par son influence destructrice.....oblige l'Aspergillus fumigatus à reprendre sa reproduction sexuée pour créer une nouvelle race résistante et capable de sauvegarder l'espèce." (see Reiss 1935). Bang (1928) was among the first to recognise that, since the cancer cell arises from the normal cell, its behaviour must be considered in relation to its environment. He also pointed out that most

carcinogenic agents probably act by injury and that pathological cellular proliferation does not of itself suffice to create malignancy. Again, Béclère (1934), in discussing the role of x-radiation in experimental carcinogenesis, gave as his opinion that it is a slow destruction of the activities and life of the cell rather than excitation of its proliferative capacity that terminates in malignancy. Somewhat similar views have been expressed, and a possible physico-chemical explanation suggested, by Crawley (1932). Parkes Weber (1936), in an essay on erythraemia, the leukaemias and Hodgkin's disease as neoplastic mutations of somatic cells, wrote as follows....."Very little seems as yet to be known about the causes of mutation in either germ-cells or somatic cells; but in regard to malignant neoplastic mutation in somatic cells, it may be surmised that hindrance to normal growth (development of cicatricial tissue etc.), together with chronic excessive physical or chemical stimulation ("irritation"), and abnormal nutrition, play an important role." Likewise Witts (1936), in a discussion of neoplasia of the blood-forming organs in relation to toxic agencies such as benzol, x-rays and radium, referred to the question "whether arrest of the normal development of the cells does not sometimes lead to the uncontrolled

proliferation of leukaemia."

With special reference to the mode of action of the carcinogenic hydrocarbons, Willmer (1935) quoted a communication from L.P. Kendal regarding evidence which suggested that the carcinogenic hydrocarbons do not act by causing a direct stimulation of growth "but rather do they slowly alter the character of the cells in such a way that they can afterwards live and proliferate on serum alone." In a recent paper Reimann and Hall (1936) clearly recognised that the stimulus to cell-division by a carcinogenic hydrocarbon may be quite indirect, and that stimulation of the rate of cell proliferat-:ion alone does not lead to neoplasia; they further suggested that tumour production is caused by "damage to the potencies of differentiation and organization." Wolbach (1936) also attempted to ascertain what processes are initiated by 1:2:5:6-dibenzanthracene antecedent to the development of tumours, and he apparently regarded the primary action as destructive.

Inhibition and the natural history of cancer.

i. inhibition and stimulation.
ii. cancer in growing tissues.
iii. influence of age.
iv. the latent period.
v. inheritance of cell stability.
vi. the process of tumour induction.

It is not surprising that the origin of cancer --- as of the non-malignant hyperplasia which frequently precedes it --- has been interpret-:ed as a primary stimulation of growth. In partic-:ular it is widely assumed (e.g., Needham 1936) that the carcinogenic hydrocarbons are growth-stimulating substances albeit of a special type. But although the application of a carcinogenic agent eventually leads to the appearance of new growth, the facts already referred to suggest rather (a) that the primary effect is an inhibition of, or interference with, the mechanism of normal growth; (b) that the end-result --- the appearance of a rapidly growing malignant tumour --- is due to the capacity of the inhibited normal cells to emancipate themselves by a process of irreversible dedifferentiation involving increase in growth-rate. It is obvious that such an explanation raises many questions

regarding the fundamental relations between stimulation and inhibition in general biology, and it is hoped to deal with these in another place.

It will be seen that these views are compatible with certain characteristic features of the naturally-occurring disease. It must be stressed that the increased division rate of the cancer cell should be regarded not as due to the acquisition of a new character but rather as an instance of variation in a property possessed by most normal cells. In Nicholson's words (1926), "excessive growth, and therefore tumour formation, is one of the physiological potencies of every healthy cell." Boveri also looked on malignancy as an unmasking of the inherent capacity of normal cells to multiply indefinitely. Thus cancer is essentially a disturbance affecting growing tissues, and in most cases its maximum incidence falls at a time when the normal growth function is or has been subjected to the progressive retardation produced by age. The importance of senescence in the induction of variants has long been recognised, particularly in bacteria, and Navashin (1933; see also Kostoff 1935) advanced the hypothesis that mutation in certain types of seed is caused by metabolic processes peculiar to ageing. An increased mutation rate from aged Datura pollen

was reported by Blakeslee, Cartledge and Murray (1935; see also Peto 1933; Gerassimova 1935). It is of considerable interest that the chief metabolic change in ageing mammalian tissues appears to consist of a decline in oxygen consumption (Pearce 1936).

Conversely, malignant rarely occurs in cells whose physiological differentiation is such that the growth function is abolished. Thus the two most highly differentiated tissues of the body --- the nerve cells and striated muscle fibres --- are those which least often become malignant, having lost once and for all their power of cellular reproduction.

It has already been noted that the hypothesis demands on the part of the affected cells a certain intrinsic capacity to vary. The conception of relative degrees of genetic or inherent stability is fundamental in the study of variation, and it finds an important parallel in cancer both on clinical and experimental grounds. It is therefore of some interest to conjecture what mechanism or mechanisms are indicated by the term "susceptibility" when used to indicate proneness to the development of tumours whether spontaneous or induced. In the former case it may mean that certain cells have an inborn instability towards

carcinogenic agents which may be present equally in a susceptible or a non-susceptible stock, or on the other hand one may imagine that spontaneous tumours do not occur in a resistant stock because carcino-:genic substances are not formed, although the cells themselves may remain essentially susceptible to the action of such agents. These are perhaps the main or simpler possibilities. Lynch (1927) compared two lines, A and B, which differed in their spontaneous tumour rates, A having a higher incidence of lung cancer than B. These tumours, however, never appeared before the age of fifteen months. By tarring of the skin she induced lung tumours before the age of thirteen months in 85 per cent. of line A and 22 per cent. of line B, the difference being highly significant. Breeding experiments indicated that susceptibility was partly determined by a number of dominant genes. The same author (1936 a, b) also studied the susceptibility of mouse strains to induction of tumours by 1:2:5:6-dibenzanthracene; she found a significant strain-difference in susceptibility to induced lung tumours but not to induced sarcoma. Andervont (1934; also 1935) produced sarcomata by the subcutaneous injection of dibenzanthracene in pure strain mice in which spontaneous tumours apparently did not develop nor-:mally. It was thus indicated that the genetic

constitution of a pure strain of mice does not prevent the cells from becoming malignant when exposed to a suitable carcinogenic agent. Rather similar were the results obtained by Reinhard and Candee (1932) when two strains of mice, one with a high and the other with a low cancer-incidence, responded to tarring with a similar yield of tumours amounting to about 25 per cent. of the animals in each strain. But in this experiment the tar tumours arising in the low cancer strain had a latent period of twelve to fourteen weeks longer than those of the high cancer strain. The latter circumstance suggests that different cells may indeed show various degrees of stability in the presence of a carcinogenic substance, a view receiving further support from the work of Kreyberg (1934). Again, two pure strains were used in tar-painting experiments, and while 69 per cent. of the animals in one strain showed benign warts or papillomas after six months of painting, in the second line no less than 95 per cent. developed similar lesions within the same length of time. In later work (Kreyberg 1935, 1936) evidence was presented which seemed to show that while there are very marked familial differences in the tendency to develop spontaneous or induced tumours, the susceptibility to develop spontaneous breast cancer,

lung tumours and induced tumours is genetically different, and that these tumours have no connection inter se. Although it is obvious that such differences need not exist, or may not be demonstrable, in any type of material (e.g., Dobrovolskaia-Zavad-:skaia 1936), sufficient has been said to show that the various possibilities are not to be regarded as mutually exclusive. In general, the end-result in any given case, namely whether malignant change takes place and if so when, must depend on a number of factors connected with (a) the stability of the cell, i.e., its capacity to withstand environmental change without undergoing discontinuous variation; and (b) the nature, intensity and duration of the environmental alteration itself.

Lastly, the hypothesis gives some indication of the significance of the latent period which is so characteristic of the induction of cancer under both occupational and experimental conditions. But it is intended to apply only to those cases in which tumour-producing agents have been shown to possess growth-inhibiting properties, viz., for x-rays, radium and the carcinogenic hydrocarbons. Other causes may operate by a different mechanism,

although it is probably significant that some of them, e.g., heat, cold and ultra-violet irradiation, are also such as might be expected to produce damage to normal cells rather than a primary stimulation. According to Juul and Kemp (1933) for instance, the effects of gamma-rays, x-rays and ultra-violet light on chick fibroblasts in tissue culture are essentially alike.

At this stage it must be emphasised, although this involves no alteration in the underlying principle, that the change from normal to cancerous is rarely the result of a single step. In most cases, (typified by the induction of skin cancer in mice), the process involves three stages..... (a) an early and reversible hyperplasia; (b) papillomatosis, which may be reversible but usually persists; and (c) the irreversible change to malignancy, involving stabilisation of the variant. Two such stages were clearly recognised by Guldberg (1931) in his study of the induction of tar tumours in mice and rabbits. In the first place, the changes induced in the skin were often found to recede when painting was discontinued, while in the second period (which included the onset of malignancy) they were quite irreversible. These morphological and physiological changes are probably reactions to corresponding degrees of damage to the cell,

partly direct and partly through its environment, which produce an interference with nutrition, and so with growth, which is first reversible but later not.

Biochemical effects of tumour-producing agents.

i. arsenic.
ii. the carcinogenic hydrocarbons.
iii. x-rays and radium.

Thus far the mode of action of cancer-producing agents has been dealt with from the physiological or functional aspect. Some attention must also be paid to the question whether these effects can be further described in terms of biochemical change.

