

## RESULTS

### General Observations

In the early months of the study, a number of animals died, or had to be killed, due to disorders of the gastrointestinal tract. The disorders took the form of enlargement and eventual haemorrhage of the caecum (Fig. 6). Histopathology of the affected tissues showed signs of inflammation and haemorrhage. This was thought to result from the anaesthetic (Avertin with Amylene Hydrate) used in the implantation procedure. In recent studies in which nembutal has been used as the anaesthetic, inflammation of the caecum has not been encountered.

Within the first nine months of the study, a few animals showed obvious signs of respiratory distress. In some of these cases it was found that the pellet had become dislodged from the lower inferior bronchiolus and had travelled up the bronchus and was blocking the trachea at, or above, the hilar junction. Where possible, the pellet was reinserted. If no pellet was located in the trachea, the animal was destroyed. In these latter cases, necropsy usually revealed that the pellet had travelled up the bronchus and trachea and had lodged itself in the nasal sinus. In a few cases of respiratory distress, it was found that the trachea had fibrosed or collapsed around the point of tracheostomy. Where possible, the situation was rectified. If this was not possible, the animal was killed.

The general health of most of the animals remained remarkably good during the two year period of the experiment. During the latter part of the study, many rats developed subcutaneous lumps. These were usually well encapsulated mammary tumours which, where possible, were 'shelled out' under anaesthesia. The histology showed that



Figure 6. Enlarged and haemorrhagic caecum from male rat killed at 159 days. Colon was also distended. This rat had received a blank metal pellet (Group 16).  
H. & E. X1.4

these tumours were invariably benign fibroadenomas, presumably of 'spontaneous' origin.

### Survival

The percentage of rats alive in the various treatment groups at 100 day intervals is given in Table 6. The deaths in this table include animals dying from all causes. Inspection of the data indicates that, apart from the 3-methylcholanthrene-treated animals, rats died or were killed at approximately the same rate in all groups. A further number of animals died, or had to be killed, before the termination of the study (726 - 732 days) and Table 7 gives the number of animals alive at the termination of the study in the chromium-containing material test groups and the negative-control groups. This table also includes a Chi-squared analysis which shows that there is no statistical difference in survival between the 14 chromium material test groups and the negative-control animals at two years from the start of the experiment.

### Body Weight Data

The body weight data for male and female rats were treated separately. The individual weights of rats at nine time intervals for the chromium material test groups and the two negative-controls were compared for homogeneity by the analysis of variance. This showed no gross changes occurring in either male or female groups at any of the time intervals during the course of the experiment. The Student's t-test performed on the individual groups of rats, comparing them to control rats, showed no significant increase or decrease in body weight between any of the test material groups and the negative-control group (Group 16). The figures for the mean body weights, standard deviations of these means, the coefficients of variation and

ANIMALS ALIVE AT 100-DAY INTERVALS AFTER PELLETT IMPLANTATION -  
PERCENTAGE OF INITIAL NUMBER

Days after implantation

Group No.	0	100	200	300	400	500	600	700
1	100	100	99	99	98	92	88	82
2	100	100	99	98	97	93	90	84
3	100	100	98	98	98	94	87	76
4	100	99	98	95	94	89	85	74
5	100	98	97	97	93	86	80	68
6	100	100	99	98	97	95	87	75
7	100	100	99	97	96	92	90	76
8	100	100	100	98	98	92	84	74
9	100	100	99	98	97	88	83	69
10	100	100	99	97	94	91	84	77
11	100	100	99	98	95	87	81	71
12	100	100	97	93	91	87	80	67
13	100	100	100	99	92	89	86	69
14	100	100	99	99	97	96	91	82
15	100	100	99	99	98	93	87	79
16	100	99	97	96	94	90	84	73
17	100	98	98	92	75	50	31	10
18	100	100	100	94	85	69	52	35
19	100	98	96	92	85	81	69	54
20	100	98	98	85	65	48	35	19

ANIMALS ALIVE AT TWO YEARS

Group No.	Initial No. of Rats in Group	Observed No. of Rats alive at 2 years (O)	Expected No. of Rats alive at 2 years (E)	Chi-squared $= \frac{(O - E)^2}{E}$
16	150	104	105.6	0.02
1	100	79	70.4	1.05
2	100	76	70.4	0.45
3	100	72	70.4	0.04
4	100	69	70.4	0.11
5	100	64	70.4	0.59
6	101	73	71.1	0.05
7	100	74	70.4	0.18
8	100	72	70.4	0.04
9	100	64	70.4	0.59
10	100	75	70.4	0.30
11	100	60	70.4	1.54
12	100	63	70.4	0.78
13	100	62	70.4	1.00
14	100	77	70.4	0.62
15	150	113	105.6	0.52
TOTAL	1701	1197	1197.5	7.88*

\* With 15 degrees of freedom, this value shows that there is no noticeable difference in survival at 2 years (P. 0.9 - 0.8)

NB. The expected number (E) of rats alive in each group was calculated from the total number of rats alive at the termination divided by the total initial number of rats, multiplied by the initial number of rats in each group.

The Chi-squared values for individual groups have been summed to arrive at a total value of 7.88, and the degrees of freedom have been calculated accordingly.

Student's t-test are given in Tables 8, 9 and 10. Although at certain times a number of groups (Tables 9 and 10) showed a significant difference from the controls by the Student's t-test, many of these were at time '0', before the treatment could have had any effect. These can, therefore, be eliminated. Further, at the 5% level of significance one would expect one in every twenty to deviate from the controls. It is thus concluded that treatment did not affect the pattern of body weight gain and maintenance during the experimental period.

