INCIDENCE OF LUNG TUMOURS IN LX MICE EXPOSED TO (1) FREE RADICALS; (2) SO_2

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Although the spontaneous incidence of pulmonary adenoma varies greatly between line-bred strains of mice, they all show a higher species susceptibility to this very characteristic tumour than other species of small rodent kept under similar laboratory conditions. Moreover, some chemical carcinogens for mouse lung, e.g. urethane and polycyclic hydrocarbons, induce similar tumours to those of spontaneous origin, but at an earlier age and in greater numbers than are found in untreated controls. Thus, the mechanism of carcinogenesis in the mouse lung appears to be very complex, involving unidentified factors responsible for the spontaneous tumours, in addition to controlled experimental factors.

A close association between the occurrence of subpleural alveolar hyperplasia and neoplasia and the presence of engorged lymphatics filled with apparently normal lymphocytes in the affected sector of the lung, has been reported (Peacock and Peacock, 1966). While the significance of this association is not clear, it might be of aetiological importance. Occasionally such engorged lymphatics are seen in the normal subpleural lymphatic sites without any epithelial hyperplasia, in apparently healthy lungs, and these may be regarded as physiological.

On the other hand, in our experience subpleural alveolar hyperplasia is rarely observed in the absence of such lymphatic engorgement. Clearly, in a single section or in a series of sections taken from a single lung, one cannot form an adequate impression of the duration of such lymphatic engorgement; but it seems possible that if it is maintained for more than a certain length of time, alveolar hyperplasia of the associated overlying epithelium may follow automatically, and this in its turn may provide a suitable site for the action of potential carcinogens.

It seemed desirable, therefore, to devise experiments which would create areas of subpleural lymphatic engorgement and maintain them for a known length of time, and observe the possible effects of such treatment on the subsequent incidence of hyperplastic and neoplastic lesions in the lung. With this in mind, we chose two non-cumulative inorganic irritants capable of being administered as airborne pollutants, namely free radicals and sulphur dioxide.

MATERIALS AND METHODS

Free radicals were chosen partly because they are present in cigarette smoke, which is associated with cancer of the lung in man, and also because free radicals are generated in the electrostatic air filter which was used in previous experiments on the possible influence of airborne soot as a potential carcinogen for the mouse lung (Peacock, 1962). Sulphur dioxide was chosen because of its presence in

urban atmospheric pollution and because it is a gaseous and non-cumulative irritant which is easily controlled.

Mice of the LX colony, bred in this laboratory from stock kindly supplied by Dr. Bloom, were used because they are known to be highly susceptible to the induction of lung adenoma in response to urethane, and because they are heterozygous though selectively bred for large size (Bloom, 1964).

To house the experimental mice, two identical perspex chambers, each measuring 50 × 40 × 85 cm. and of approximately 180 litres' capacity, were used. These chambers were equipped with two perspex shelves, each of which could carry four standard mouse boxes; 12 boxes in all. The chambers were closed by sliding doors and had input ventilation tubes of 5 cm. diameter and similar output openings on one side of each cage. No intercommunication was possible between the two chambers.

The mice were segregated by sex and were kept in galvanised boxes each containing not more than five mice, usually members of one litter. Food pellets (Diet 41) and tap water in plastic bottles, were constantly available.

Group 1: 41 Male and 39 female, three-month-old LX mice born at about the same date as the experimental groups, were kept in the main animal house in similar boxes.

Group 2: 30 male and 30 female LX mice, three months old, were kept in Chamber 1, which was equipped with thin copper sleeve electrodes, 1·25 cm. broad and 6 mm. apart, fitted around the input ventilation duct. The lower electrode was connected to the active terminal of a diathermy unit giving an output of 1·5 kw at 2 kv. The upper electrode was earthed.

The technique for free radical reproduction was that of radio-frequency discharge (Shaw, 1960). The apparatus was switched on for 3 hours daily from Monday to Friday inclusive throughout the experiment.

Group 3: 35 male and 30 female LX mice, three months old, were kept in Chamber 2 under similar conditions to those in Group 2, except that the ventilation duct was equipped with a side tube through which SO_2 could be added to the input air.

By preliminary pilot trials a rate of 20 ml. per minute for 5 minutes (500 p.p.m.) was found to be well tolerated, and this dosage was given daily from Monday to Friday throughout the experiment.

All animals were inspected daily by the technical staff throughout the experiment, and sick animals were reported, and were examined by one of us (P.R.P.) and if considered to be seriously ill were killed by chloroform and immediately autopsied. Despite this, some animals were found dead and were autopsied as soon as possible after discovery. Most survivors over two years old were killed and immediately autopsied.

The procedure adopted was described in detail in a previous communication from this laboratory (Peacock and Peacock, 1966). Suspicious lesions in any organ were processed for histology. The lungs were inflated with 1% formalin and removed intact for further inspection. The heart was removed and all obvious lung lesions noted. The lungs were then compressed between the lid and inverted base of a plastic petri dish and examined from both sides by transmitted light. In this way, lesions of less than 1 mm. diameter can be seen by the narked eye as translucent spots. All such lesions were processed for serial sectioning, stained by Van Geison or Mallory and Gomori's aldehyde fuchsin elastic stain, in

order to distinguish between alveolar hyperplasia and neoplasia, in which condition the elastica is defective.

RESULTS

Only mice that survived for three hundred days or more are considered in assessing the results because no primary tumours of the lung were seen in LX mice below this age. The essential details of experimental data, autopsy and histology for each mouse, and the site and degree of neoplasia, when present, are given in Tables I to III.

In order to give some numerical value to the degree of neoplasia in the lungs, one (+) was awarded for each mm. of tumour diameter for the largest tumour in any one mouse. A mouse with one tumour of 3 mm. and several smaller tumours, scores (3), and so on. Admittedly this method under-estimates the differences between a single tumour of 1 mm. or less diameter discovered during histological examination of an apparently normal lung and a mass of tumours which caused death or serious respiratory embarrassment. However, the method of attempting to count individual tumours is thought to be less satisfactory because a number of small tumours may become confluent and so the number of recorded tends to grow less as the tumours grow larger.

The incidence of primary lung hyperplasia and neoplasia is shown in Tables IV and V. Since the degree of neoplasia is debatable all neoplastic lesions are included under Adenoma in Table IV. (see Discussion). It will be seen that in the mice exposed to SO_2 there is an increased incidence of primary neoplasia in the lung of both sexes, from 31% to 54% in males and from 17% to 43% in females.

In those exposed to free radicals there was an increase of approximately 10% in males and 6% in females. Primary lung tumours in males occurred about twice as frequently as in females in groups 1 and 2.