From the literature it already appears that many agents associated with cancer production resemble each other in their effects on living protoplasm. The case of arsenic is of particular interest. In 1928 Barry, Bunbury and Kennaway drew attention to the fact that cancer of the skin might be induced by two wholly different chemical means, namely by arsenites and by high-boiling hydrocarbons. In order to investigate this matter they carried out experiments on the effect of arsenic on various oxidations-reduction systems (hypoxanthine and the xanthine-oxidase of rat or mouse skin, acetaldehyde and colloidal platinum, and propaldehyde or acetaldehyde with glycine and phosphate). Arsenite, in concentrations similar to those of arsenic in the skin in arsenical hyperkeratosis, was found to have

a retarding action on these systems. As a result it was suggested that arsenic induces cancer not by any direct action but by causing the accumulation in the tissues of some organic compounds which would otherwise be oxidised or reduced to other forms. The subject was carried a step further when Boyland (1933) showed that arsenite inhibited lactic dehydrogenase, and that derivatives of carcinogenic hydrocarbons, chemically quite unrelated to arsenite, had a similar effect on this enzyme. Boyland also quoted the work of Szent-Gyorgyi (1930) on the inhibitory effect of arsenite on respiration in liver and yeast. There is however no specificity in the inhibitory action of these carcinogenic agents on lactic dehydrogenase, since Quastel and Wheatley (1931) found that certain dyestuffs had a similar effect. Thus malachite green and acriflavine in dilutions of 1 in 5000 inhibited 90 per cent. of the respiration of B. coli in the presence of lactate. Boyland further found that 1:2:5:6-dibenzanthracene required activation before an effect could be demonstrated; thus a solution which previously had no effect on lactic dehydrogenase had a marked inhibitory action after ultraviolet irradiation. Solutions irradiated anaerobically remained inactive, and it was concluded that carcinogenic substances are capable of oxidation to acidic substances which can then inhibit dehydrogenating enzymes such

as the lactic and succinic dehydrogenases of yeast and muscle extracts. Activated 1:2:5:6-dibenz-:anthracene proved sixty times as active an inhibitor as arsenite when compared in presence of lactate. Moreover, the oxidised hydrocarbon destroyed or inactivated a definite amount of enzyme and therefore differed in its effect from arsenite which competes with the substrate. In preliminary experiments (Boyland 1932) the compounds were dissolved in benzene and the inhibitory action seemed to run parallel to the carcinogenic activity of the substance oxidised. But further work (Boyland 1933) with more than fifty compounds in another solvent (cyclohexane) showed that the parallelism was subject to many exceptions, since some of the non-carcinogenic compounds, including anthracene, were oxidised to powerful inhibitory products. In continuation of these experiments, Boyland and Boyland (1934) studied the effect of a water-soluble oxidation product of 1:2:5:6-dibenzanthracene on the respiration and glycolysis of various rat and mouse tissues both normal and malignant: an inhibitory effect was observed in all the cases tested. Pourbaix (1933) also found that 1:2:5:6-dibenz-:anthracene reduced the respiration of guinea-pig liver <u>in vitro</u>. With regard to carcinogenic substances other than the hydrocarbons, it is of

interest that Dickens (1936), studying the action on metabolism of the trypanocide styryl 430, (Browning, Gulbransen and Niven 1936), found that this substance produced a large increase in the respirat-:ion and glycolysis of rat brain; the effect on other tissues was less marked.

Of rather less direct interest though none the less fundamental in the study of metabolism and growth, are the important experiments of Clowes and Krahl (1936) and Krahl and Clowes (1936). These workers, in describing the stimulation of oxygen uptake in fertilised sea-urchin eggs (Arbacia punctulata) by the nitrophenols, made the important observation that a reversible inhibition of cell division commenced at the point of maximal respirat-:ory stimulation. 4:6-dinitro-o-cresol gave an effect at a concentration as low as 10^{-6} molar, and the response remained reversible up to concentrations one hundred to five hundred times those required for initial inhibition. They regarded the phenomenon as due either to (a) activation or inactivation by the nitro compounds of one or more substances connected with metabolism and cell division, which they looked on as more likely, or, (b) an accumulation, in high local concentration, of an intermediate metabolite unfavourable to division. A similar principle applied to the effect produced by o-cresol indophenol, methylene blue, neutral red, dimethyl-p-phenylene

diamine, tetramethyl-p-phenylene diamine, and pyocyanine, although in these cases the inhibition of division was irreversible and death of the cells resulted. The investigation was extended so as to include the dihalo- and trihalophenols and phenols containing both halo- and nitro- substituents in the same molecule (e.g., o-nitro-p-chlorphenol, 2:6-dichloro-4-nitrophenol, 2:6-dibromo-4-nitrophenol), and additional compounds such as 2:4-dichloro-naphthol, 2:4-dichlorothymol, 2:4-dibromothymol, 4:6-dibromocarvacrol, and 5:7-dibromo-8-hydroxy-quinoline. Essentially comparable results were obtained, although interesting points of difference emerged between the halophenols and nitrophenols. All the experiments supported the likelihood that biological activity was due to the presence and properties of the phenolic OH group.

In view of the similarity between gamma rays and the tumour-producing hydrocarbons in respect of their carcinogenicity and inhibitory properties, it becomes of importance to compare the inhibiting effects of the hydrocarbons with those obtained in similar experiments with x- and gamma-rays. Havard (1934) found that large doses of radiation (up to 20,000 r) did not inactivate the "respiratory enzyme" or indophenol oxidase, although it had been shown by others that a similar dose would inhibit the respiratory processes of living cells. In a later paper (1935) he showed that similar doses had no destructive effect on the lactate, glucose or citrate dehydrogenases, although similar experiments with succinic dehydrogenase gave a partial inhibition, confirming a previous result obtained by Crabtree (1933). Havard concluded that the inhibition by x-rays of the respiration of living cells is probably not due to their effect upon the enzymes employed in respiration but to some other factor, possibly to an effect upon the properties of the interphase surfaces surrounding and within the cell. Holmes (1935), in experiments on the action of radium on the metabolism of tissues growing in culture, obtained inhibition of the lactic dehydrogenase of young though not of older embryos. Harker (1936) reported work on the influence of radium upon the inverting capacity of yeast. The radiation --- which however consisted of some

penetrating beta-radiation in addition to the gamma-rays --- produced a considerable reduction in inverting power. On the other hand, irradiation had scarcely any effect on the inversion produced by yeast treated by toluene; the inference being that the action is due to some effect upon the living cells rather then upon the enzyme. Crabtree (1936) described an increase in the formation of ammonia by tissues subjected to the action of radium in vitro, and suggested that this is due to a secondary utilisation of protein as a result of impairment of carbohydrate metabolism. In a discussion of the effects of ultra-violet light on living cells Heyroth and Loofbourow (1934 a, b) regarded the destruction of cell enzymes as unlikely to account for lethal action, but they suggested that destruction of compounds of the cell nucleus, such as thymus nucleic acid, adenine and uracil, might be of great importance. In the case of x-rays the further possibility has been considered (Erdmann 1935) that the inhibition of cell division is due rather to a decrease of the swelling power of living tissue which is necessary for growth. The problem has been well summarised by Crabtree (1935). "Many attempts have been made to attribute the action of radiation to its predominating effect on some single cell structure or physico-chemical equil-:ibrium within the cell. Any such specific action

seems improbable on general grounds, but it seems clear that damage to certain vital structures, with low powers of recovery, would lead to general impairment of cell function. In this sense it is possible to speak of selective damage to definite cell systems of paramount importance in cell economy."

Ischaemia in tumour induction.

Orr (1934, 1935) found that the induction of tumours of the skin in mice by coal tar or 1:2:5:6-dibenzanthracene was appreciably accelerated by interference with the vascular supply by subcutaneous fibrosis or by the repeated injection of adrenalin. These findings are of further interest in relation to special cases of cancer development as for instance in cicatrices and the scars of burns (e.g., Treves and Pack 1930, Roffo and Gandolfo 1933). In later experiments Orr (1937) employed intra vitam injection of phenol red as a method for the demonstration of local ischaemia during the course of carcinogenesis with tar, 1:2:5:6-dibenzanthracene and 3:4-benzpyrene. The earliest change was an increase in the intensity of staining throughout the area treated with the carcinogens, but at a later stage foci in the treated skin stained yellow instead of red, and tumours began to appear at the same time. The progress of this staining phenomenon was closely associated with the rate of tumour induction, and a high proportion of the tumours first appeared in relation to yellow areas. A further observation of the greatest significance was made in these experi-:ments, namely, that rapid growth of tumours was in general associated with disappearance of the yellow colour, while the persistence of the colour was frequently accompanied by retarded growth or actual

regression of warts. In Orr's own words, "the conflicting relationship of yellow staining, on the one hand to the appearance of tumours and on the other to their subsequent development, is of interest in that it supports the view that the conditions leading to the inception of the cancerous process are not all the same as those favouring its spread in the body. The provisional interpretation of the present results is that the yellow colour is brought about by functional ischaemic conditions which interfere with the removal of acid metabolites from certain regions treated with carcinogens. It is argued that these ischaemic zones are the most likely places for tumours to arise, i.e., for the neoplastic change to take place in the cells. Persistence of the ischaemic conditions may make it impossible for the tumour to grow, so that regression or "spontaneous cure" takes place. For the further increase of the tumour, it appears that the establishment of better circulatory conditions is an advantage."

When taken together, such results give at least some indication of the biochemical changes which may be expected to play a part in tumour induction, whether by gamma-rays or the carcinogenic hydrocarbons. In particular they appear compatible

with the principle enunciated by Warburg (1930 a) that "interference with the respiration of growing cells is, from the standpoint of the physiology of metabolism, the cause of tumours."

Recapitulation.

The present position may be recapitulated briefly as follows. The malignant cell is a discontinuous and irreversible variant. When produced by x-rays, radium or the carcinogenic hydro-:carbons, variation of this type apparently represents a mechanism whereby the cell effects a release from long-standing interference with growth and metabolism, characters in which the new race shows pronounced differences from the normal. There can be no doubt that the increased growth-rate is the most striking feature of the change from normal to malignant, and it is reasonable to suppose, on the argument already pursued, that this is the cellular reaction to chronic inhibition of the normal growth mechanism. The latter process must in turn be dependent on some disturbance of metabol-:ism which precedes or accompanied it. While x- and gamma-rays have been shown to produce interference with the metabolism of normal cells, it is not yet clear which aspects of metabolism are most directly connected with growth. It must be pointed out for instance that interruption of growth may be accomplished by the use of x-rays without any accompanying change in respiration or glycolysis. Crabtree (1935) regarded the action of short-wave radiation on rate of growth as a complex process

which he described in three phases.....(a) the primary physical effects of atomic excitation, ionisation and disintegration, with resultant physico-chemical and chemical changes; (b) the primary biological effects such as altered permeability or changes in viscosity, pH, state of protein aggregation and enzyme activity; (c) the final response of the affected tissues.