#### Macroscopic Lung Tumours

The first macroscopic lung tumour was found after an implantation period of 217 days and Figure 7 shows the number and time at which all lung tumours occurred. The lung lesions were mainly found in the 3-methylcholanthrene-treated groups of rats, but similar lesions occurred in a number of chromium-containing test groups. The majority of lung lesions were large and occupied and expanded the major part of the left lung. Most of the rats in which they occurred eventually showed signs of respiratory distress such as hyperpnoea and rib retraction, at which time they were killed.

Post mortem examination often revealed metastases to local lymph nodes and occasionally to one or both kidney. Figures 8 and 9 illustrate the type of finding seen. Direct invasion of tissues in and around the thoracic cavity was not uncommon. The majority of these lung tumours resulted in the death of the animals in which they occurred. A few similar, but smaller, lung tumours were found in rats during the 'terminal kill'. These occupied at most the lower third of the left lung.

Microscopic examination of the lung lesions proved that the vast

**MEAN BODYWEIGHTS OF MALE AND FEMALES RATS IN ALL TREATMENT  
GROUPS AT 84-DAY INTERVALS AFTER PELLET IMPLANTATION**

Days after Implant- ation	No. of Rats Male	Mean Wt.(g) of Rats ( $\pm$ S.D. of mean)	C.V.* %	No. of Rats Female	Mean Wt.(g) of Rats ( $\pm$ S.D. of mean)	C.V.* %
0	817	210 (18)	8.6	884	189 (17)	9.0
84	816	392 (37)	9.4	882	238 (17)	7.1
168	807	446 (47)	10.5	876	252 (20)	7.9
252	796	485 (53)	10.9	867	263 (24)	9.1
336	783	521 (64)	12.3	858	283 (33)	11.7
420	766	550 (64)	11.6	839	302 (44)	14.6
504	738	566 (67)	11.8	803	322 (54)	16.8
558	693	578 (69)	11.9	756	343 (59)	17.2
672	605	581 (73)	12.6	644	361 (62)	17.2

\* C.V. - Coefficient of Variation [S.D. as a % of mean]

STUDENT'S t-TEST VALUES FOR FEMALE RAT BODYWEIGHTS IN CHROMIUM-MATERIAL TEST GROUPS AT 84-DAY INTERVALS AFTER PELLET IMPLANTATION - COMPARISON WITH BLANK PELLET GROUP

days after implantation

Group No.	0	84	168	252	336	420	504	558	672
1	1.84	1.45	1.57	1.46	1.29	0.64	0.48	0.21	0.10
2	0.50	0.03	0.15	0.38	0.48	1.17	0.38	0.39	0.23
3	1.03	0.37	0.24	0.61	0.31	1.00	0.30	0.68	0.05
4	1.00	0.91	1.33	0.63	0.03	0.85	0.32	0.47	0.90
5	1.42	0.12	0.11	0.19	0.03	1.39	0.46	0.57	0.43
6	0.03	1.49	1.64	1.59	1.37	2.08	1.59	0.13	1.07
7	1.84	0.12	0.33	0.47	1.26	0.14	2.32	0.20	0.71
8	2.04	0.78	0.51	0.13	0.29	1.10	0.80	1.40	1.30
9	2.30	0.71	0.43	0.62	0.96	0.37	0.14	0.38	0.41
10	1.38	0.70	0.29	0.78	0.48	1.42	0.32	1.05	1.36
11	0.54	1.89	1.94	1.75	0.45	1.47	0.34	0.88	1.19
12	0.68	2.47	2.14	1.57	0.79	1.54	0.73	0.67	0.71
13	1.48	0.92	1.17	1.20	0.57	1.90	1.20	1.74	1.19
14	2.11	0.11	0.55	0.41	0.33	1.61	0.84	1.37	1.72
15	0.89	0.37	0.59	0.05	0.03	0.80	0.06	0.31	0.44

□ Denotes a significant difference from blank pellet controls (Group 16) at the 5% level.

Maximum number of animals per group = 78 (Group 15 at day 0)  
 Minimum number of animals per group = 34 (Group 5 at day 672)

STUDENT'S T-TEST VALUES FOR MALE RAT BODYWEIGHTS IN CHROMIUM-MATERIAL TEST GROUPS AT 84-DAY INTERVALS AFTER PELLET IMPLANTATION - COMPARISON WITH BLANK PELLET GROUP

days after implantation

Group No.	0	84	168	252	336	420	504	558	672
1	1.38	0.22	1.21	1.29	1.01	1.12	0.96	0.86	0.70
2	2.30	0.33	1.38	1.48	0.75	1.08	1.32	0.37	0.54
3	1.29	2.41	3.23	1.77	1.18	1.31	0.72	0.13	0.10
4	2.50	0.15	0.64	1.16	0.31	0.88	0.56	0.30	0.69
5	1.98	1.00	0.21	0.62	0.56	0.52	0.15	0.29	0.35
6	1.58	0.31	1.55	1.54	2.51	1.45	1.20	0.55	0.46
7	2.60	0.30	0.48	0.29	0.45	0.18	0.32	0.68	0.48
8	1.52	1.67	1.30	1.25	1.99	1.28	1.69	2.04	2.30
9	2.54	1.80	0.64	0.08	0.51	0.15	0.11	0.46	0.40
10	2.17	0.20	0.73	1.40	0.43	1.03	0.63	0.08	0.30
11	0.77	0.34	0.14	0.34	0.19	0.17	0.28	1.01	0.08
12	3.14	1.70	0.85	0.54	0.85	0.57	0.83	1.27	0.55
13	2.26	0.81	0.24	0.10	0.40	1.14	0.94	1.39	0.90
14	1.02	0.68	0.34	0.75	0.39	0.19	0.11	1.17	0.56
15	1.88	0.54	0.02	0.29	0.01	0.11	0.02	0.40	0.40