DISCUSSION

The anatomical and histological grading shows about equal distribution between tumours of subpleural and other origin in males of groups 1 and 2, and in females of group 3; in the other groups subpleural exceeded other-site tumours.

A number of factors which cannot easily be quantitated must be considered in assessing these results. Thus, the grading of neoplasia as carcinoma or adenoma is not always easy. Lung tumours in mice rarely metastasise outside the thorax and even large tumours replacing a whole lobe may show no invasive tendency. We classify as carcinoma only those tumours which invade blood vessels (fig. 6) or other organs, though in many adenomata atypical growth suggesting malignancy may be found.

For this reason all primary tumours of the lung are included under the heading " Adenoma " in Table IV and some of these also appear under " Primary Carcinoma " and under " Hyperplasia ".

All the " Primary carcinoma " mice also had adenoma, but some " Hyperplasia " mice had no neoplastic lesions.

The distinction between hyperplasia and neoplasia of the pulmonary alveolar epithelium is somewhat arbitrary since both types of lesion are often found together or in different parts of the same lung. Inevitably therefore subjective judgment plays a part in assessing the degree of neoplasia.

In general, the group exposed to SO_2 had more and large primary lung tumours

than untreated controls, and at an earlier age. Those exposed to free radicals showed an incidence of lung tumours intermediate between the SO_2 and control group.

The occurrences of primary carcinoma of the lung in females was limited to the SO_2 group. Fewer of the mice exposed to SO_2 were free from detectable primary neoplastic lung lesions than in the other two groups. In many animals several stages of hyperplasia and neoplasia were present together at autopsy. In heterozygous animals, genetic factors may determine variation in the effects of environmental carcinogens and might be held responsible for the higher incidence of lung tumours in the SO_2 group. Against this probability is the fact that tumours of other organs, notably the liver in males and the lymphatic system in females, show no such group distinction. In Table VI a comparison is made of the actual and percentage incidence of hepatoma and lymphomatoses (including leukaemia and lymphosarcoma), on the same basis as that used in Table V. For ease of comparison the results for lung and liver tumours are shown in the form of a histogram (Fig. 1).

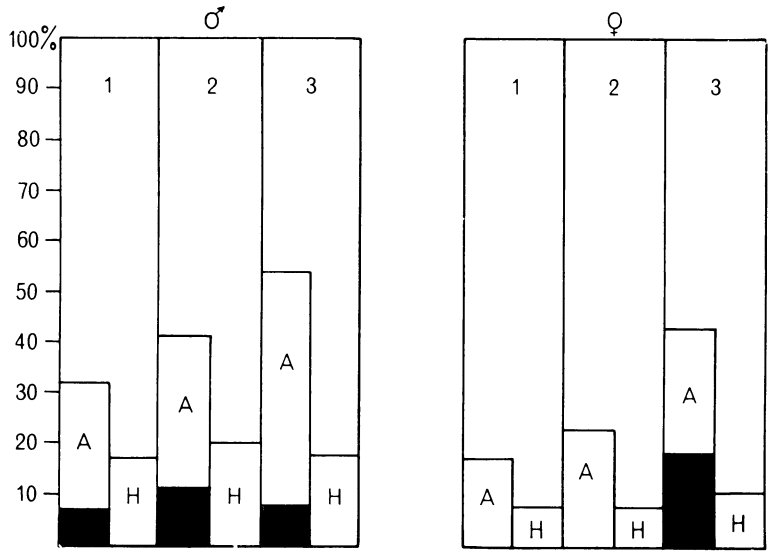


FIG. 1.—Histogram showing percentage distribution of pulmonary papillary adenoma A and hepatoma H; histologically malignant tumours shown in black. Figures at head of columns correspond with Groups 1, 2, and 3.

It is clear that there is no significant variation in the distribution of hepatoma between the three groups, but that the incidence in the males is more than twice that in the females. The distribution of lymphomatosis shows a greater susceptibility of females in all groups, but no apparent relationship to experimental conditions. The higher incidence of lymphomatosis in male control mice may be related to their lower incidence of lung tumours, but with the small number involved (5/35) it is not thought to be aetiologically significant.

Independent statistical analysis of the figures in Tables IV–VI by our colleague Dr. S. Iversen shows that such a distribution of tumours could be a matter of chance.