Irritation in relation to the induction and inhibition of tumours.

It is now widely acknowledged that, while types of chronic irritation may lead to the development of cancer, irritation is not in itself the fundamental factor. This is shown by the fact that the majority of irritant substances are not carcinogenic and that the most powerful carcinogenic compounds have little or no irritant action. In the present discussion stress has been laid on certain conditions which appear to be necessary for tumour induction, the most important being that the preliminary inhibiting effects must be such as to allow the survival of most of the affected cells. It seems probable that while a certain degree of chronic irritation may fulfil this condition and so permit malignant variation to occur, irritation of an excessive or more acute type will entail damage and death of the cells to such an extent as to restrict or even prohibit the occurrence of variation or to hinder the survival of variant cells. As early as 1923 Murray drew attention to the fact that an intensity of irritation greater than the optimum is as detrimental to the production of tumours as an insufficient intensity. A similar result was described by Schürch and Winterstein (1935) who found that while painting with one per

cent. benzpyrene in benzene thrice weekly produced considerable local injury to the skin with ulceration but no tumours, and high mortality from toxic action, half this concentration twice weekly produced carcinoma in 70 per cent. of the animals, while with 0.1 per cent. twice weekly only 26 per cent. developed tumours. The best concentration range appeared to be from 0.2 to 0.5 per cent.

Reference has already been made to the finding of Berenblum (1930) that the successive application of two different tumour-producing agents (tar and repeated freezing) during two consecutive periods produced a summation of carcino-:genic effect. But it was also found that when repeated mild freezing was applied simultaneously with tar treatment an inhibition was produced and not acceleration as might have been expected. Incidentally it is of interest that the destructive action of carbon dioxide snow has been employed in the treatment of skin cancers (e.g., Epstein 1933). In the course of further experiments along these lines it was discovered by Berenblum (1929) that ββ'-dichlorodiethylsulphide, (mustard gas), when applied in concentrations which produced only a mild reaction free from ulceration, inhibited the inception of tar tumours of the skin in mice. (In a recent study Dorffel and Popping (1935)

described the effects of mustard gas on the skin of rabbits and young pigs. The changes involved local hyperaemia and oedema, followed by vesication, leucocytic infiltration, necrosis, ulceration and slow healing. These workers likened the lesion in man and animals to a third degree roentgen burn.)

The inhibitory effect was further shown by Berenblum to be a local one limited to the period of time during which the mustard gas acted, and to be due to a direct action on the tissues and not to any chemical interaction involving inactivation of the tar. When once a wart appeared, however, its continued development was not significantly affected by subsequent applications of mustard gas in a concentration of 0.1 per cent. Several compounds closely related to mustard gas gave pronounced inhib-:ition of induction, while others had no such effect. Among a group of irritants not related to mustard gas, cantharidin alone was found to possess this property. Berenblum (1931) considered the possibility that the effect was due to superoptimal irritation but thought that some of his experiments failed to support this view. He stated (1935) that of the substances tested, all those which inhibited tumour induction were irritants, although all skin irritants did not necessarily inhibit induction; and he regarded it as not improbable

that irritation plays an essential part in the process of anti-carcinogenic action, provided the irritation is of a particular kind. According to Berenblum, Kendal and Orr (1936) ββ′-dichlorodiethyl-sulphide, ββ′-dichlorodiethylsulphone and cantharidin, which inhibit the induction of tar tumours, reduce the glycolysis of minced Jensen tissue in vitro more than its respiration, while ββ′-dichlorodiethyl-sulphoxide, thiodiglycol and croton oil, which have no inhibitory effect on carcinogenesis, do not reduce glycolysis more than respiration. On the other hand ethylene-bis-β-chloroethylsulphide, which inhibited the induction of tumours, had no selective action on glycolysis.

Nature of the relation between induced tumours and their causal factors.

According to the general argument pursued thus far, cancer cells arise and commence their career of proliferation under conditions which impair the life of normal cells. This they probably accomplish in virtue of their altered metabolism, which apparent-:ly has distinct survival value and in effect confers a biological advantage upon those cells which show 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. In the study of variation in bacteria attention has been drawn to the fact that variants arising by dedifferentiation are frequently more resistant than the parent form (as in the S----> R transformation) and it is possible that a not dissimilar relation exists between corresponding normal and malignant cells. It has been stated for instance that "though there are many examples of the greater viability of cancer cells as compared with the normal, there is no evidence as yet available of a single property in which they are more vulnerable." (see 29th. Ann. Rept. Imperial Cancer Research Fund,). Such statements obviously refer to a condition of general or non-specific resistance, and no information is yet available as

to whether more specific relationships may exist. It would thus be of interest to know the radio-sensitivity or radio-resistance of a number of experimental tumours induced by x- or gamma-radiation. The evidence from human cases of roentgen carcinoma is unfortunately quite inadequate for a number of reasons. First, it is usually impossible to assess the relative importance of the radiation itself and of the secondary radio-dermatitis in the causation of such tumours. Secondly, proof of the true malignancy of the lesions is often absent. Third, the effect of treatment is often complicated by the poor healing powers of the damaged normal tissues. It is probably for the last reason that several authors (e.g., Bordier 1926, 1927; Nicolas 1930; Holfelder 1933) have recommended diathermy or electro-coagulation in the treatment of these cases, although Laborde (1931), Roffo (1932) and Roffo and Aravena (1932) have reported the cure by radium of tumours arising on the basis of radio-dermatitis. Such data are obviously insufficient to yield an answer to this important question, and the problem is essentially one for experimental approach, through the study (a) of the radio-sensitivity or otherwise of radiation-induced tumours, or (b) of the relative sensitivity or resistance of the cells of tumours induced by

individual carcinogenic hydrocarbons when exposed to the inhibitory action of the same and related compounds. If examples of resistance should be found, further problems will of course arise regarding the degree of specificity in any given case and the permanence or otherwise of the resistant state during the course of propagation of the tumour by transplantation.

Sensitization and tumour induction.

Before passing on to discuss the relation of the present position to other theories, mention must be made of yet another aspect of the mode of action of tumour-producing agents. While most of the changes involved are probably due to a direct action of the carcinogenic agent on the cell, some attention has been devoted to the possibility that sensitizat-:ion processes may be involved. Thus in the opinion of Hueper (1936), "allergic as well as neoplastic cells originate in most instances from normal cells which have acquired under the repeated and prolonged influence of certain agents new biological qualities, as a result of which they react in a manner fundamentally different from normal cells to specific and non-specific stimuli." According to present conceptions of the causative mechanism of allergy, it appears necessary to assume that these chemicals first combine in the body with proteins, thereby forming antigens, of which the aromatic amine is the hapten, before histogenic sensitization can occur. Hueper discussed the allergic hypothesis mainly in connection with the occupational bladder tumours produced by aniline dye intermediates, and he recognised of course that the great majority of allergies have no relation to neoplasia. It is of considerable interest in

the same connection that Landsteiner and Jacobs (1935), in experiments on the sensitization of guinea-pigs with simple chemical compounds, obtained positive results after the administration of small amounts of substances such as 1:2:4-chlorodinitrobenzene, p-nitrosodimethylaniline, 1:2:4-trinitrobenzene, picryl chloride, four dichlorodinitrobenzenes and a number of other aromatic compounds, but not with 1:2:5:6-dibenzanthracene. In a later study of azoproteins derived from aromatic hydro-:carbons Jacobs (1937) described the serological reactions of such substances from p-aminodiphenyl, β-naphthylamine, β-anthramine and some compounds containing two benzene rings (aminodiphenylmethane etc.).

Whether the cellular reaction to cancer-produc-:ing substances is relatively direct or involves sensitization, it is of possible significance that, according to Bradley (1936), the type of molecular arrangement found in the carcinogenic hydrocarbons is also effective in determining the dyeing properties of organic substances, being present in "substantive" dyes, that is, dyes which can be employed without a mordant. Thus the type of relation between the structure of an anthraquinone derivative and the affinity of its leuco-form for textile fibres is analogous to that between chemical

structure and carcinogenicity in the corresponding polycyclic hydrocarbons; and the same angular orientation of fused rings appears to be equally significant in determining the dyeing properties of anthraquinone derivatives.

Cellular inhibition in relation to other theories.

I. The theories of Virchow and Cohnheim.

i. limited validity of the irritation hypothesis.

ii. embryonal tumours.

According to Murray (1933), "the experimental confirmation of Virchow's chronic irritation theory in the last twenty years has established its validity as a concise description of the emergence of cancer after prolonged, localised slight irritation of the tissues by a variety of agents, and nothing more." The effects of irritation have been described above with regard both to the induct-:ion of cancer and to inhibition of its appearance, and emphasis must once more be laid on the essential conditions which govern the association of irritation and malignancy, namely, that the irritation must be of long duration and optimal intensity. But even when these limitations have been appreciated, little attempt has been made in the past to analyse the immediate physiological consequences of such irritation, and a primary stimulation of growth has been widely assumed. Such is probably the reverse of the truth, and, further, the great advances in the chemistry of cancer-producing substances have

made it clear that irritation in the usual sense is non-essential to tumour induction and is not in fact produced by the most actively carcinogenic compounds in highly purified form. But where it is effective the process of chronic irritation is usually easily appreciated and intimately associated with the cause of the cancer which later results. It is suggested that it represents a variety of low-grade destructive processes of such an order as to permit the survival of a sufficient number of affected cells and so allow the emergence and survival of an advantageous variant. In the case of induction by the carcino-:genic hydrocarbons the gross or lethal effects of irritation are absent, and it is suggested that these pure compounds produce their effect by a much more delicate interference with growth and metabolism without the proportionate toxic and lethal effects which follow less specific types of damage. Cellular inhibition is thus the significant common factor in those irritative processes which Virchow defined, on account of their prominent clinical and pathological associations with the onset of cancer, as the main cause of the disease.