Denotes a significant difference from blank pellet controls (Group 16) at the 5% level

Maximum number of animals per group = 72 (Group 15 at day 0)  
 Minimum number of animals per group = 34 (Groups 5 and 12 at day 672)

CUMULATIVE CURVES OF LUNG TUMOUR YIELDS IN MALE  
AND FEMALE RATS

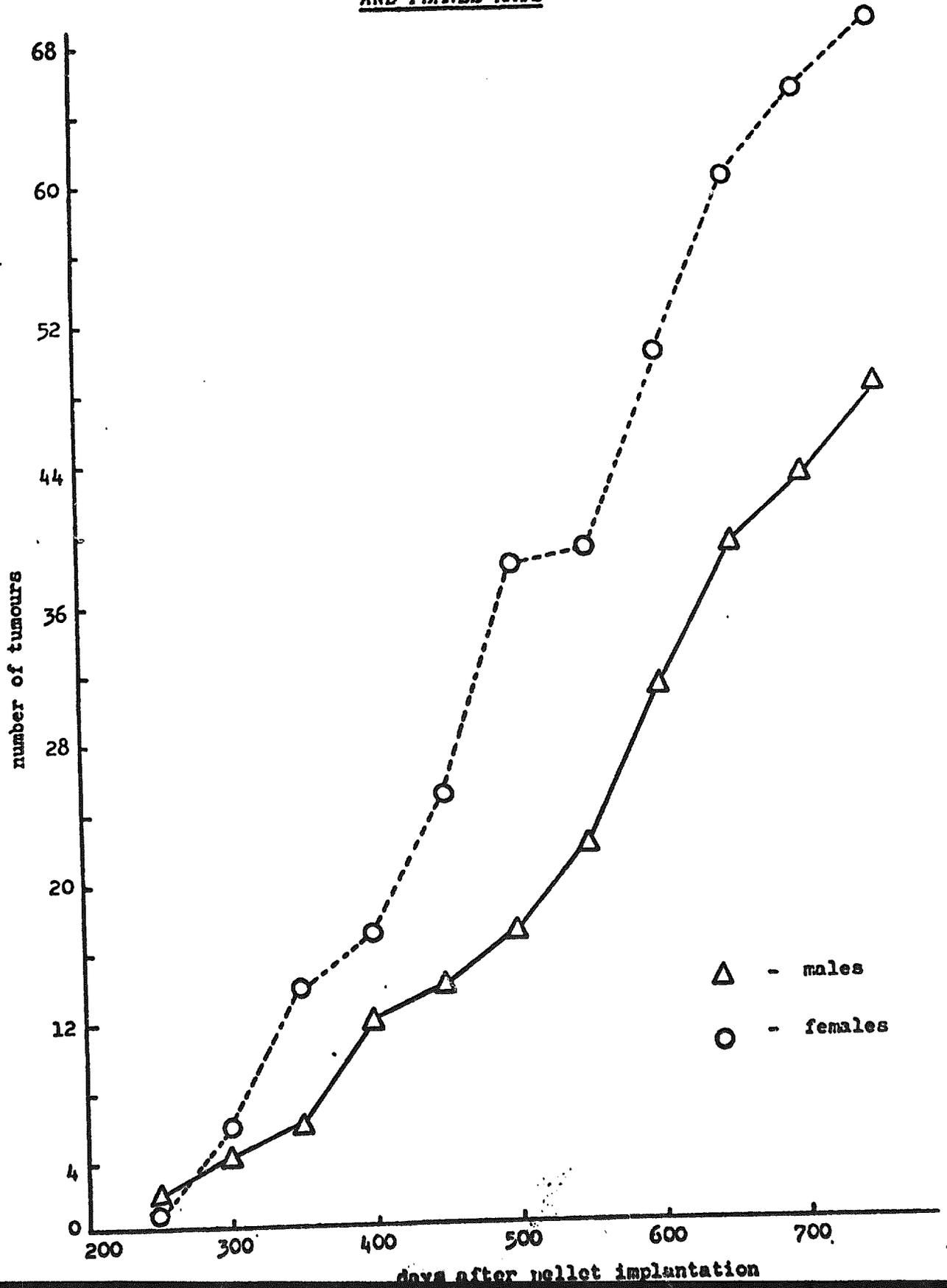




Figure 8. Lung tumour (bronchial carcinoma) involving all of left lung and azygous lobe of right lung (X1.4). Metastases are seen in hilar lymph nodes. Female rat killed 493 days after receiving pellet containing 100% 3-Methylcholanthrene (Group 17).

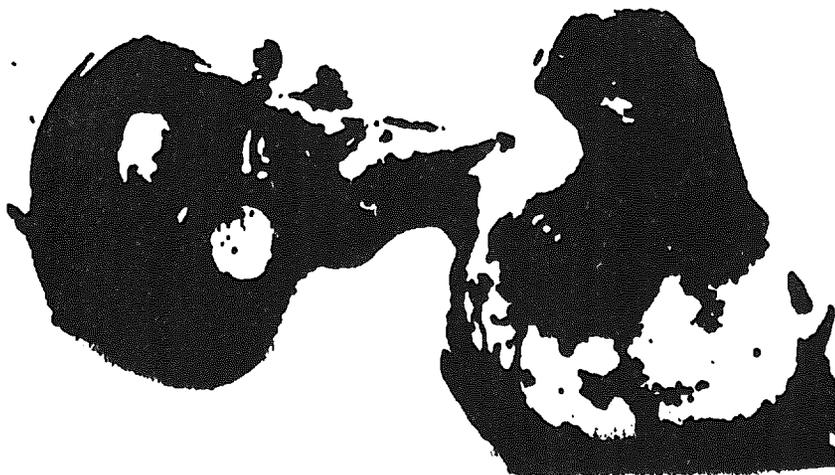


Figure 9. Metastases to both kidneys from squamous-cell carcinoma of left lung (X1.5). Male rat killed 647 days after receiving pellet containing calcium chromate (Group 11).