TABLE I.—*Controls*

No.	Weight (g.)	Age at death (days)		Lung* 1	2	3	4	Autopsy (A) and Histology (B)
Males:								
1459/2	45	323		—	—	—	—	(A) Haemorrhagic mass in right axilla. (B) Haemorrhagic lymphoma.
1461/1	45	360		—	—	—	—	(A) and (B) Haemorrhagic enteritis; N.A.F. in lungs.
1425/1	30	393		—	—	—	—	(A) and (B) Enteritis and pneumonia.
1328/4	50	411		—	—	—	—	(A) Large pale liver. (B) Lymphomatosis.
1392/2	80	K.426		—	—	—	—	(A) Perirenal tumour; multiple metastases. (B) Anaplastic sarcoma.
1452/2	35	K.460		—	—	—	—	(A) Poor condition; tumour in liver; stone in bladder; pneumonia. (B) Hepatoma; pneumonia.
1452/3	30	K.494		—	—	—	—	(A) and (B) Collapsed and congested lung.
1328/5	45	495	(1)†	+	—	+	—	(A) Ulcer of right ear; multifocal lung tumours. (B) Squamous carcinoma *in situ* of ear; subpleural papillary adenoma and alveolar hyperplasia.
1452/4	40	K.499	(12)	+	+	—	—	(A) Poor condition; large central liver tumour; large tumour left lobe of lung, small tumour right azygos lobe. (B) Hepatoma; multifocal papillary adenoma.
1461/2	35	K.511	(5)	+	+	—	—	(A) Abscess of right eye; large tumour invading heart and diaphragm. (B) Papillary adenoma and carcinoma.
1450/1	42	534		—	—	—	—	(A) Multiple nodules in liver. (B) Lymphomatosis.
1539/1	45	K.548		—	—	—	—	(A) Very large liver. (B) Leukaemia.
1542/2	40	571		—	—	—	—	(A) Subcutaneous abscess of left groin; liver tumour; lung congested. (B) Hepatoma; lung congested.
1450/2	28	571	(12)	+	+	—	—	(A) Multifocal tumour of right middle lobe of lung. (B) Multifocal papillary adenoma and carcinoma.
1539/2	65	K.594		—	—	—	+	(A) Good condition, N.A.F. (B) Other site alveolar hyperplasia in lung.
1329/5	45	596		—	—	—	—	(A) Large central liver tumour (5 g.); lung congested. (B) Hepatoma; lung congested.
1539/3	56	K.608	(3)	+	+	—	—	(A) Good condition; tumour (5 mm.) right middle lobe of lung. (B) Multifocal papillary adenoma.
1457/5	75	K.640		—	—	—	—	(A) Good condition but distended bladder (20 ml.). (B) Lungs N.A.F.
1459/3	24	K.645		—	—	—	—	(A) Kyphosis; left lobe collapsed. (B) Interstitial penumonia.
1327/1	47	K.645		—	—	—	—	(A) and (B) Good condition; N.A.F.
1327/2	45	K.645		—	—	—	—	(A) and (B) Good condition; N.A.F.
1459/4	48	651		—	—	—	—	(A) and (B) Autolytic; N.A.F. in lung.
1459/5	45	K.658		—	—	—	—	(A) and (B) Distened bladder; N.A.F. in lung.
1450/3	50	K.679	(2)	+	—	—	—	(A) Multifocal tumours in lungs. (B) Subpleural papillary adenoma.
1450/4	65	K.679	(1)	+	—	—	—	(A) Tumour (4 mm.) right upper lobe of lung; tumour (1 cm.) in liver. (B) Subpleural papillary adenoma; hepatoma.
1450/5	70	K.679	(3)	—	+	+	—	(A) Tumour (3 mm.) right middle lobe of lung and smaller lesions. (B) Other site papillary adenoma and subpleural alveolar hyperplasia.
1450/6	70	K.679	(2)	—	+	—	—	(A) Multifocal tumour right middle and right azygos lobes of lung. (B) Other site papillary adenoma.
1542/3	35	K.696		—	—	—	—	(A) Mesenteric tumour; soft stones in bladder. (B) Haemangioma.
1456/2	38	708	(2)	+	+	—	—	(A) Retention; stone in bladder; tumour in left lung. (B) Multifocal papillary adenoma.
1539/4	52	714		—	—	—	—	(A) and (B) Good condition; N.A.F. in lungs.
1456/3	40	730	(1)	+	—	—	—	(A) Stone in bladder; hydronephrosis; tumour right lobe of lung. (B) Subpleural papillary adenoma.
1456/4	50	730		—	—	—	—	(A) and (B) Stone in bladder; N.A.F. in lung.
1456/5	40	730		—	—	—	—	(A) and (B) Stone in bladder; N.A.F. in lung.
1329/1	45	K.813		—	—	—	—	(A) Tumour on right side of neck; tumour in liver. (B) Lymphoma; hepatoma.
1329/4	35	873		—	—	—	—	(A) and (B) Traumatic rupture of heart; lung collapsed.

No.	Weight (g.)	Age at death (days)		Lung* 1	2	3	4	Autopsy (A) and Histology (B)
Females								
1453/1	30	317		—	—	—	—	(A) and (B) Haemorrhagic gastroenteritis; autolytic; N.A.F. in lung.
1541/1	20	367		—	—	—	—	(A) and (B) Autolytic; lung congested.
1451/1	52	436		—	—	—	—	(A) and (B) Right side pyometra.
1330/1	35	473		—	—	—	—	(A) Cystitis; many soft stones. (B) Chronic cystitis.
1460/1	45	K.491		—	—	—	—	(A) and (B) Poor condition; ruptured pymetra; mesenteric abscesses; hepatoma.
1330/2	45	K.517		—	—	—	+	(A) Multiple tumours in subcutaneous tissue and muscles of thorax and limbs. (B) Lymphosarcoma and other site alveolar hyperplasia.
1462/2	25	532	(11)	+	+	—	—	(A) Poor condition, large tumour in left lobe of lung. (B) Multifocal papillary adenoma.
1540/1	45	535		—	—	—	—	(A) and (B) Pyometra; lungs N.A.F.
1453/2	30	K.552		—	—	—	—	(A) Multifocal infarcts; large mass in left uterus. (B) Septic infarcts and septic endometritis.
1462/3	55	561		—	—	—	—	(A) Left pyometra; tumour in left ovary; nodules in liver. (B) Lymphosarcoma.
1538/1	50	K.578		—	—	—	—	(A) and (B) Purulent cystitis; lungs congested.
1331/5	45	K.581	(1)	+	—	—	—	(A) Ascites; multifocal tumour of liver omentum lymph nodes; ?lung tumour. (B) Lymphatic leukaemia; lymphosarcoma; subpleural papillary adenoma.
1541/3	40	K.606		—	—	—	—	(A) Large liver tumour right lobe. (B) Hepatoma.
1460/2	40	K.615		—	—	—	—	(A) and (B) Good condition; N.A.F.
1330/3	50	K.622		—	—	—	—	(A) Haemorrhagic ascites; large nodular liver; tumour of right uterus. (B) Lymphatic leukaemia; lymphosarcoma.
1541/2	20	626		—	—	—	—	(A) and (B) Lungs congested.
1455/3	50	K.631		—	—	—	—	(A) Large tumours involving abdominal viscera and mediastinum. (B) Multifocal lymphosarcoma.
1541/4	25	K.632	(1)	+	+	—	—	(A) Enteritis; tumour in right lower lobe of lung. (B) Multifocal papillary adenoma.
1458/1	33	K.641		—	—	—	+	(A) Bilateral hydrosalpynx; ?tumour of right lower lobe of lung. (B) Small other site alveolar hyperplasia.
1455/4	50	K.646		—	—	—	—	(A) Good condition; large uterus: congested lung. (B) Endometriosis.
1458/2	35	K.648		—	—	—	—	(A) Good condition; tumour in left ovary and uterus. (B) Squamous carcinoma of uterus.
1458/3	45	K.648		—	—	—	—	(A) and (B) Good condition; hydrosalpynx.
1540/2	45	K.658		—	—	—	—	(A) and (B) Good condition; very fat; N.A.F.
1538/3	47	K.678		—	—	—	—	(A) and (B) Good condition; hydrosalpynx; papilloma of forestomach; ?lung lesions. (B) Papilloma of forestomach; desquamative alveolar hyperplasia.
1451/3	53	K.679	(4)	+	—	+	—	(A) Good condition; hydrosalpynx; tumour in right upper lobe of lung. (B) Subpleural papillary adenoma and alveolar hyperplasia.
1330/5	52	689		—	—	—	—	(A) Anasarca; multifocal liver tumours; haemorrhagic salivary gland. (B) Lymphomatosis.
1538/4	50	K.724		—	—	—	—	(A) Tumour of back and ulcerated tumour of right groin. (B) Multifocal myxosarcoma.
1540/3	30	724		—	—	—	—	(A) and (B) Good condition; lungs congested.
1540/4	35	724	(4)	+	—	—	—	(A) Good condition; tumour (4 mm.) in right lower lobe of lung. (B) Subpleural papillary adenoma.
1330/4	18	761		—	—	—	—	(A) and (B) Autolytic; N.A.F.