Shaw (1932) regarded cancer as "the process of growth in cells which have either failed congenitally to come under those influences which determine cell

function and form, or which, after being subject to such differentiating influences, have become independent of them owing, apparently, to a prolonged change in environment." There are therefore a number of cases in which the cancer cell is not derived from cells of normal differentiation but is rather the descendant of embryonic cells which failed to differentiate and which therefore retain their early rate of growth. Such an origin obvious-:ly involves no process of transformation, and Shaw referred to it under the head "continuing", "in which an embryonic cell group fails to come under the influence of the mechanism of differentiation and continues to grow in its natural primitive state..... .....The outstanding example is the renal blastoma or embryoma, which, often, cannot be accurately classified as carcinoma or sarcoma because the stage of differentiation into germinal layers has not been attained." The renal embryomata were studied in particular detail by Nicholson (1926) who regarded them as congenital cell rests or enormously enlarged remnants of the renal blastoma of the embryo. He concluded that their cells have always remained at an early stage of embryonic development and have never proceeded beyond this stage of differentiation, and that these cells retained the primitive physiol-:ogical function because they were prevented from undergoing the process of differentiation on account

of an environmental fault at an earlier stage of their life history.

Notwithstanding the importance of these views there can be little doubt that there may also exist cell rests of higher grades of differentiation which find themselves arrested in an essentially foreign environment. Tumour formation in these cases must obviously involve a certain degree of conversion which it would appear is quite absent in the purely embryonal tumours already discussed. It is reasonable to conclude that Cohnheim's views still possess limited value, although their extension to include all manifestations of the cancer process can receive little support.

II. Genetic theories.

i. Boveri's theory.
ii. somatic mutation theory.
iii. chromosome mutation.
iv. gene mutation.
v. cytoplasmic alterations.
vi. technical objections.

The conceptions of genetics have an all-important application in any attempt to decide the exact biological nature of the relationship which has been shown to exist between a malignant cell and the normal somatic prototype from which it is derived. The development of such an application may be traced from the observations of Boveri on multipolar mitosis in double-fertilised sea-urchin eggs. The unequal distribution of the chromosomes to the daughter cells in some cases was followed by abnormal growth, resulting, he thought, either from an excess or a deficiency of the normal amount of chromatin. In 1902 he was led to postulate what may be called the aberrant chromosome theory of cancer causation (see Boveri 1929), although the initial causes responsible for the assumed unusual distribution of the chromosomes in tumour formation were at no time made clear. Thereafter the malig-:nant cell came to be regarded as a variant of the

normal produced by a definite chromosomal re-arrangement or by gene mutation (e.g., Bauer 1928, 1932). Although such theories probably expressed, albeit imprecisely, a large part of the truth, and in any case represented a stimulating contribution, they are open to certain objections which may now be discussed.

The cytological examination of tumour material has revealed a large variety of conditions of the chromosomal apparatus, varying from apparent normality to extreme degrees of disorganisation. Ludford (1930 a) described a striking abnormality of mitosis, consisting of chromosome formation without spindle development, in cells of the Jensen rat sarcoma, the mouse sarcoma 37-s, Fibiger's tar sarcoma and the mammary tumour 63, although it was absent in other tumours. He also found (1930 b) a considerable variation in the shape of the chromosomes of transplantable tumour cells. In chromosome counts the normal diploid number was found, as well as much higher and lower numbers. The extent of the deviation from normal varied in the tumours studied, and repeated rapid transplant-:ation failed to eliminate these differences. In a study of the malignant cells of the Walker rat sarcoma 338 Lewis and Lewis (1932) found that these differed cytologically from normal fibroblasts in that they were larger, their cytoplasm denser and

more granular, and the mitochondria smaller and more numerous. There were more cells with two or more nuclei than are found in cultures of normal fibroblasts, and the number of atypical, abortive and abnormal mitoses was far greater than in cultures of normal cells. The chromosome number was subject to great variation, while aberrant chromosomes were common and tended to form vesicles. According to Politzer (1935) there are undoubtedly carcinomata the cells of which possess the normal chromosome content. On the other hand, polyploid values are often found and hypodiploid and hypotetraploid forms are most frequent. But since abnormal mitoses can be found in tissues which are not malignant and since abnormality is not constant even in cancer tissue, such morphological changes are by no means diagnostic. Politzer nevertheless regarded them as suggestive, since they are much more frequent in cancer than in rapidly-growing but non-malignant tissues. Ishibashi and Haroda (1933) counted and tabulated the number of chromosomes in a series of human and animal tumours, and found a wide range of variation. These findings they discussed in connection with Boveri's theory of chromosome changes and somatic characters, and they reached the general conclusion that tumour development is in some way related to chromosome disharmony.

Many have been impressed by the fact, already referred to, that agents --- and particularly gamma-radiation --- which are employed experimentally in the induction of mutations are frequently associated with the development of cancer. Mottram (1934) studied the effects of certain cancer-producing substances on chromosomes, although he was unable to show more than fragmentation and delayed migration in late anaphase, changes which are non-specific and which occur, as he pointed out, under a variety of other experimental conditions (exposure to ether, carbon dioxide, etc.) not necessarily connected with cancer induction. The relation of radiation to genetics and the mutation theory of cancer was discussed by Desjardins (1934), and among studies on the effects of x-rays on chromosomes may be mentioned those of Huskins and Hunter (1935) and Marshak (1935). It is also of importance that Lewis (1935) drew attention to abnormalities of mitosis (chiefly due to inter-:ference with the mitotic spindle) occurring in her work on the influence of some of the carcinogenic hydrocarbons on cell growth <u>in vitro</u>. Similarly Hearne (1936) found that cultures of mouse fibro-:blasts after three days' growth in a medium contain-:ing approximately 0.005 mg. methylcholanthrene per c.c., contained a high percentage of abnormal mitotic figures of various types.

Apart from mutation affecting a whole chromosome, gene-mutation must also be considered. Jones (1936) has summarised the evidence to show that there exist growth-regulating genes, capable of nor-:mal transmission according to the known rules of heredity. The infrequently occurring mosaics in both plants and animals show that changes in the genetic constitution in different parts of the organism may result in differences in growth, of which the fowl mosaics described by Roberts and Quisenberry (1935) and some of the plant chimeras are examples. Again, tumour-formation in Drosophila may be the result of mutant genes or some similar genetic change (Wilson 1924; Stark and Bridges 1926). Gene multiplication may result from aberrant chromosome distribution producing a duplication of parts or of whole chromosomes. There is some tendency for duplication to augment size but little to indicate that unregulated growth may result from this process. If on the other hand a gene loses the ability to propagate itself without stopping chromosome- and cell-division this gene will be lacking in all but one of the resultant cells. Losses of single genes in this way by inactivation may bring about no visible change in the chromosome, and in most material such losses may seldom or never be detected by present means of investigation. In Jones' opinion these small losses

are more likely to permit growth and development than visible deficiencies or other chromosome aberrations. That the change in the cancer cell is a gene-mutation as opposed to a chromosome-mutation must be regarded as a possibility in those cases in which there is apparently no alteration in the chromosome complex. On the other hand, the assumption of gene-mutation has certainly made the general mutation hypothesis invulnerable, in Murray's words (1933), "by withdrawing it from the possibility of experimental proof or disproof." Murray regarded the main objection to the gene-mutation theory to be the necessity involved of assuming a large number of constituent units in each gene to allow for the great number of slight but permanent modifications presented by the new growths of any one tissue. But it seems that this objection may very easily disappear as our knowledge of determination by genes increases.

Modern cytological work shows that normal diploid cells, sub-diploid cells, and the most irregular polyploid cells up to real giant cells may occur in one and the same tumour, whether spontaneous or induced. According to Mohr (1934) it is just this irregularity of the chromosome equipment which seems difficult to bring into accord with any aspect of the mutation theory. "Both in

the individual organism and when transplanted, each tumour exhibits an astonishing degree of constancy and specificity. As will be understood, this pronounced uniformity of the tumour tissue as regards general phenotypical characteristics is just the opposite of what we would expect from the exceedingly variable chromosome relations of the tumour cells. It seems more likely that the chromosome irregular-:ities are a consequence of the change that has occurred in the cancèr cell rather than its cause. On the whole I fear that in the somatic mutation theory of cancer development we are dealing with rather superficial conclusions from analogy which hardly lead us any further as long as our knowledge of the growth and cell differentiation processes proper is as limited as it is to-day."

Lewis and Lewis (1932) suggested that malignancy may be bound up with permanent alteration of the centrosomal apparatus rather than with abnormal chromosome complexes, the latter being regarded rather as a result of the former. Later, Lewis (1935) gave it as his opinion that malignancy is due to alterations of the cytoplasm rather than to changes in the chromosomes or genes. In this connection it is of some interest that Whitaker (1934), in describing spontaneous tumours occurring in the F1 hybrids between Nicotiana glauca and

N. langsdorffi, ascribed them to a cytoplasmic disturbance caused by the introduction of chromosomes of the latter plant into the cytoplasm of the cells of the former. Certain results obtained by Moore (1933) may also be of great importance in deciding whether cleavage rate is a function of the cytoplasm or of the nucleus. Advantage was taken of the fact that the eggs of Dendraster can readily be fertilised with the sperm of Strongylocentrotus, although the eggs of these echinoderms possess widely different segmentation rates. Cross-fertilised eggs had the cleavage rate of their own species, and were unaffected in this respect by the sperm. It was concluded that neither sperm nor egg nucleus has any effect on segmentation tempo, but that the reactions of the cytoplasm alone determine it. The suggestion was further made that the cleavage reaction depends upon a substance of granular character in the cytoplasm.