majority were highly keratinising squamous-cell carcinoma. Many were necrotic, or at least partly so, but this did not preclude a firm diagnosis in any case. The typical microscopic appearance of these tumours is illustrated in Figures 10 and 11. The morphological appearance of these tumours is remarkably similar to squamous-cell carcinoma as seen in man. The typical microscopic appearance of lymph node and kidney metastases is shown in Figures 12 and 13. Direct invasion of the tissues in and around the thoracic cavity included the ribcage itself (Fig. 14), the diaphragm (Fig. 15) and occasionally the myocardium (Fig. 16). It was commonly found that the right lung had been invaded in the hilar region. This presumably was the cause of much of the respiratory distress. The lung tumour findings will be given under the headings:- negative-control groups, positive-control groups, and test-residue groups.

#### Negative-Control Groups

In the 300 rats in the two negative-control groups (Groups 15 and 16), which received either blank material pellets or cholesterol-loaded pellets, no bronchial tumours were detected either during the course of the study or at the 'terminal kill'.

#### Positive-Control Groups

Lung tumours found in the four groups of rats which were treated with 3-methylcholanthrene-loaded pellets are recorded in Table 11. This table gives histopathology of the lesion as well as the occurrence of metastases or direct invasion. With one exception, all these tumours were primary squamous-cell carcinoma of the left lung. The tumour site of origin was presumably the zone of bronchial epithelium surrounding the pellet. Direct invasion of the right lung was usually

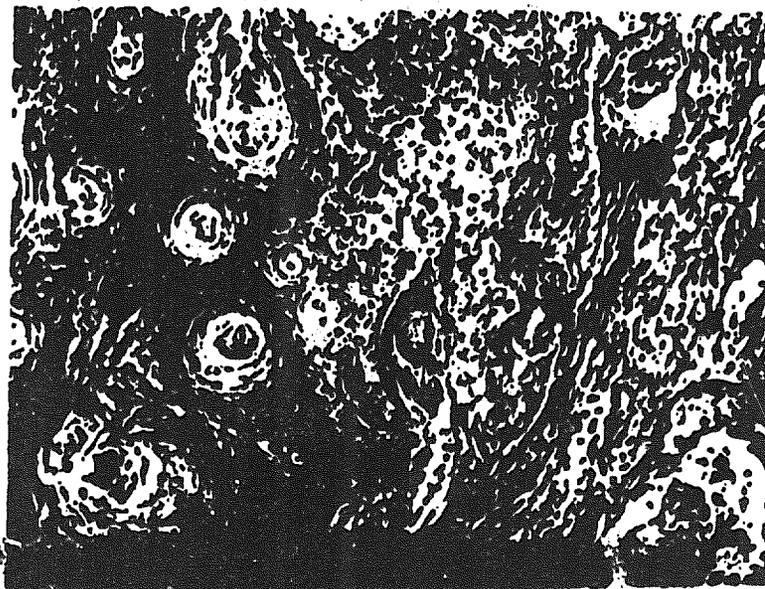


Figure 10. Squamous-cell carcinoma of left lung from female rat killed 649 days after receiving pellet containing calcium chromate (Group 11). Keratin filled nests are typical of this tumour type. H. & E. X 128

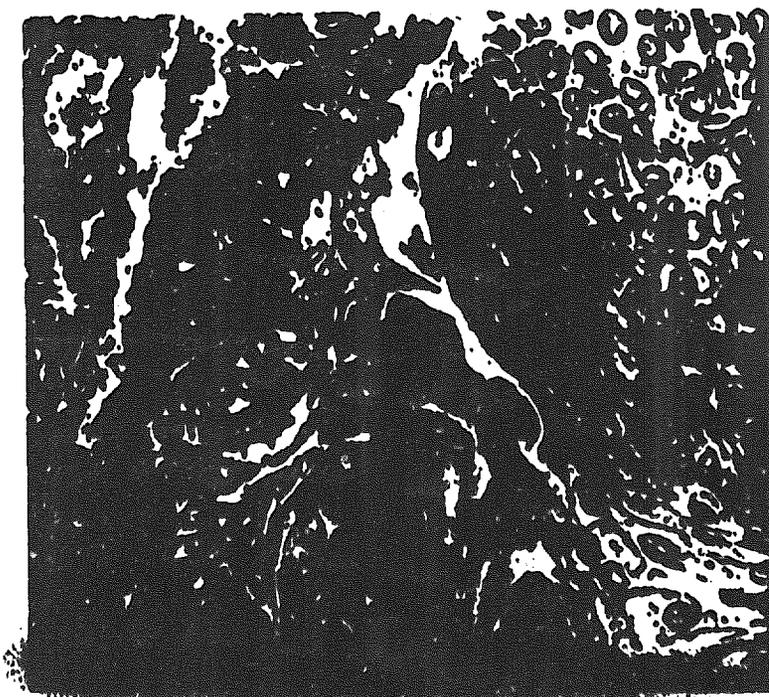


Figure 11. Squamous-cell carcinoma of left lung from male rat killed 730 days after receiving pellet containing 100% 3-Methylcholanthrene (Group 20). H. & E. X 512