* Primary Lung Lesions:—Column 1—Papillary ademona of subpleural origin.
2—Papillary adenoma of other sites.
3—Alveolar hyperplasia of subpleural origin.
4—Alveolar hyperplasia of other sites.

† Figures in brackets show diameter in mm. of largest tumour.

TABLE II.—*Free Radicals*

No.	Weight (g.)	Age at death (days)	Duration of experiment (days)		Lung* 1	2	3	4	Autopsy (A) and histology (B)
Males:									
1321/2	75	319	234		−	−	−	−	(A) Large hepatic tumour; stone in hypertrophic bladder. (B) Hepatoma.
1357/1	35	421	385		−	−	−	−	(A) and (B) Necrotic penis, cystitis, pyelitis.
1323/4	50	K.435	347	(3)	+	+	−	−	(A) Tumour of antrum and orbit; multifocal lung tumours. (B) Squamous carcinoma of antrum; papillary adenoma.
1361/1	50	440	422	(2)	+	+	−	−	(A) Liver tumour; large multifocal lung tumours. (B) Hepatoma; subpleural and other site papillary adenoma and carcinoma.
1325/5	65	464	316		−	−	−	−	(A) and (B) Autolytic dark lung; patchy pneumonia.
1360/1	65	470	441		−	−	−	−	(A) Generalised lymphatic tumours; nodes liver and spleen. (B) Leukaemia.
1323/3	40	477	389						(A) Aytolytic; N.A.F. in lungs.
1323/1	50	495	417		−	−	−	−	(A) Intestinal obstruction by caecal tumour. (B) Anaplstic adeno-carcinoma of caecum.
1325/2	60	K.574	486		−	−	−	−	(A) and (B). Traumatic ulcers (fighting); N.A.F. in lung.
1325/4	70	K.574	486		−	−	−	−	(A) and (B). Traumatic ulcers (fighting); oedema of lung.
1360/2	60	584	534	(1)	−	+	−	−	(A) Necrotic penis; distended bladder; pale mottled liver; lung tumour. (B) Tyzzer's disease; other site papillary adenoma.
1325/1	35	609	524	(11)	+	+	−	−	(A) Large tumours in right lower lung; pneumonia. (B) Multifocal papillary adenoma and carcinoma.
1321/1	55	610	525	(5)	+	+	−	−	(A) Stone in distended bladder; multifocal lung tumour. (B) Subpleural and other site papillary adenoma.
1325/3	55	K.636	548		−	−	−	−	(A) Abdominal swelling, torsion of distended bladder; liver tumour. (B) Hepatoma.
1357/2	25	669	633		−	−	−	−	(A) Huge liver tumour. (B) Hepatoma.
1360/3	40	K.669	640	(3)	+	+	−	−	(A) Skin tumour right ear; tumour right middle lobe. (B) Necrotic squamous carcinoma; subpleural and other site papillary adenoma.
1361/2	40	684	616	(10)	+	+	−	−	(A) Multifocal lung tumour invading ribs and diaphragm. (B) Multi-focal papillary adenoma and carcinoma.
1361/3	60	691	673	(2)	+	−	+	−	(A) Bladder obstructed by stone; huge liver tumour. (B) Hepatoma.
1323/2	30	694	606		−	−	−	−	(A) and (B) Uretheral stone obstruction; N.A.F. in lung.
1361/5	55	K.714	696	(2)	+	−	+	−	(A) Traumatic ulcers (fighting); bladder stone; multifocal tumour in lung. (B) Subpleural papillary adenoma and alveolar hyperplasia.
1361/4	50	K.746	728	(3)	+	−	+	−	(A) Multifocal lung tumour. (B) Subpleural papillary adenoma and alveolar hyperplasia.
1321/5	33	K.750	662		−	−	−	−	(A) and (B) Nasal abscess; N.A.F. in lung.
1360/4	35	K.757	728		−	−	−	−	(A) and (B) N.A.F.
1360/5	48	K.757	728		−	−	−	−	(A) and (B) N.A.F.
1357/3	27	K.773	737	(8)	+	+	+	+	(A) Huge tumour right middle lobe of lung and multifocal tumours in other lobes. (B) Subpleural and other site papillary adeoma and alveolar hyperplasia.
1357/4	28	K.773	737		−	−	−	−	(A) Liver tumour. (B) Hepatoma.
1357/5	29	K.773	737		−	−	−	−	(A) N.A.F. (B) Lungs: perivascular lymphatic engorgement.
1321/3	50	K.805	720	(3)	+	+	−	−	(A) Ulcer of right axilla; multifocal tumour right upper and right lower lobes of lung. (B) Subpleural and other site papillary adenoma.
1321/4	35	847	762	(2)	−	+	−	+	(A) Chronic nephritis; tumour right lower and ?left lung. (B) Other site papillary adenoma and alveolar hyperplasia.