It can be postulated in view of these facts that the extent of dedifferentiation in the cancer cell may range from a stage involving no apparent morphological derangement of mitosis to one in which the chromosome mechanism is completely disorganised although the cells divide with considerable vigour and continue to transmit their specific characters to their descendants with great precision. It must also be remembered however that the most

abnormal cells of a tumour may well have lost their viability, and on this account the problem is possibly less difficult than actually appears.

Considerable objections to the use of the term "mutation" in cancer have been raised by the geneticists. According to Haldane (1933) "Boveri's hypothesis, that the difference between a cancer and a normal cell is of the same character as that between the cells of two different varieties, that is to say, due to chromosomal aberration or gene mutation, cannot be proved or disproved by genetical methods; for cancer cells do not reproduce sexually, and it is only by sexual reproduction that the geneticist can distinguish nuclear from plasmatic changes or virus infections." Much of the difficulty arises from the use of the term --- which after all need only signify change, and has no other necessary implications --- in a limited sense without adequate definition. Confusion has therefore arisen through inadequate appreciation of, or respect for, the technical use of the term, with all its added implications derived from plant and animal genetics. Further, many geneticists appear disinclined to allow the use of the term in any but its artificial or technical sense. Thus Haldane took particular exception to a statement (Lockhart-Mummery 1934) that "the proof of a mutation

having occurred in a cell, is that all the descendants of that cell will exhibit the changed characters, or will transmit the character to their daughter cells." In his actual words (see Jour. Path. Bact., 1934, 38, 507), "no geneticist would accept this criterion. The proof that a change is due to a gene mutation can only be given if the altered gene segregates from its normal allelo-:morph at meiosis according to Mendel's laws, and this proof is clearly inapplicable to malignant tissue." Haldane also pointed out that each of several types of tissue cell and leucocyte reproduces its kind, and would therefore satisfy the above criterion of mutation, although there is no evidence that the changes involved in the normal processes of differentiation are due to the alteration of a single gene. It seems clear that the term cannot be used in carcinogenesis with the connotation which it has in genetics, and in the opinion of certain workers (e.g., Foulds 1934) it is wiser on that account not to use it at all.

The relation between the normal and malignant races of a given cell lineage has an accurate parallel in the case of stable bacterial races which may appear discontinuously in the propagation of a pure culture. Certain biochemical, serological and physiological

differences may distinguish these races, (as, for instance, constant differences in division-rate in standard media), yet while there may be little doubt as to the fact of the emergence of one form as a true variant of the other, and although the variant on account of discontinuity and subsequent stability may be dubbed "mutation" with a certain practical justification, a completely precise proof of the real relationship is impossible. The very absence of sexual reproduction in the bacteria, which enables one to exclude hybridisation as the source of these variants, in turn prevents any satisfactory demonstration of the truth of factor mutation.

It must again be stressed that the frequency of tumour formation is increased by physical and biolog-:ical agents which increase the frequency of gene mutation and chromosome aberration. There is little evidence which refutes the possibility of a genetic change in the cell as the starting point of malignancy, and much evidence that points directly to the alteration or loss of growth-regulating genes as the primary factor (Jones 1936). In particular there are few facts giving serious support to the idea that the malignant cell represents a purely plasmatic variation from the normal; even so the change is obviously no temporary modification, since the individual structural and physiological

characters of most tumour strains have proved beyond doubt to possess remarkable stability. There remains the possibility that the malignant cell is a pathological variant induced or even maintained by the action of a virus. While certain facts in support of this view are admittedly striking, at present they still fall short by far of proving the truth of the infective hypothesis. To the writer's mind the most probable relationship seems to be that between a somatic cell and a true somatic mutation. There can be no justification for the use of the term without adequate definition, but the word mutation may be of great service in cancer if it is used, in stressing the undeniable fact that the malignant cell is a variant, to indicate its most probable biological type. It may be noted that a definition of mutation sufficiently wide to include micro-organisms was given by Gates (1915).

In cancer as in the whole field of general biology more attention has been paid to the differences between variant cells than to the precise conditions of their origin. It is suggested that the most significant environmental precursor of the origin of cancer is an interference with the power of growth, and it is hoped that the conception of inhibition may prove of service in a wider application to the origin of variation in general.

III. Warburg's hypothesis.

Warburg (1930 a) suggested that the origin of tumours might be referred to the development of a non-oxidative type of metabolism in tissues partially asphyxiated by various morbid processes. "If the respiration of a growing cell is disturbed, as a rule the cell dies. If it does not die, a tumour cell results. This is no theory, but a comprehensive summary of all the measurements at present available." Thus far such views are compatible with the present hypothesis, interference with growth being stressed in the latter case rather than inhibition of metabol-:ism. But in the presentation of his hypothesis Warburg attributed undue importance to selective processes and later appeared to under-estimate the essential factor of transformation and to confuse its role with that of selection. According to Cannan (1927), "Warburg regarded a tissue as a population of cells having wide individual differences in their respiratory and fermentative activities and contain-:ing some few with all the metabolic characteristics of neoplastic cells. In the economy of the whole tissue their high fermentative activity would make no appreciable contribution owing to their very limited number. Should, however, a local condition of chronic anoxaemia occur, the majority of the cells would be killed, since they would be unable to

respire, but the few cells which possessed sufficient glycolytic activity to derive their energy from fermentation processes would survive and proliferate, and from them might develop a malignant growth." Thus Warburg appeared to assume cancer cells as present in all normal tissues, and to regard the exciting cause for their proliferation as a sustained local lack of oxygen, "anoxaemia" taking the place of "irritation" in the older theories. Some confusion between selection and conversion is apparent in the following passage (Warburg 1930 a). "It is expedient to start from the fact that every tissue in a stationary condition has a slight glycolytic action. There is nothing to prevent us from assuming an irregular distribution of the glycolysis, and ascribing to some cells embryonic glycolytic action, and to the bulk of the cells no glycolytic action whatever. If such a mixture of cells is subject to lack of oxygen --- owing to pressure, sclerosis of the vessels, presence of bacteria, or other circumstances, --- those cells which have no glycolytic action <u>must</u> perish, while those cells which exert glycolytic action <u>may</u> survive. Let us assume that some of them actually do so, and are consequently in a position to make use of the energy set free by glycolysis, and to grow at its expense. Then, if the lack of oxygen is chronic, tissue will develop which possesses the glycolytic

action of embryonic tissue, but, since it has grown under conditions of lack of oxygen, its respiration is inadequate; it is in fact tumour tissue. We have seen.....that it is mainly the respiration that is impaired by lack of oxygen." In a later note (1930 b) Warburg again drew attention to the fact that when cells are injured so that they die, respiration is decreased before glycolysis, and a stage of aerobic glycolysis results. But he also recognised that while the asphyxiation of embryonic tissue may produce a type of metabolism resembling that of tumours by an irreversible injury to respiration without corresponding interference with glycolysis, the tissue does not behave like tumour tissue on transplantation. He classified the biological effects of respiratory injury as follows: (a) death of the affected cells; (b) temporarily inco-ordinate growth with aerobic glycolysis, as for example in granulomata; or (c) cell growth and continued division, with inheritance of the abnormal metabolism, i.e., tumour growth. The characteristic of the latter is therefore that the capacity for cell-division persists in spite of an abnormality of respiration and that both properties are transmitted to the daughter cells unchanged.

Warburg's hypothesis is of greatest significance in the present connection for the stress it lays on functional inhibition of the cell as an essential prelude to the emergence of a metabolic variant. It was perhaps natural that Warburg regarded the preliminary asphyxiation as operating by mere selection of those cells most highly endowed with glycolytic properties. Some of the objections to such an interpretation have already been cited, but it must again be emphasised that when the subject is regarded from the aspect of growth rather than metabolism, the difficulties in the acceptance of a purely selective mechanism are greatly increased. While all tissues may contain a small proportion of individual cells with highly developed glycolytic powers --- and at present this must remain as an assumption even when its feasibility is granted --- the mere persistence of tissue normality over a sufficient period of time is obviously enough to prove that the tissue did not at the beginning of that period contain even one cell with a fission rate as high as that of a malignant tumour. Even if it be assumed that normal cell populations contain individuals with the power of intermittent growth at rates considerably higher than the mean, it is far from certain that the pathological states leading to cancer formation are such as to favour their survival: on the contrary, as has been shown, the

local conditions leading to the inception of malignancy are not such as to encourage rapid growth in general, but have primarily the reverse effect.

IV. Infective hypotheses.

i. the filterable avian sarcomata.
ii. possible interpretations.
iii. viruses and proliferation in general.
iv. viruses and mammalian tumours.
v. objections to the virus hypothesis.
vi. current views.

No discussion of experimental work on the etiology of cancer could be complete without reference to the infective hypothesis in its modern aspect. It seems clear that the older parasitic views of cancer have completely failed to stand the test of time, partly on account of lack of positive evidence to support them and still more because of the rapid accumulation of unassailable evidence in other fields, a process which has tended to throw doubt on the truth of any infective hypothesis. Indeed, but for the study of the filterable avian sarcomata commenced by Rous in 1910 and stimulated by the work of Gye in 1925 and later years, it is possible that this hypothesis would have attracted but a small share of attention at the present time. It will be recalled that the filterable mesoblastic fowl tumours (of which the Rous I spindle-celled sarcoma is the most widely known) differ from mammalian tumours in that they may be transmitted

experimentally by the inoculation of cell-free filtrate or of desiccated tumour material similarly devoid of living cells. The present problem is first to define the nature of the filterable agent (and particularly to determine whether it is an extrinsic and independent living virus), and, secondly, to assess the importance of these facts in relation to tumour causation as a whole, with special reference to those purely biological theories already discussed.

A most comprehensive review of the problem was given by Andrewes (1934), who set himself to show that while difficulties in the way of an infective hypothesis undoubtedly existed, many of the features of cancer were consistent with causation by a virus, and moreover that certain facts in his view were difficult to reconcile with any other theory.