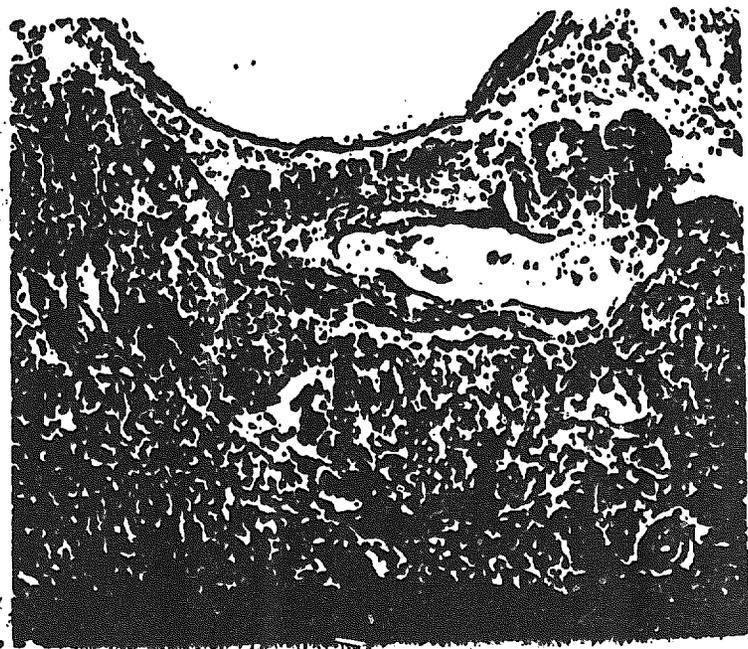


Figure 12(a). Hilar lymph node with metastatic deposits of squamous-cell carcinoma from left lung tumour. Female rat found dead 473 days after receiving pellet containing calcium chromate (Group 11). H. & E. X128

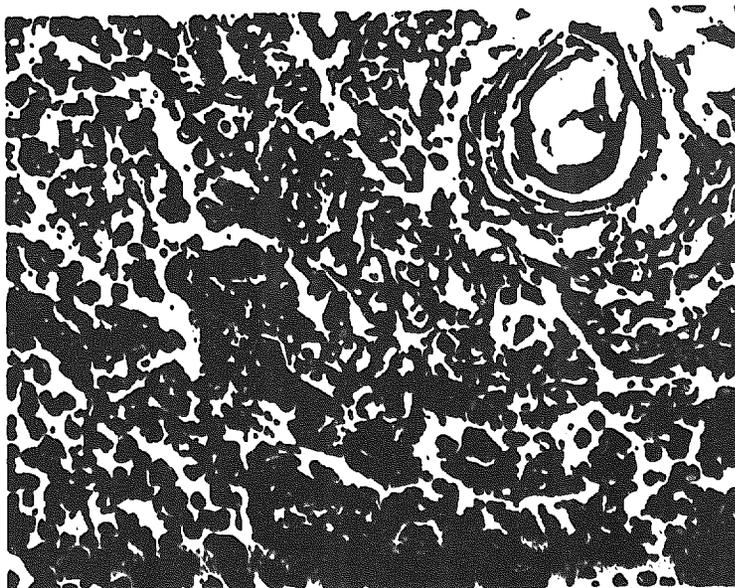


Figure 12(b). Detailed zone from above showing pleomorphic nature of squamous-cell tumour cells and the formation of a keratin nest. H. & E. X512



Figure 13(a). Kidney showing metastase from left lung squamous-cell carcinoma. Female rat killed 317 days after receiving pellet containing 3-Methylcholanthrene (Group 20). H. & E. X128

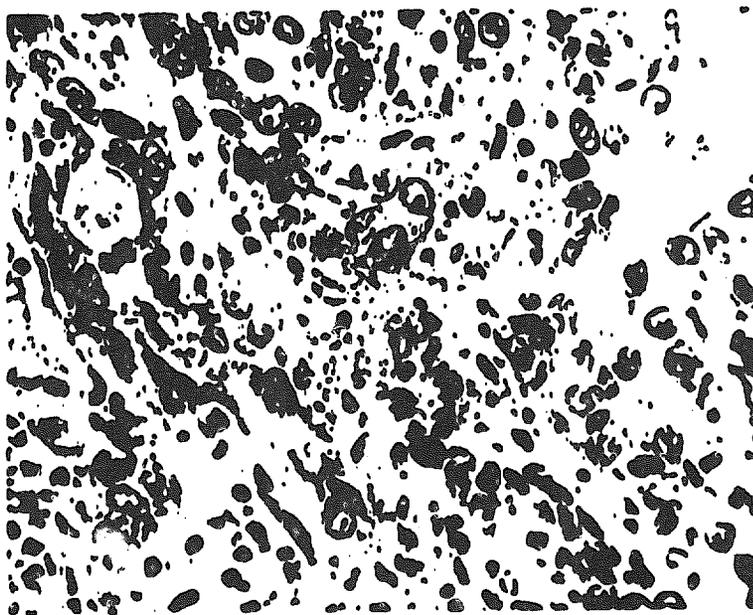


Figure 13(b). Detail from above secondary tumour clearly showing the pleomorphic nature of the tumour cells. H. & E. X512



Figure 14. Metastatic nodule of squamous-cell carcinoma attached to ribcage wall. Female rat killed 473 days after receiving pellet containing calcium chromate (Group 11). H. & E. X128