No.	Weight (g.)	Age at death (days)	Duration of experiment (days)		Lung* 1	2	3	4	Autopsy (A) and histology (B)
Females:									
1324/3	35	396	311	(2)	–	+	–	–	(A) Large tumour right upper lobe of lung; tumour right mandible; tumour of thymus. (B) Well differentiated papillary adenoma; lymphoma; squamous carcinoma.
1322/2	55	K.403	318		–	–	–	–	(A) Anasarca; diffuse pelvic tumour; metastases in liver. (B) Anaplastic sarcoma of subcutaneous tissues; lymphosarcoma of parametrium.
1358/1	30	408	372		–	–	–	–	(A) and (B) Acute enteritis and hepatitis. N.A.F. in lung.
1356/1	25	434	399		–	–	–	–	(A) Bilateral hydronephrosis; vesical stone.
1359/1	35	478	449		–	–	–	–	(A) Cyst left side of neck; liver tumour. (B) Hygroma in lymph node; trabecular hepatoma.
1326/1	38	491	403		–	–	–	–	(A) Ascites; pneumonia. (B) Pneumonia—autolytic.
1324/2	40	536	446		–	–	–	–	(A) and (B) Haemorrhagic ascites and visceral lymphomatosis.
1356/2	40	K.539	504		–	–	–	–	(A) Proptosis right side; large bladder stone; pelvic tumour. (B) Spindle cell sarcoma of vagina; metastasis in orbit.
1326/2	52	545	452		–	–	–	–	(A) Ascites and pleural effusion; lung collapsed. (B) Lymphomatosis; leukaemia.
1324/5	50	K.649	564	(1)	+	+	–	+	(A) Ascites; huge liver and spleen; pyometra; multifocal lung tumour (B) Leukaemia; subpleural and other site papillary adenoma; other site alveolar hyperplasia.
1359/3	43	695	667		–	–	–	+	(A) Chronic peritonitis and pyometra; ?tumour left lung. (B) Other site alveolar hyperplasia.
1356/3	35+	761	666		–	–	–	–	(A) ?tumour left lung. (B) Desquamating alveolar hyperplasia; perivascular lymphangitis.
1326/3	23	714	629	(1)	+	–	+	–	(A) Enteritis; ?lung tumours. (B) Subpleural papillary adenoma in area of alveolar hyperplasia.
1358/2	45	716	680		–	–	–	–	(A) and (B) Pyometra; multifocal liver abscesses; N.A.F. in lung.
1324/1	55	K.721	633		–	–	–	–	(A) Mammary tumour R.2. (B) Pleomorphic carcinoma.
1359/4	35	K.724	695		–	–	–	–	(A) Tumour right sacral origin; metastases in liver and lung. (B) Osteogenic sarcoma.
1359/2	15	725	696		–	–	–	–	(A) and (B) Autolytic; N.A.F. in lungs.
1359/5	36	K.725	696		–	–	–	–	(A) and (B) Septic endometritis.
1324/4	39	749	661		–	–	–	–	(A) and (B) Chylous ascites; viceral lymphomatosis.
1358/3	30	751	715		–	–	–	–	(A) Pendulous tumour right liver lobe; ?lesions in lungs. (B) Trabecular hepatoma; oedema of lungs.
1322/3	55	754	666		–	–	–	–	(A) Ascites; large mottled liver. (B) Leukaemia.
1358/4	25	K.773	737	(1)	+	–	–	–	(A) Subpleural lung tumours. (B) Small subpleural papillary adenoma.
1358/5	40	K.773	737		–	–	–	–	(A) and (B) Good condition; no lesions found.
1322/5	40	783	695	(6)	+	–	–	–	(A) Haemothorax; tumour left lung; uterine tumours L > R. (B) Subpleural papillary adenoma; adenocarcinoma of uterus; metastases in lung.
1356/4	27	K.792	757		–	–	–	–	(A) and (B) Good condition; no lesions found.
1356/5	28	K.792	757		–	–	–	–	(A) Good condition; lesion left lung. (B) Perivascular lymphatic engorgement.
1322/4	25	K.798	710		–	–	–	–	(A) Uterine tumour; ovarian cysts; mediastinal lymphoma. (B) Adenocarcinoma of uterus; lymphomatosis.
1322/1	45	K.833	745		–	–	–	–	(A) Tumour right side vulva; paraplegia. (B) Squamous papilloma and carcinoma of vulva.
1326/5	50	882	797	(3)	+	+	–	–	(A) Multifocal tumour right middle and left lobes; liver enlarged; right ovarian cyst; endometriosis. (B) Subpleural and other site papillary adenoma; leukaemia; endometriosis.
1326/4	35	K.896	808	(5)	+	–	–	–	(A) Tumour in left lung. (B) Subpleural papillary adenoma.

TABLE III.—SO_2

No.	Weight (g.)	Age at death (days)	Duration of experiment (days)		Lung* 1	2	3	4	Autopsy (A) and Histology (B)
Males:									
1355/1	100	346	311	(2)	+	−	−	−	(A) Very fat; bladder stone; fibrinous peritonitis; 2 tumours left lung. (B) Subpleural papillary adenoma.
1355/2	25	390	325	(2)	+	+	−	−	(A) Autolytic; tumour right middle and left lung. (B) Pneomonia and papillary adenoma.
1355/3	35	444	379		−	−	−	−	(A) and (B) Autolytic; lung congested and oedematous.
1351/1	45	498	463	(3)	+	+	−	+	(A) Bladder distended; turbid urine; liver tumour; multifocal lung tumour. (B) Trabecular hepatoma; multifocal adenoma and hyperplasia.
1319/1	60	515	430		−	−	−	−	(A) and (B) Acute oedema of lung; pyelonephritis; stones in bladder.
1351/2	55	535	500		−	−	−	−	(A) and (B) Autolytic ; N.A.F. in lung; soft stones in bladder; pyelonephritis.
1372/1	40	548	489		−	−	−	−	(A) and (B) Autolytic; N.A.F. in lung.
1353/1	40	567	532	(2)	+	+	−	−	(A) Tumour of liver; distended bladder; tumour of left lobe of lung. (B) Trabecular hepatoma; multifocal papillary adenoma.
1319/2	30	600	512	(2)	+	+	−	−	(A) Multifocal liver tumours; tumours right lower lobe of lung. (B) Trabecular hepatoma; papillary adenoma.
1319/5	55	603	515		−	−	−	−	(A) Traumatic ulcers (fighting); distended bladder; liver tumour. (B) Trabecular hepatoma.
1355/4	25	K.603	538	(3)	+	−	+	−	(A) Poor condition; ?lung lesions. (B) Subpleural lymphangitis; alveolar desquamation; subpleural papillary adenoma and alveolar hyperplasia.
1355/5	30	K.603	538		−	−	−	−	(A) and (B) Poor condition; N.A.F. in lungs.
1353/2	50	K.632	597		−	−	−	−	(A) and (B) Severe trauma (fighting); N.A.F. in lungs.
1372/2	30	K.658	601		−	−	−	−	(A) and (B) Left pyonephrosis; right pyelonephritis; lung congested.
1315/4	50	674	589		−	−	−	−	(A) Turbid pleural effusion; mediastinal tumours. (B) Lymphomatosis.
1353/3	60	K.686	651	(8)	+	+	−	−	(A) Distended bladder; stone in urethra; liver tumour; tumour left lobe of lung. (B) Trabecular hepatoma; multifocal papillary adenoma and carcinoma.
1315/5	40	K.701	616		−	−	−	−	(A) Phimosis; full bladder; ?lesions in lung. (B) Desquamating alveolar hyperplasia.
1319/3	30	719	631		−	−	−	−	(A) Autolytic. (B) Oedema of lung.
1315/3	35	K.741	706	(5)	+	−	−	−	(A) Trauma (fighting); cystitis; lung tumours. (B) Subpleural papillary adenoma.
1351/4	47	K.741	706	(4)	+	−	−	−	(A) Trauma (fighting); tumour in left lung. (B) Subpleural papillary adenoma.
1319/4	44	K.749	664	(3)	+	−	+	−	(A) Nasal and subcutaneous abscess; multifocal lesions in lungs. (B) Subpleural papillary adenoma; alveolar hyperplasia.
1372/3	36	K.756	700	(4)	−	+	−	−	(A) Patchy pneumonia; tumour right upper lobe of lung. (B) Large other site papillary adenoma.
1372/4	32	K.756	500		−	−	−	−	(A) Poor condition. (B) Subpleur l lymphangitis.
1353/4	50	K.791	756	(5)	+	+	+	+	(A) Good condition; tumour right middle lobe of lung. (B) Subpleural and other site papillary adenoma; alveolar hyperplasia.
1353/5	50	K.791	756	(2)	+	+	+	+	(A) Good condition; tumour right lower and left lobes of lungs; mesenteric tumour. (B) Papillary adenoma and alveolar hyperplasia; angioma.
1351/5	30	K.791	756	(5)	+	+	−	−	(A) Huge tumour right lower lobe of lung; cystic mesenteric nodes. (B) Multifocal papillary adenoma and carcinoma.
1315/1	35	K.896	808	(3)	+	−	−	−	(A) Good condition; tumour left lobe of lung. (B) Subpleural papillary adenoma.
1315/3	30	K.896	808		−	−	−	−	(A) and (B) Good condition; N.A.F.