It was pointed out by the writer (Haddow 1933) that the phenomenon of filterability was open to several theoretically alternative interpretations, but from the practical point of view there is no doubt that the process of cell-free transmission of these sarcomata shows a striking although perhaps superficial similarity to the transmission of some of the infections acknowledged to be caused by an independent living virus. Discussion therefore

centres round the question whether the filterable agent is a similar virus, in the usual sense of the word.

The whole question has become so involved that little more can be done than simply to enumerate and discuss the main points for and against this orthodox view. The evidence which tends to indicate that the agent is a virus depends mainly on (a) the unlimited capacity for multiplication which the agent shows, as in the case of undoubted viruses, in the presence of susceptible living cells either in vivo or in vitro; (b) the fact that cell-free filtrates of certain of the fowl tumours have been shown to induce similar tumours in other members of the class Aves; (c) the size of the Rous agent, which, in the opinion of Andrewes, tends to indicate its living nature. In addition to these directly suggestive features, more circumstantial support is given by the fact that other proliferative conditions in man and animals are associated with or caused by filterable agents the living nature of which there is no reason to deny.

In discussion of such evidence it may be pointed out first that, highly suggestive as is the increase in amount of agent in contact with live susceptible cells, this phenomenon in itself can no longer be regarded as indicating the living nature

of the material showing increase. Thus treatment of an undifferentiated (R) pneumococcus with the type-specific capsular polysaccharide derived from a specific S type results in the differentiation of the former into the latter, which then continues to breed true (within the environmental limits) and to produce further large amounts of specific type-determining substance (Dawson 1928, 1930; Alloway 1932, 1933).

Further evidence of the same type is furnished by recent work on plant viruses. Takahashi and Rawlins (1932) demonstrated that the tobacco mosaic virus in infective plant juice might be composed of submicroscopic rod-shaped particles capable of causing stream double refraction, and found (1935) that under certain conditions the concentration of tobacco mosaic virus in plant juices showed a high positive correlation with the intensity of the stream refraction (see also Takahashi and Rawlins 1937). Stanley (1936; see also Vinson and Petre 1929, and Loring and Stanley 1937), described the preparation of a crystalline protein possessing the properties of mosaic virus from the sap of infected tobacco and tomato plants. Confirmatory results were obtained by Bawden, Pirie, Bernal and Fankuchen (1936) who found in addition that by subsequent purification the protein in neutral aqueous solution could be obtained in liquid crystalline states. Using three

strains of the virus, those causing common tobacco mosaic, aucuba mosaic and enation mosaic (see also Stanley 1937 a) it was found that although each purified agent reproduced its characteristic disease in susceptible plants, no gross physical or chemical differences could be detected between the three proteins, which further proved to be related serologically. Bawden et al. also discussed the possible identity of the protein molecules and virus particles, a conclusion they regarded as reasonable and probable but still not proved. According to the ultracentrifugal analyses of Wyckoff, Biscoe and Stanley (1937; see also Stanley and Wyckoff 1937) the sedimentation constants of the tobacco mosaic virus proteins are the largest yet found for any protein, indicating molecular weights in excess of 10,000,000. There was at first no proof that the crystalline protein was indeed the purified virus, but these workers further found that the proteins of healthy plants do not contain a demonstrable amount of molecules with weights as great as 30,000, and they also adduced physical evidence to suggest that the molecules of the purified virus protein are identical with those present in the infected plant itself. According to a later paper (Stanley 1937 b), "as a whole, the experiments with tobacco mosaic protein and with aucuba mosaic protein demonstrate that, as negatively or positively charged protein

ions are centrifuged from solution, there is a loss of virus activity and that the decrease in virus activity is proportional to the decrease in the concentration of the high molecular weight protein. This proves that under various conditions the virus and the high molecular weight protein sediment at the same rate and that they are the same size. The results indicate, therefore, that the virus and the protein are identical." In a recent summary of the significance of these results (see Nature, 1937, 139, 203) Stanley wrote....."In view of the properties which this protein possesses, the borderline between the living and the non-living tends to become non-existent, for, although it possesses properties which have been regarded as characteristic of living things, such as specificity of host range and the ability to reproduce and to mutate, it is nevertheless a protein molecule and as such may be regarded as non-living. It is possible that by virtue of its size, it is enabled to possess sufficient organisation within the molecule to endow it with such properties. As such, it would represent a link between the type of organisation within the atom or molecule with which chemists have concerned themselves and the type of organisation within the cell with which biologists have been concerned."

At different times many observers have been impressed by the biological analogies between the cell-free transmission of avian sarcomata and transmissible bacterial lysis, and opposed schools have sought to define bacteriophage as an independent living entity on the one hand or on the other as an intrinsic product of the bacterial cell itself. Controversy in this field has accordingly followed the same lines as with tumour viruses, and it is of great importance that Northrop (1936), in experiments on the concentration and partial purification of bacteriophage from lysed staphylococcal cultures, obtained a protein preparation showing the properties of phage itself. The loss in activity of a solution of the protein was proportional to denaturation of the protein at various temperatures and different pH. The protein was also adsorbed by phage-susceptible bacteria (living or dead) but not by resistant organisms. Schlesinger (1936) has also recorded the observation of intensive staining of the bacteriophage substance in the Feulgen reaction.

While much significance has been attached to the transmission of the Fujinami fowl myxosarcoma to ducks and of the Rous I sarcoma and MH-2 endothelioma by filtrate to pheasants (Andrewes 1932), the writer pointed out (Haddow 1934) that transmission in both of these cases is still within the limits of blood relationship as judged by the precipitin reaction. Moreover, propagation to the pheasant is within the limits not only of blood-relationship but also of bastardisation. (Apart from modern examples of phasianus x gallus crosses, it is of interest that Darwin made reference to the work of Mr Hewitt on these hybrids in Chapter IX of the "Origin of Species"). These facts indicate that transplantation between fowl, pheasant and duck should be regarded as within the limits of relative tissue-compatibility. The successful transmission of a fowl tumour to pheasant or duck does not therefore make it less likely that the filterable agent is a cell-derivative on the one hand, or more likely that it is an independent parasitic virus on the other.

With regard to the size of the active agent, an important contribution to the whole subject was made through the work of Elford and Andrewes (1935). By filtration of partially purified Rous I agent through graded collodion membranes these workers obtained results indicating that the active particles

had a diameter of about 0.1μ, while a similar figure was also obtained for the Fujinami agent whether obtained from fowl or duck tumours. Both these results received additional support from preliminary centrifugation tests. It thus appeared that as far as size was concerned the fowl sarcoma agents resembled the larger rather than the smaller filterable viruses. It is of interest that the estimated size should bring the sarcoma agents just within the limits of resolution by ultraviolet light. As a result of this work Andrewes rather inclined to the view that the size of the agent supports its living nature. While this can hardly be conceded on strictly logical grounds, there is no doubt that this accurate determination of size will cast considerable doubt on the conception of the agent as an enzyme-like or relatively simple chemical substance.

Lastly, one must consider the facts which associate several proliferative although non-malignant lesions in man and animals with virus infection. With considerable ingenuity, and provisionally including tumours as a group of virus diseases, Andrewes (1934) showed how an ordered and apparently continuous series might be described for both the epithelial and connective tissues, starting with the virus diseases associated with necrosis, proceeding through similar conditions characterised

by necrosis and proliferation, and ending in the purely proliferative tumours. In the epithelial series the range may be said to extend from foot-and-mouth disease (necrotic) through fowl-pox (epithelioma contagiosum), sheep-pox (molluscum contagiosum), the filterable warts of man, dogs and cattle, infectious papillomatosis of rabbits (Shope), non-filterable papilloma and rodent ulcer to malignant epithelioma; in the connective tissues a similar series extends from canary-pox, possibly vaccinia, infectious myxomatosis of the rabbit, Shope's infectious fibroma of the rabbit, the Fujinami sarcoma (in duck and fowl), and such tumours as the Rous I sarcoma and Mill Hill I sarcoma to the non-filterable fowl and mammalian sarcomata. These comparisons are certainly striking, and, if the premise is correct, might be taken to indicate a continuous relationship between mammalian tumours and conditions acknowledged to be due to independent parasitic viruses.

The subject of viruses in relation to mammalian tumours has now received a fresh impetus from the work of Rous and his collaborators on the rabbit papillomatosis originally described by Shope (1933) as due to undoubted virus infection. It was shown by Rous and Beard (1934) that the cells of the Shope papilloma when implanted within the subcutan-

:eous tissues, muscles and organs of the host underwent steady proliferation so as to simulate the appearance and behaviour of a malignant tumour. Later (1935 a, b; also Rous, Kidd and Beard 1936, and Rous, Beard and Kidd 1936) the same authors reported the spontaneous development of carcinoma in skin papillomata (induced by the virus) in a considerable proportion of domestic rabbits, and this has also been confirmed by other workers (e.g., Andrewes, Selbie, in unpublished experiments). In these cases biopsy showed various appearances ranging from an invasive papillomatosis to squamous cell carcinoma with metastases to the regional lymph nodes. These tumours manifested active proliferation and invasion when transplanted intra-muscularly in the host. According to Rous and Beard (1935 b), "the virus that gives rise to the rabbit papillomas must be looked upon as the primary cause of the cancers developing therefrom. Whether it is the proximate cause has yet to be determined."