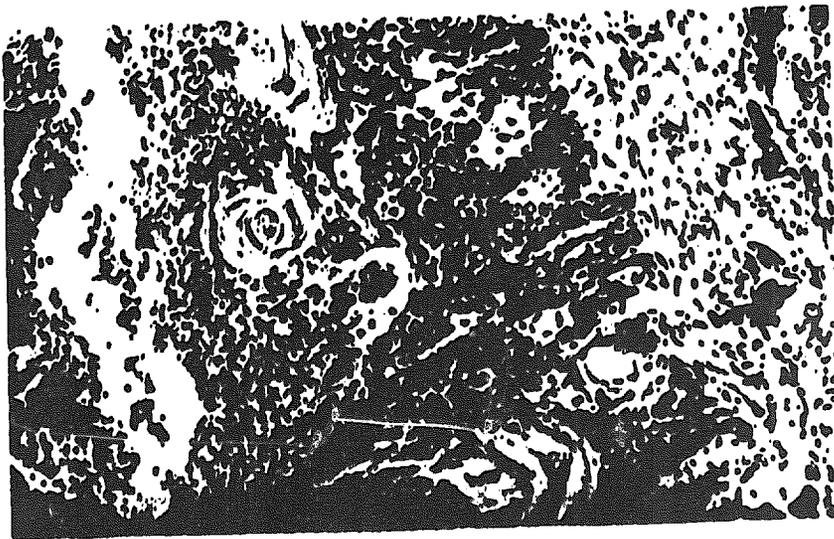


Figure 15. Squamous-cell carcinoma invading diaphragm. Keratin is clearly shown, as well as necrosis in zone behind advancing tumour front. Rat treated as above. H. & E. X128

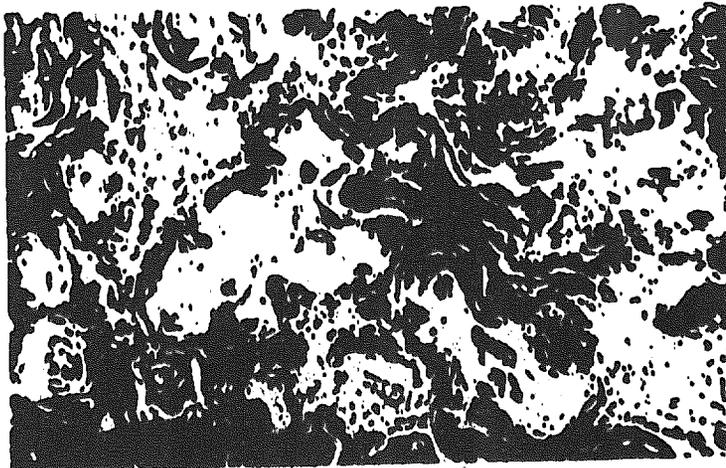


Figure 16. Squamous-cell carcinoma invading and replacing myocardium. The calcification is widespread. Rat was killed 621 days after receiving pellet loaded

BRONCHIAL CARCINOMAS\* IN RATS EXPOSED TO 3-METHYLCHOLANTHRENE

Group No.	% 3-MCA	No. of Rats in Group	No. of Rats with Left Lung Tumours		No. of Bronchial Tumours invading Right Lung	Invasion to Other Organs and Tissues	Metastases to Other Organs †	Mean Induction Period in days (± S.D. of mean) [C.V.%]**	
			F	M				F	M
19	25	48	11	2	2	1xDiaphragm 1xMediastinum	2xKidneys	525.4 (143.3) [27.3]	473.5 (126.6) [26.7]
18	50	48	12	6	10	1xDiaphragm, Liver & Ribcage 1xHeart 1xMediastinum 1xMediastinum & Hilar Lymphnode	1xKidneys	458.6 (119.7) [26.1]	497.7 (135.2) [27.1]
17	100	48	20	17	11	2xDiaphragm 1xDiaphragm & Ribcage 1xRibcage & Mediastinum 1xDiaphragm, Ribcage & Mediastinum	4xLeft Kidney	479.6 (124.6) [26.0]	535.5 (113.6) [21.3]
20	100	48	17	16	8	1xDiaphragm & Ribcage 2xMediastinum	2xKidneys	481.5 (141.0) [29.3]	496.8 (183.8) [37.0]

\* all these, apart from one anaplastic carcinoma, were squamous-cell carcinoma

\*\* Coefficient of Variation [S.D. as a % of mean]

† Metastases to the left hilar lymph node were seen in about 40% of lung tumour-bearing rats.

in the hilar region, but direct invasion of the azygous lobe of the right lung was not an uncommon finding.

A summary of the lung tumour incidence in the 3-methylcholanthrene-treated groups is presented in Table 12. At 25% and 50% concentration of the carcinogen, there were more tumours seen in female compared to male rats. This effect is not apparent in the two groups of rats treated with 100% carcinogen. The average induction periods, i.e. from implantation of pellet to discovery of tumour, for the three dose levels of carcinogen appear very close together, as do their coefficients of variation. The concentration of carcinogen has been plotted against the percentage of induced tumours (log. scale) and results appear in Figure 17. Although only three dose-levels were used, it would seem that the log. of the percentage tumour yield is proportional to the concentration of carcinogen over the range used. The square on Figure 17 is that of Group 20. This is the group of rats treated with 100% 3-methylcholanthrene, but set up four months after the other carcinogen-treated rats. The Group 20 animals are therefore not from the same pool of animals and, strictly speaking, should not be compared to the other carcinogen-treated rats. They have, however, been included in this section as the number of tumours induced and the mean induction period is notably close to that seen in the other group of rats treated with 100% 3-methylcholanthrene (Group 17).