No.	Weight (g.)	Age at death (days)	Duration of experiment (days)		Lung* 1	2	3	4	Autopsy (A) and Histology (B)
Females:									
1320/4	70	316	231		-A	—	—	—	(A) Large thymus. (B) Lymphomatosis.
1316/1	60	K.325	237		—	—	—	—	(A) Large mammary tumour R.3. (B) Cystic mammary adenocarcinoma.
1318/3	55	371	286		—	—A	—	—	(A) Anasarca; huge liver; tumour of uterine cervices. (B) Lymphomatosis.
1318/4	25	403	318	(18)	+	+	—	—A	(A) Huge tumour right upper lobe of lung compressing heart. (B) Multifocal carcinoma of lung.
1320/3	65	406	321		—	—	—	—	(A) and (B) Left pneumonia.
1352/1	40	445	380		—	—	—	—	(A) and (B) Autolytic; lungs congested.
1318/5	40	454	366		—	—	—	—	(A) Pyometra; ?lung lesions. (B) Oedema of lung; pyaemic agscesses.
1316/2	70	558	470	(2)	—	+	—	—	(A) Anasarca; haemorrhage in peritoneum; huge liver tumour; right uterine tumour. (B) Sarcoma of uterus; other site papillary adenoma.
1320/5	27	K.593	508	(5)	+	+	—	—	(A) Multifocal tumour of right lower lobe of lung. (B) Subpleural and other site papillary adenoma and carcinoma.
1354/1	30	K.622	587	(3)	+	—	—	—	(A) Paraplegia; large tumour right middle lobe of lung; mesenteric cysts; hygroma. (B) Subpleural papillary adenoma.
1352/2	35	625	560	(2)	+	+	—	—	(A) Tumour in left lung; pedunculated liver tumour. (B) Haemangiohepatoma; multifocal papillary adenoma.
1350/5	50	641	551	(1)	+	+	—	—	(A) Multifocal lung tumour. (B) Multifocal papillary adenoma.
1318/1	65	642	554		—	—	—	—	(A) Small scarred liver. (B) Hepatocellular carcinoma; metastases in lung.
1350/1	35	K.643	553	(12)	+	+	—	—	(A) Huge tumour left lung. (B) Multifocal papillary adenoma and carcinoma.
1352/3	20	K.655	590		—	—	—	—	(A) Enlargement of mediastinal nodes. (B) Lymphatic engorgement.
1316/4	55	671	586	(3)	—	—	+	+	(A) Large abdominal tumour involving uterus; lungs congested. (B) Lymphomatosis; congestion and alveolar hyperplasia.
1350/3	40	733	643	(1)	+	—	+	—	(A) Small tumours right middle lobe of lung; right ovarian cyst. (B) Subpleural papillary adenomas.
1320/1	40	757	672	(5)	—	+	—	—	(A) Tumour right hind leg; papilloma of forestomach; large tumour right middle lobe of lung. (B) Fibrosarcoma of leg; other site papillary adenoma.
1352/4	50	K.757	692		—	—	—	—	(A) Large liver tumour (4g); lungs congested. (B) Heptoma.
1350/2	59	760	670		—	—	—	—	(A) Multifocal liver tumour; tumour left uterine horn. (B) Adenocarcinoma of uterus; metastasis in liver.
1320/2	45	K.771	686	(1)	+	+	—	—	(A) Turbid ascites; liver tumour; mediastinal tumours; small lung tumour. (B) Hepatocellular carcinoma; mediastinal metastases; subpleural and other site papillary adenoma.
1354/2	65	K.776	741		—	—	—	—	(A) Ascites; tumour of pylorus; tumour right upper lobe of lung. (B) Lymphosarcoma.
1316/5	25	K.779	693	(1)	—	+	—	—	(A) Poor condition; tumour right azygos and left lobes of lung. (B) Other site papillary adenoma.
1354/3	27	789	754		—	—	—	—	(A) Massive pneumonia.
1354/4	35	K.791	756		—	—	—	—	(A) and (B) Good condition. N.A.F.
1354/5	45	K.791	756	(2)	+	—	+	+	(A) Huge purple liver; tumour right azygos lobe of lung. (B) Extramedullary haemopoesis; papillary adenoma and alveolar hyperplasia.
1352/5	45	K.791	756		—	—	—	—	(A) Good condition; uterine tumours. (B) Endometriosis.
1318/2	40	792	707	(10)	+	+	—	—	(A) Ovarian cyst; huge tumour right azygos and right middle lobes of lung. (B) Multifocal papillary adenoma and carcinoma.
1350/4	20	K.847	757		—	—	—	—	(A) and (B) Poor condition; pale kidneys; N.A.F. in lung.
1316/3	30	K.896	808		—	—	—	—	(A) and (B) Uterine mucocele; N.A.F. in lung.