It was pointed out by Rous (1936) that the cancers arose in domestic rabbits inoculated with strains of virus which could not be recovered from the papillomatous tissue, nor was virus recovered from the cancers themselves. Such findings suggest-:ed the possibility that the virus might do no more than start the papillomas, and might have no closer

etiological relation to the cancers which later developed. A study was therefore made of the serological reactions (a) of the blood of animals carrying the papillomata (Kidd, Beard and Rous 1935, 1936) and (b) of the blood of animals bearing resultant cancers (Kidd, Beard and Rous 1936). It was found that while virus-neutralising power was wholly absent from the serum until after the papillomas had begun to develop, it then increased in proportion as the growths enlarged, the inference being that the virus persists and thrives in the papillomatous tissue. In the second case the serum of a rabbit, with large cancers resulting from the transplantation of a squamous cell carcinoma that had arisen from a virus-induced papilloma, possessed the power to neutralise the virus, although to a much less extent than the blood of rabbits carrying papillomas; in this experiment an axillary metastasis was utilised as material for the transplantations, thus ruling out the possibility that papilloma cells had been accidentally transferred to the new host. The sera of rabbits carrying tar papillomas or the Brown-Pearce carcinoma proved wholly devoid of effect on the virus. Rous clearly recognised (1936) that this demonstration means no more than that the Shope papilloma virus underwent transfer with the cancer, and that it therefore existed in such cancers, although not necessarily as their cause (see also

Rous, Beard and Kidd 1936).

It is taken as proved throughout the work of Rous and Beard that the virus itself is highly specific for the epithelium of the skin. It is true that it may be recovered from the cells of implantation growths in the muscle and viscera of wild rabbits, but, on the other hand, Rous and Beard themselves were unable to demonstrate any infective affinity of the virus towards embryonic or regenerating cutaneous epithelium. Although not specifically drawn attention to in the papers of Shope or of Rous and Beard, it seems clear from the descriptions given that mere application of virus to intact cutaneous epithelium (normal or regenerating) or to embryonic epithelium <u>in vitro</u> is insufficient to produce infection, but that scarification or tattooing seem necessary before the virus is applied. This suggests, as Handley (1931 a, b) pointed out in speaking of other experiments, that successful transmission of such papillomata by virus depends on a technique which allows injection of the skin lymphatics to occur. Handley also reviewed with completeness the evidence suggesting an infective lymphangitis as a probable cause of common warts in man, and it is at least a possibility that the Shope virus first produces an obliterative process in the skin lymphatics which is then followed by papilloma formation as a secondary effect of the

changes in local nutrition. This hypothesis is merely suggested as one which will require consideration. If verified it would show the papilloma to be the outward though rather indirect evidence of the virus infection, and the onset of malignancy as possessing only a non-essential relation to virus infection, and forming an example rather of the well-known fact that malignant change is a possible sequel to papillomatosis arising from any cause (as, for instance, syphilis, tubercle, schistosomiasis of the bladder, chronic non-specific ulceration, or in intestinal polyposis).

At this stage we may enumerate what may be regarded as the salient objections to the virus hypothesis. First must be considered the lack of evidence suggesting an infective process. There is neither clinical nor experimental evidence to suggest that cancer ever develops in any individual as a sequel to contact with individuals already affected. Although a disease of parasitic origin need not be demonstrably infectious, the complete lack of epidemiological evidence in the case of tumours is in marked contrast to the condition obtaining in undoubted virus infections. The second objection rises out of the strict cytotropic specificity which is shown by the fowl sarcoma agents, by which each

transmits to the new host the strictly specific characters of that particular tumour alone from which it was obtained. Any simple virus hypothesis would therefore entail such a multiplicity of individual viruses as to throw serious doubt on the whole conception. The question of the specificity of fowl-tumour agents has also been approached by a number of workers using immunological methods, but Andrewes (1934) concluded that serologically the various agents were neither identical nor entirely distinct, but rather constituted a group including various degrees of relationship analogous with the group and specific affinities shown by certain related bacteria and with the similar type of antigenic relationship shown by the bacteriophages.

Lastly, as a further objection to an infective view of cancer causation as a whole must be placed the failure to demonstrate cell-free transmission in tumours other than avian. Till this is done, as Murray pointed out, "it is dangerous to transfer conceptions arising out of the study of the filterable new growths of the fowl to the elucidation of the nature and causation of the new growths of other animals and man."

It is clear that no definite statement of fact can as yet be made regarding the true nature of these agents. Following up older and inconclusive experiments by Murphy and Landsteiner (1925) and Carrel (1925), work is being continued on the behaviour of fowl sarcomata artificially induced by the injection of carcinogenic substances. Of the work published to date, only that of McIntosh (1933) supplies a sufficiently large amount of evidence to show that artificially produced fowl sarcomata may prove to be filterable in the same way as are some of the spontaneous sarcomata already discussed. Unfortunately, even in these experiments any conclusion requires to be guarded in view of the circumstances: in the first place, not all the tumours ultimately found to be filterable originated at the site of tar injection; secondly, many of the fowls showed a coincident leukaemia. It must be remembered that the Mill Hill I fibro-:sarcoma (Imperial Cancer Research Fund) was propagated for about three years before filterability was established (Begg 1929), and Gye and Andrewes (1926) described the loss of filterability shown by the Rous I sarcoma during phases of relatively poor growth. Accordingly, as Foulds (1934) remarked, "it will surprise no one whose acquaintance with fowl tumours extends beyond the Rous sarcoma I if the demonstration of cell-free transmission should

be difficult or long delayed." But the experiment is by no means final, and there is a curious differ-:ence of opinion as to the ultimate significance to be attached to any such demonstration. While the induction of filterable tumours by chemical means is thought by some to indicate, if not to prove, the essentially intrinsic or non-viral nature of the agent, others incline to the opposite or a different conclusion.

In the absence of all the necessary data various attempts have been made to correlate or interpret the facts already known and to reconcile those which show apparent contradiction. The suggestion has been made in the first place that the filterable element is a chromosomal particle carry-:ing a malignancy-determining gene which may (on penetration) induce permanent or specific changes in differentiation and fission-rate in the normal cell of the same lineage as that to which the tumour belongs. It must constantly be realised that the avian and mammalian tumours present a marked contrast in filterability, and any successful theory must (at least eventually) include an explanation of this difference in behaviour. Unorthodox as the gene-filtration view undoubtedly is, it is the only theory which indicates a possible solution of this difficulty. Since the source of the contrast may depend on an

inherent cellular difference in mammals and birds, it is of great interest that some such difference may indeed exist. White (1932) studied the somatic chromosomes in 40-hour embryo chicks, and drew attention to the great range in size of the individ-:ual elements in the somatic and metaphase plates, the smallest ones being absolutely on the limit of resolving power of the microscope (about 0.2 μ in diameter). This range in size of the chromosomes he regarded as characteristic of birds in general and quite unparalleled elsewhere. It seems just possible that this distinctive cytological finding may be linked with the filterability of avian neoplasms. It is of added interest that this hypothesis is again the only one, excepting the orthodox virus theory, which allows a good fit with the estimated size of the filterable agent (0.1 μ).

Impressed by the parallel between the transmissible effects produced by the fowl-tumour agents and by the specific soluble substances which determine physiological characters in bacteria (and particularly in the pneumococci), Murphy (1931) applied the term "transmissible mutagens" to substances possessing this type of activity. Looked on in this light, the sarcoma agents and the type-determining bacterial polysaccharides are relatively simple chemical products of their respective cells,

and under suitable circumstances may impress the characters of their cell of origin on its undifferentiated prototype. Attractive and stimulating as this suggestion undoubtedly is, two points of criticism may be applied: it gives no hint of explanation of the absence of filterability in mammalian sarcomata and, in the second place, it allows little room for the knowledge we already have that the agent is a particle comparatively large in size.

Intermediate between the extreme viral and non-viral interpretations is the rather less simple conception of Gye and Purdy (1933). Although directly derived from the original claims of Gye in 1925, this hypothesis is now dependent on evidence mainly of a serological kind, and seeks to show the infective agent as a complex of two or three components: (a) a non-specific extrinsic virus inducing neutralising antibodies acting in absence of complement; (b) an intrinsic fowl-derived fraction inducing antibodies acting only in the presence of complement; and (c) a viral element inducing the appearance of an antibody acting in the presence of complement although not absorbed by fowl-embryo tissue emulsions. According to Gye (1936) "there is an antibody against the tumour agent in these antisera obtained by injecting extracts of

normal (fowl) tissues, but the antibody acts only in the presence of complement. There is no explanation of this peculiar behaviour but at the present time the conclusion is inescapable that normal fowl tissues do contain an antigenic substance the antibody to which is capable of neutralising to a very large extent, though never completely, the activity of a fowl tumour agent.....The element is integrally part ofthe agent, since filtrates which have been freed of all detectable protein are neutralised as readily as the plain filtrate. Such antisera neutralise equally well the agents of all the filterable fowl tumours. It is probable, though by no means proven, that the antigen is derived from nuclear material.....The antibodies are easily absorbed from these immune sera by means of minced embryonic tissue." On the other hand, "when filtrates of a fowl tumour are injected in animals of alien species, and especially in large animals like the goat and horse, the blood serum of the injected animals acquires the power to neutralise active filtrates of the same tumour and of other filterable fowl tumours, and this neutralisation occurs in the absence of complement. The neutral-:ising antibody is not removed from the serum by absorption with an emulsion of normal fowl tissue." While Andrewes recognised that neutralisation of filtrates by antisera prepared against normal fowl

tissues indicated an unusually intimate association of virus with fowl protein, he did not believe the latter to be an essential component of the tumour-producing agent. But to the writer this circumstance and other features, such as specificity, seem to indicate that the conception of the agent as an intrinsic cell-product may more than possibly prove correct. Such a view received support from Boycott (1933), and as Foulds pointed out, three different constitutions of the agent have been deduced from serological experiments: (a) an extrinsic virus and an intrinsic "specific factor" (Gye and Purdy); (b) an extrinsic virus but no intrinsic specific factor (Andrewes); and (c) a specific intrinsic factor but no virus (des Ligneris 1934).

A more direct approach to the nature of the agent (Ledingham and Gye 1935) has recently been made with the use of methods which have been employed with success by Ledingham and others in the study of vaccinia and other virus diseases. Ledingham and Gye showed that high-speed centrifugalisation (15,000 r.p.m.) will deposit the particles of the agent in the form of "elementary bodies". In addition, it was shown that the serum of tumour-bearing fowls might develop agglutinins --- detectable in dilutions varying from 1 in 4 to 1 in 50 --- for these bodies. One bird possessed agglutinins

before tumour inoculation, and the authors suggested that such a fact might be correlated with the occasional presence of agent-neutralising properties in the serum of normal fowls. Sedimentation of the Rous agent was also obtained by McIntosh (1935) in experiments with an air-driven centrifuge capable of speeds of 40,000-60,000 r.p.m. Stained films showed the presence of particles with the appearance of elementary bodies.