#### Chromium-containing Test Materials

Amongst the groups of rats exposed to 14 chromium and chromate residues and compounds under test, tumours were found in the lungs of animals in six groups. Table 13 describes the compounds involved, the number of tumours, their histology and induction periods. As

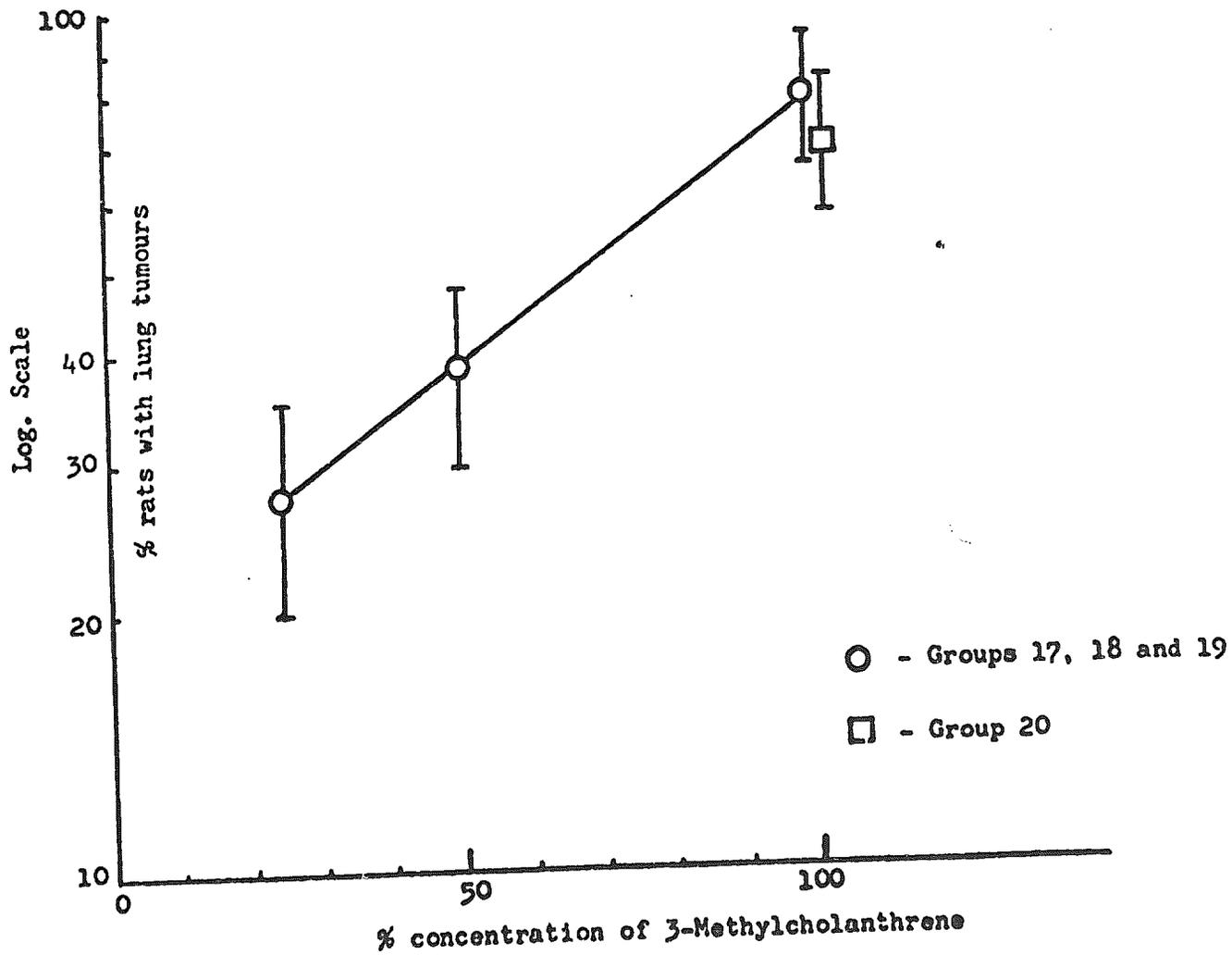
SUMMARY OF BRONCHIAL CARCINOMAS IN RATS EXPOSED TO PELLETS  
CONTAINING THREE CONCENTRATIONS OF 3-METHYLCHOLANTHRENE

Group No.	% 3-MCA	No. of Rats in Group (n)	No. of Rats with Tumours (o)		Mean Induction Period in days ( $\pm$ S.D. of mean)	C.V. %
19	25	48	13 (27%)	7.5%°	517.4 (137.2)	26.5
18	50	48	18 (38%)	8.8%°	472.3 (122.6)	26.0
17	100	48	37 (77%)	12.7%°	504.3 (121.1)	24.0
20**	100	48	33 (69%)	12.0%°	487.9 (160.5)	33.0

° 1 S.D. Confidence Limits, calculated from :-  $\sqrt{\frac{o}{n}} \times \frac{100}{1}$

\*\* This group was a repeat of Group 17

INCIDENCE OF BRONCHIAL CARCINOMA WITH THREE  
CONCENTRATIONS OF 3-METHYLCHOLANTHRENE



Vertical bars = confidence limits of  $\pm 1$  S.D. calculated from

$$\text{S.D.} = \sqrt{\frac{\text{observed number of tumours}}{\text{number of rats in Group}}} \times 100$$

LUNG TUMOURS FOUND IN THE 14 CHROMIUM-CONTAINING  
MATERIAL TEST GROUPS

Group No.	Test Material	No. of Rats in Group	No. of Rats with Lung Tumours (F,M)	Histology of Tumour	Invasion and Metastases	Induction Period in days ( $\pm$ S.D. of mean) [C.V.%]
11	Calcium Chromate	100	8 (5,3)	7x Squamous-cell Carcinoma 1x Anaplastic squamous-cell Carcinoma	3x Invasion of right lung 4x Invasion of diaphragm 1x Invasion of ribcage and mediastinum 3x Metastases to hilar lymph node 1x Metastases to both kidneys	603.75 (97.9) [16.2]
13	Zinc Potassium Chromate	100	3 (1,2)	3x Squamous-cell Carcinoma	-	708.0 (44.17) [6.2]
9	Chromic Acid	100	1 (0,1)	Squamous-cell Carcinoma	-	560
12	Chromic Chloride	100	1 (1,0)	Lymphoma (localised)	-	587
7	Sodium Dichromate	100	1 (0,1)	Fibro-sarcoma	-	683
6	Residue from Slurry Tank,	101	1 (1,0)	Anaplastic Carcinoma	Invasion of thymus and diaphragm	232

N.B. The induction periods for the tumours in Group 11 were examined by the third and fourth moment method. This showed the distribution to be 'normal' for both sexes. A 'normal' distribution is assumed for Group 13. As there are only 3 rats (2 female, 1 male) it was not possible to use the above method.

these results are of importance, it is necessary to describe and discuss these findings in detail.