TABLE IV.—*Actual Number and (%) of LX mice over 300 Days Old With or Without Primary Lung Neoplasia or Hyperplasia*

	No lung lesion	Primary carcinoma	Adenoma	Hyperplasia
Group 1 (Control)				
	♂ 24/35 (69%)	2/35 (6%)	11/35 (31%)	3/35 (9%)
	♀ 23/30 (77%)		5/30 (17%)	3/30 (10%)
Group 2 (Free radical)				
	♂ 16/29 (55%)	3/29 (10%)	12/29 (41%)	4/29 (14%)
	♀ 22/30 (73%)		7/30 (23%)	3/30 (10%)
Group 3 (Sulphur dioxide)				
	♂ 13/28 (46%)	2/28 (7%)	15/28 (54%)	5/28 (18%)
	♀ 16/30 (53%)	4/30 (18%)	13/30 (43%)	3/30 (10%)

TABLE V.—*Site Incidence of Tumours/Mice at Risk*

	Subpleural	Other sites
Group 1 (Control)		
♂	9/35 (26%)	7/35 (20%)
♀	5/30 (17%)	2/30 (7%)
Group 2 (Free radical)		
♂	10/29 (35%)	10/29 (35%)
♀	6/30 (20%)	3/30 (10%)
Group 3 (Sulphur dioxide)		
♂	14/28 (50%)	9/28 (32%)
♀	10/30 (33%)	10/30 (33%)

TABLE VI.—*Actual Numbers and (%) of LX Mice Over 300 Days Old with Hepatoma and/or Lymphomatosis*

	Hepatoma	Lymphomatosis
Group 1 (Control)		
♂	6/35 (17%)	5/35 (14%)
♀	2/30 (7%)	8/30 (27%)
Group 2 (Free radical)		
♂	6/29 (20%)	1/29 (3%)
♀	2/30 (7%)	9/30 (30%)
Group 3 (Sulphur dioxide)		
♂	5/28 (18%)	1/28 (4%)
♀	3/30 (10%)	4/30 (13%)

EXPLANATION OF PLATES

FIG. 2.—♂ LX 1317/2, age 96 days. Died after accidental exposure for 5 hours to SO_2, 500 p.p.m. Lungs showed patchy congestion. H. & E. ×100.

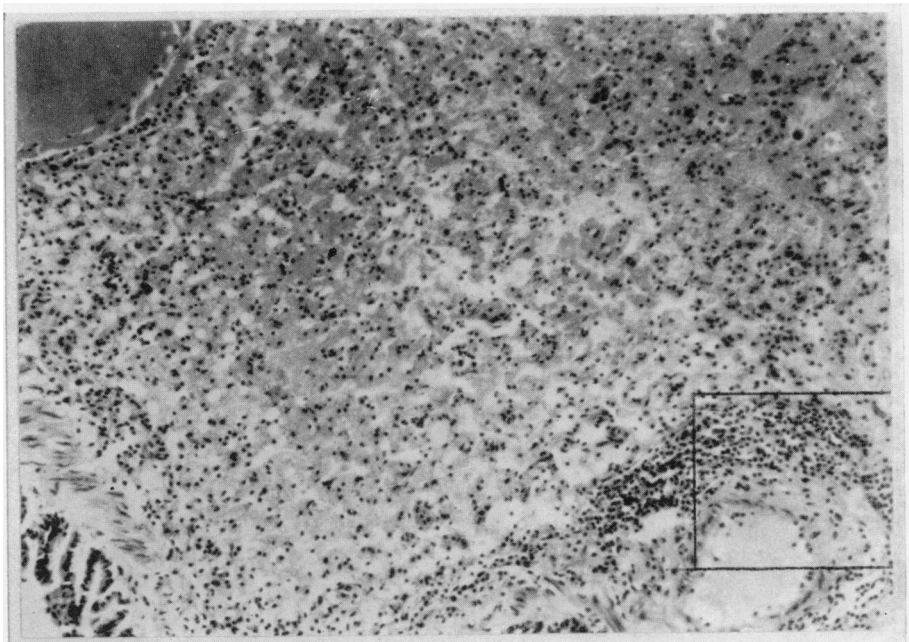
FIG. 3.—Same mouse as above marked are enlarged. A small branch of bronchial artery showing oedema and periarterial lymphangitis. H. & E. ×350. (Nuclear detail emphasized by red filter).

FIG. 4.—♀ LX 1320/5, age 593 days; SO_2, 508 days. Killed in poor condition. Depressed sternum; multifocal tumour (5 mm. diameter) in base of right lower lobe. Section shows adjacent foci of alveolar hyperplasia (R) and papillary adenoma (L), separated by compressed alveoli and lymphatics engorged with lymphocytes.
Mallory and Gomori's aldehyde fuchsin. ×85.

FIG. 5.—Enlargement of above, shows intact elastin in hyperplastic focus (R) and defective or absent delastin in neoplastic focus (L). Note lymphatics engorged with apparently normal lymphocytes and absence of other leucocytes. Contrast with Fig. 3. ×250.

FIG. 6.—♀ LX 1318/4, age 403 days: SO_2, 318 days. Killed in poor condition. Right side of thorax distended by huge tumour in right upper lobe compressing other right lobes and displacing heart and depressing and invading right dome of diaphragm. No remote metastases found. Section shows anaplastic papillary adenocarcinoma invading venule. H. E. ×250.

All above photographed on Pan F film with green filter.



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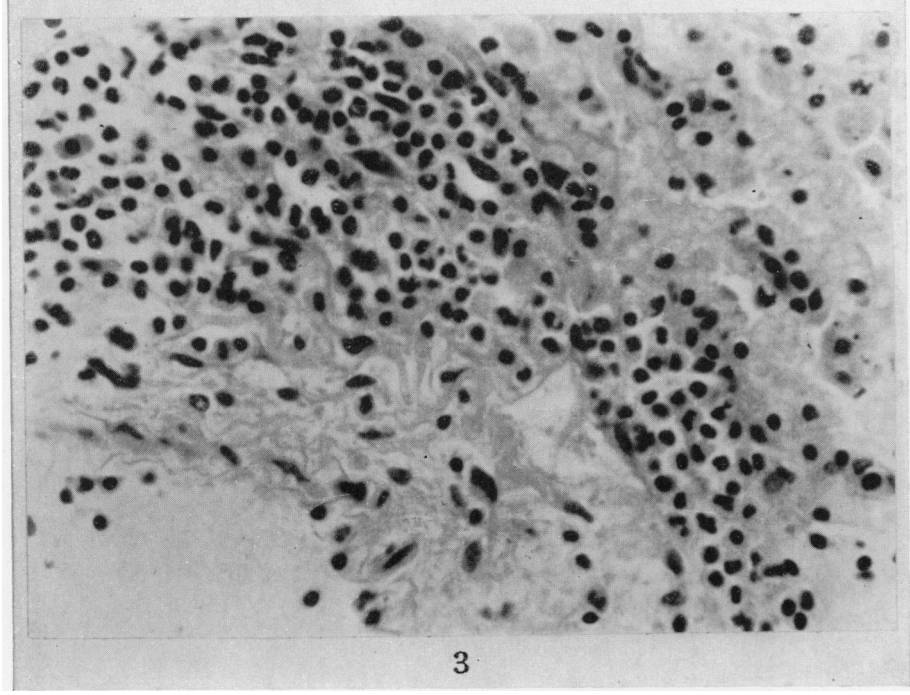