In similar studies by Amies (1937) it was found that a centrifugal force of 20,000 to 30,000 times the force of gravity will deposit the whole of the tumour-exciting agent from highly active cell-free extracts of the Rous I and Fujinami sarcomata. By employing the method of repeated fractional centrifugation it was possible to prepare highly active suspensions which were apparently free from fowl protein, and microscopic examination of these purified suspensions revealed the presence of large numbers of particles of fairly uniform size and appearance but below the limits of optical resolution. According to Amies "the available evidence suggests that the tumour agents exist in the form of these particles, which are of the nature of virus elementary bodies." It was further found that these purified tumour agent suspensions might be specifically agglutinated by the sera of fowls

bearing the corresponding tumour, and that some degree of cross agglutination occurred between Rous and Fujinami suspensions and the corresponding antisera. Agglutinins for both Rous and Fujinami tumour agent suspensions were demonstrated in the serum of apparently normal adult fowls but not in normal young chickens, and sera which showed a strongly positive agglutination reaction also possessed the property of neutralising the tumour agents. It is of the greatest interest that purified tumour agent suspensions which were apparently free from fowl protein were still neutralised by hyperimmune rabbit anti-fowl sera, and that the presence of complement did not appear to be essential for the reaction. In Amies' own words "this fact apparently implies that these tumour agents have an antigenic constituent which is the same or at least closely related to one that is normally present in fowl tissues. Presumably in this antigenic similarity is to be found the explanation for the lack of an efficient immune response on the part of the host. This phenomenon has no counterpart in any of the recognised virus infections. A potent goat anti-rabbit serum, for example, has no inhibiting effect upon a suspension of vaccinia elementary bodies prepared from lapine. On account of this property alone the avian sarcoma agents must remain in a class of their own." It must

be conceded that these findings are, to quote a recent annotation in the Lancet (1937, i, 277), "difficult to reconcile with the view that the tumour virus is an independent organism, unless it is a parasite which in some curious way takes on a stamp from its host."

In apparent contrast to these results it will be recalled that the intrinsic fowl-derived fraction described by Gye and Purdy (1933) induced the formation of antibodies acting only in the presence of complement. Amies referred to this difference, and regarded it as due to the use of purified tumour agent suspensions in place of the crude tumour filtrates employed by Gye and Purdy, since his own antisera produced only partial and irregular neutralisation of crude tumour extracts when complement was omitted from the mixtures, although they neutralised the same extracts efficiently when fresh complement was added.

In a recent note Fraenkel and Mawson (1937) stated that while they succeeded in depositing the Rous agent by centrifugation they were unable to obtain a satisfactory correlation between the number of elementary bodies in different active preparations and the infectivity of the extracts, and they emphas-:ised that the possibility of adsorption of active agent on inactive particles could not be excluded.

In addition to the hypotheses already noticed, consideration must be given to the view, supported by Andrewes, which represents the cancer virus as ubiquitous as a symbiont within the normal cell. This suggestion implies that chemical carcinogenic agents act indirectly on the cell through virus activation. Unlikely as such a process may seem to be, there is a curious parallel, as Andrewes pointed out, in the work of Boak, Carpenter and Warren (1934) on the frequent occurrence of herpes following the artificial induction of pyrexia. Typical lesions developed in thirty-six to seventy-two hours after a fever of 40-41.5 C. in 122 of 200 patients. Filtrates from vesicle fluid produced a fatal and transmissible encephalitis in the rabbit, and immunised animals showed cross-immunity to a known herpetic strain. But analogy with this phenomenon, however striking and ingenious, is lacking in conviction, and it must be stressed that the onus of demonstrating such a process must rest on those who interpolate virus action as a stage in tumour induction by tar, the carcinogenic hydrocarbons, or any other physical or chemical agent.

The bulk of present information indicates that the causation of the majority of neoplasms is independent of any process of infection. Yet there

is evidence, additional to that already considered, which suggests that a certain proportion of tumours is either due to, or associated with, the presence of a virus. In particular, Russell (1932; see also Wolf and Orton 1933) described the occurrence of intranuclear inclusion bodies in a considerable proportion of human gliomata, and prominent acidophile inclusions were also observed by Lucke (1934 a, b; see also Reports of the International Cancer Research Foundation) in the cells of a widely distributed kidney tumour of the leopard frog. It must also be borne in mind however that the morphological appearance of intranuclear inclusions is possibly not a specific indication of infection (e.g., see the description of intra-nuclear inclusions in the epithelium of the human male genital tract by Gilmour 1937).

The underlying biology of malignant change is undoubtedly so fundamental that it is extremely improbable that infection is necessarily a causal factor. Yet it may readily be conceded that a cellular variation of this kind, which is known to be caused by a considerable variety of physical and chemical agents, may in certain instances be brought about by a process of cell infection and particularly by virus action. Yet even in these cases it would appear that the greatest promise of effective under-

:standing lies in an attempt to express the phenomenon analytically in terms of cellular variation.

Little (1931) recognised that any explanation of neoplastic growth must depend on our knowledge of normal growth and its deviations, and while the whole subject still presents a multitude of formidable problems, it is significant that progress is most marked, and is likely so to continue, along lines which describe the pathological event in terms of physiology.

S U M M A R Y.

Attention is drawn to the striking multiplicity of carcinogenic agents, which, although they are sufficiently diverse as to have no physical or chemical features in common, nevertheless produce identical end-results in cells exposed to their action. Similar examples are quoted from other fields and the basis of biological non-specificity is examined. Arguing from the general biology of variation, it is suggested, in the special case of tumour-producing agents, that these operate by producing interference with certain normal functions of the cell, and particularly with growth, in such a way as to induce variation in the characters affected. The detailed evidence in support of this view derives from a certain degree of parallelism between optimal growth-inhibiting power and tumour-producing capacity which is shown by radio-active agents and the carcinogenic hydrocarbons. In a discussion of the biological nature of this cellular change, emphasis is concentrated on its irreversibility. A summary is given of the known biochemical effects of tumour-producing agents, including a reference to the possible significance of ischaemia in tumour induction. The conception of somatic cell variation in general, and of

cellular inhibition in particular as its cause, is discussed in relation (1) to the salient features of the natural history of cancer and (2) to the theories of Virchow and Cohnheim, to allied genetic theories, to Warburg's hypothesis, and to the various infective hypotheses of tumour causation.

R E F E R E N C E S

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G E N E R A L S U M M A R Y

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Sections I and II.

Intraperitoneal administration of a number of carcinogenic hydrocarbons, including 1:2:5:6-dibenz-:anthracene, 5:6-cyclopenteno-1:2-benzanthracene, and 3:4-benzpyrene, produced considerable inhibition in the rate of growth of the Jensen and Walker tumours. On the other hand, a series of related non-carcinogenic compounds (anthracene, phenanthrene, 1:2-cyclopentenophenanthrene, dodecahydro-1:2-benz-:anthracene, pyrene, fluoranthene, triphenylene, dehydronorcholene, perylene, 1:9-benzanthrone and diphenylene oxide) gave no inhibition of tumour growth when tested under the same conditions. Of the synthetic oestrogens 1-keto-1:2:3:4-tetrahydro-:phenanthrene and 9:10-dihydroxy-9:10-di-n-propyl-9:10-dihydro-1:2:5:6-dibenzanthracene, the latter proved to be moderately inhibitory and the former quite inactive. Inhibitory activity was also shown to a variable extent by chrysene and by 1:2-benz-:anthracene and certain of its derivatives (3-, 4- and 7-methyl-), the carcinogenicity of which is either very feeble or nil.

In comparative experiments on body-growth, x-radiation, lead and colchicine were found to

produce a temporary interference which was followed by recovery to the normal growth-rate, with or without compensation. Although the carcinogenic hydrocarbons must be regarded as toxic substances, their growth-inhibiting action is apparently independent of toxicity in any non-specific sense, since manifest poisons evoke an entirely different response.

The inhibition produced by the carcinogenic compounds was extremely prolonged, even after a single injection. This feature is discussed in relation to the rate of excretion of such substances. It was further noted that 1:2:5:6-dibenzanthracene produced a diminution of both male and female fertility accompanied, in certain cases, by histological changes in the gonads.

There is thus a correlation in compounds of this type between carcinogenicity and growth-inhibitory power. It is suggested that the mode of action of these substances is indirect, and that they operate by producing a prolonged retardation of the growth of normal cells, which eventually react by a process of discontinuous variation to give a new cell race with a greatly increased fission rate.

Section III.

Attention is drawn to the striking multiplicity of carcinogenic agents, which, although they are sufficiently diverse as to have no physical or chemical features in common, nevertheless produce identical end-results in cells exposed to their action. Similar examples are quoted from other fields and the basis of biological non-specificity is examined. Arguing from the general biology of variation, it is suggested, in the special case of tumour-producing agents, that these operate by producing interference with certain normal functions of the cell, and particularly with growth, in such a way as to induce variation in the characters affected. The detailed evidence in support of this view derives from a certain degree of parallelism between optimal growth-inhibiting power and tumour-producing capacity which is shown by radio-active agents and the carcinogenic hydrocarbons. In a discussion of the biological nature of this cellular change, emphasis is concentrated on its irreversibility. A summary is given of the known biochemical effects of tumour-producing agents, including a reference to the possible significance of ischaemia in tumour induction. The conception of somatic cell variation in general, and of

cellular inhibition in particular as its cause, is discussed in relation (1) to the salient features of the natural history of cancer and (2) to the theories of Virchow and Cohnheim, to allied genetic theories, to Warburg's hypothesis, and to the various infective hypotheses of tumour causation.