#### Group 11 - calcium chromate

Eight lung tumours were found in this group (5 in females, 3 in males), all of which were diagnosed as squamous-cell carcinomas of bronchial origin. In all respects, these tumours were indistinguishable from those encountered in the 3-methylcholanthrene-treated animals, i.e. the same size, the same site of origin around the implanted pellet and the same histological appearance (Fig. 18). Further, in one of the animals in this group, metastases to both kidneys were found, similar to those seen in some of the carcinogen-treated animals. All but one of the 8 tumours resulted in the premature death of the rats in which they appeared. The mean induction period was 585 days (range 473 - 733 days).

#### Group 13 - zinc potassium chromate

In this group, three lung tumours were found (1 in a female, 2 in males), but only one directly resulted in the death of the rat in which it occurred. All three tumours were classified as squamous-cell carcinoma of bronchial origin and with the same characteristics as that seen in the 3-methylcholanthrene-treated animals. The two tumours which did not appear to affect the health of the rats in which they appeared were both of approximately the same size, occupying the lower third of the left lung around the pellet area, but not distorting the outline of the lung in any way (Fig. 19). Both tumours, however, were easily distinguished during the 'terminal kill' necropsy. The three tumours had a mean induction period of 708 days (657, 733 and 734 days).

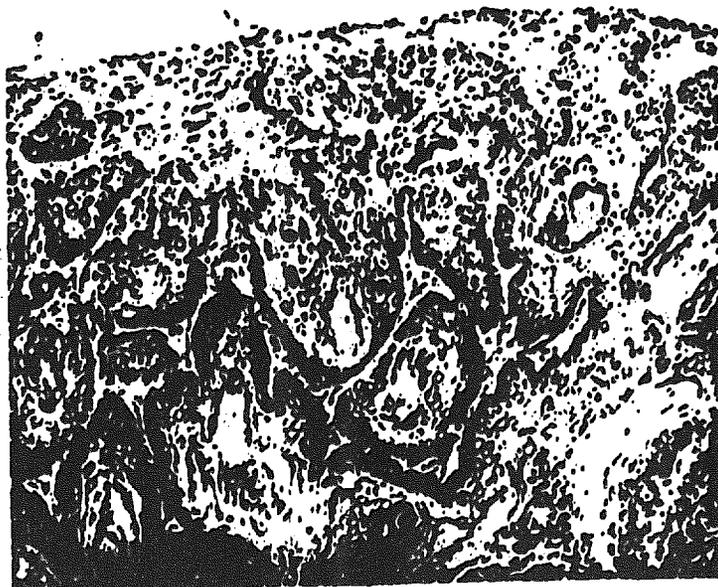


Figure 18(a). Squamous-cell carcinoma involving left lung of female rat killed 473 days after receiving pellet containing calcium chromate (Group 11). H. & E. X128



Figure 18(b). Detail from above tumour showing marked variation in nuclear forms, mitotic figures and formation of keratin nests. H. & E. X512



Figure 19(a). Squamous-cell carcinoma from left lung of male rat killed 733 days after receiving pellet containing zinc potassium chromate (Group 13). This lesion occupied the lower third of the left lung and did not appear to affect the health of the animal. H. & E. X128

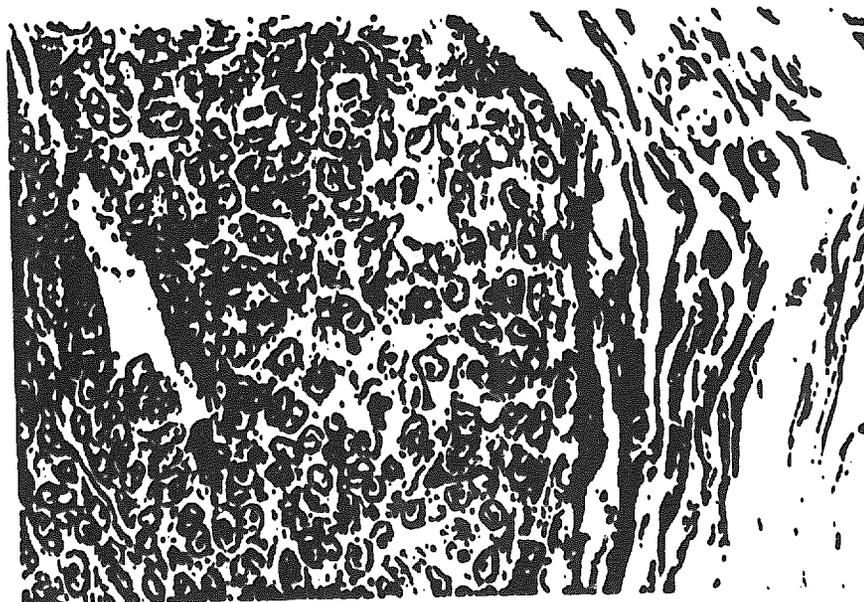


Figure 19(b). Detail of squamous-cell carcinoma from left lung of female rat killed 734 days after receiving pellet containing zinc potassium chromate (Group 13). The proportion of left lung involved in this lesion was similar to that of the above male rat. Mitotic figures are frequent. H. & E. X512