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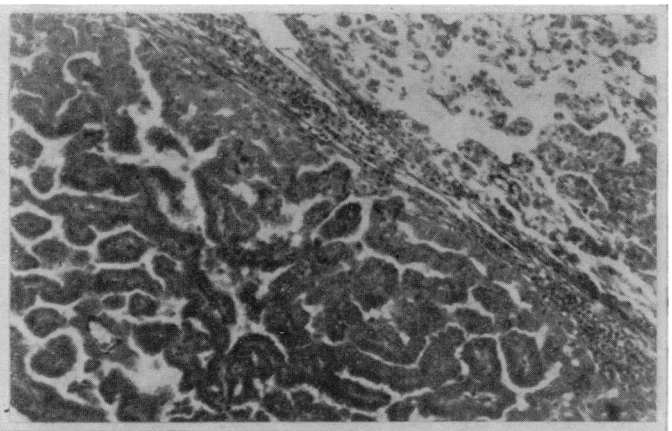

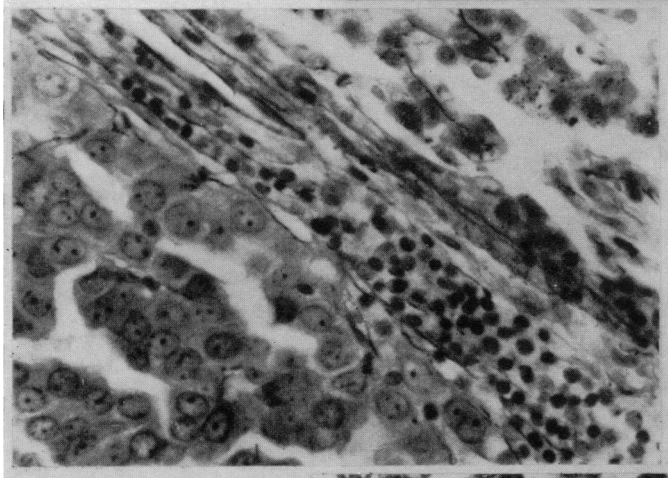

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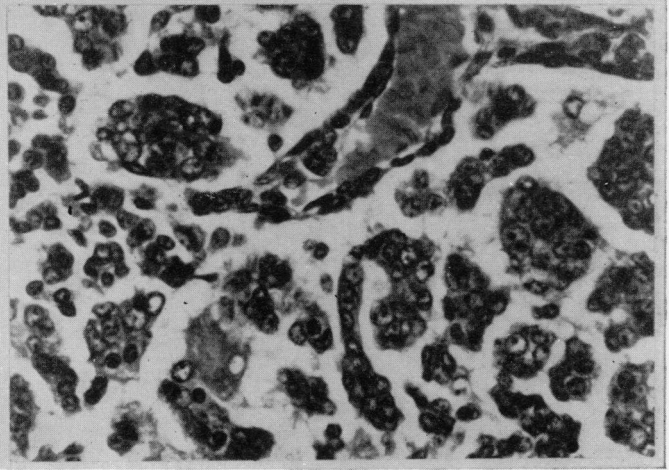

Peacock and Spence.

However, tumours occurred earlier in both experimental groups, the incidence of lung tumours was about twice as high in mice of both sexes exposed to SO_2 as in controls, and in the females there were as many malignant tumours in the SO_2 group as there were adenomata in the controls.

Malignancy was observed only in the larger tumours and presumably occurred as a progressive development in a tumour which had already reached a diameter of about 5 mm., and by implication had been growing for some time. From the presence of such large tumours in the SO_2 group, generally at an earlier age than in the controls, it is concluded that the experimental conditions accelerated the onset of neoplasia in susceptible mice. The results are consistent with a positive effect of exposure to SO_2 and to a lesser extent, of exposure to free radicals.

Thus it appears that the LX population contains a resistant moiety of about 50% which is unaffected by the experimental conditions.

In the controls about 70% of males and 80% of females live to old age without developing spontaneous lung tumours while the remainder show varying susceptibility.

How does the chemically simple gas SO_2 induce primary lung tumours in susceptible mice?

Early pilot experiments showed that with toxic exposure to SO_2 death might occur within an hour or two from acute oedema and congestion of the lung. In less severe cases which survived for several days or were killed at various intervals from hours to weeks after exposure, the most constant features were inter-alveolar congestion and oedema and intra-alveolar exudate accompanied by lymphangitis of subpleural and interstitial lymphatics. In the early acute inflammatory stages many polymorphs were present along with lymphocytes in these lymphatics (Figs. 2 and 3), but in later stages apparently normal lymphocytes persisted in the engorged lymphatics, unaccompanied by evidence of chronic inflammatory reaction and particularly in immediate juxtaposition to areas of alveolar epithelial hyperplasia and neoplasia (Fig. 4 and 5).

It has been observed previously in other experiments that such lymphatic engorgement is found fairly regularly associated with subpleural alveolar hyperplasia and neoplasia (Peacock and Peacock, 1966). This association of lymphatic engorgement with local alveolar hyperplasia and neoplasia has not yet been explained. It might represent a defence reaction which, in these cases, must have failed to prevent progressive neoplasia; or it might be an aftermath of inflammatory lymphangitis which favours, in some way, local hyperplasia of adjacent alveolar epithelium.

It is suggested that clinically subtoxic exposure to SO_2 causes such lymphatic engorgement and associated alveolar hyperplasia which, in the mouse, appear to predispose to further progression to neoplasia (Fig. 4 and 5). The action of free radicals is inconclusive.

It is concluded that the increased incidence of primary lung tumours in LX mice of both sexes in Group 3 is a consequence of the initial essentially inflammatory reaction to SO_2, followed by a state of apparent tolerance, which accelerates the inherent tendency of these mice to develop lung tumours spontaneously but does not justify the classification of SO_2 as a chemical carcinogen as generally understood.

SUMMARY

Three comparable groups of LX mice of both sexes were examined for primary lung tumours and other lesions. Group 1, untreated controls; Group 2 exposed to inhalation of free radicals; and Group 3 exposed to inhalation of SO_2.

No lung tumours were observed in mice below 300days of age and only those which survived this age are considered in assessing the results.

An increased incidence of primary lung tumours in both sexes exposed to SO_2 was approximately doubled as compared with controls; carcinoma of the lung in females was observed only in those exposed to SO_2. There was a slight increase in lung tumours in both sexes exposed to free radicals. The incidence of hepatoma and lymphomatosis, the next most frequent tumours in controls, was unaffected by the experimental conditions. There was an association between persistent lymphatic engorgement and alveolar hyperplasia and the development of progressive neoplasia, papillary adenoma and carcinoma, in the lungs of mice in all groups. Repeated exposure to SO_2 apparently accelerated the unexplained sequence of events which leads to the growth of spontaneous lung tumours in the mouse.

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