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EFFECTS OF PRECIPITATED SILICA AND OF IRON OXIDE ON THE INCIDENCE OF PRIMARY LUNG TUMOURS IN MICE

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Although since the discovery of production of skin cancer experimentally by tar full attention has been given to the importance of the time factor for this type of malignant growth similar care has not been taken when testing various substances for their carcinogenic potency in another organ—namely, the lung. In man the mean age at death from lung cancer is about 55 years—that is, in the last quarter of his average life. In our strain of mice lung tumours are very rare before the tenth month and are not numerous until the last quarter of the animal's average existence. Many experiments have been conducted to test the effects of various dusts upon the lung tissue. Usually guinea-pigs or rabbits have been employed, and the animals have been dusted for a few hundred days and then killed almost immediately after cessation of the dusting. Most of these experiments have given negative results so far as lung tumours are concerned; and it is obvious that such negative results carry little weight if the animals to begin with are not susceptible to lung tumours or if they have not been allowed to live to the age at which these tumours are most common. In my experiments I have used mice which are susceptible to lung tumours, and in practically all cases the animals have been allowed to fulfil their allotted span of life—that is, to live to the age for prevalence of lung cancer.

In industry man is exposed to a dust hazard usually for a quarter or a half of his life, so that in experiment on animals it is necessary to subject them to dust for a similar period of their lives. As mice live only about two to three years they are the most suitable animals to employ. Guinea-pigs and rabbits live so much longer that an experiment properly conducted for the time factor for cancer of the lung is much too prolonged.

In previous researches it was recorded that road dust containing tar greatly increases the incidence of primary lung tumours in mice exposed to clouds of the dust six times a day for five days of the week over a total period of a year (Campbell, 1934). It was also found that removal of the tar from the dust as completely as possible with benzene does not render the dust impotent; it still definitely increases the incidence of the primary lung tumours, but not to so great an extent as when the tar is also present (Campbell, 1937). The composition of the inorganic fraction, which formed 90 per cent. of the total weight, is as follows: SiO_2, 44; CaO, 14; Fe_2O_3, 4.5; MgO, 2.5; Al_2O_3, 12 per cent. As many of the tumours produced by this tar-free dust reveal a malignancy very rarely seen amongst the controls—that is, mice not specially dusted—it is considered that some of the above inorganic constituents such as silica or a soluble silicate might have played some part in their production. In view of the importance of the dust hazard in industry and on the streets, and also in view of the rather confused condition of the "silicosis" problem at the moment, it was decided that all the other constituents should also be tested. Some of these experiments have been concluded—namely, those with silica and with iron oxide—and their results are given below. Some results are also given for a dust containing nickel and obtained from the Mond Nickel Company. Certain of their workers have revealed an increase in lung cancer (Bridge, 1936).

A dust from Czecho-Slovakia has also been tested, and proved to be rather innocuous. As this dust did not contain uranium or arsenic in any quantity it is not regarded as a sample of the dust which is held responsible for the cancer of the lung amongst the Schneeberg and Joachimsthal miners. Professor J. Löwy has kindly given me a sample of the Joachimsthal dust, which is said to contain 55 per cent. of uranium oxide and some 2 per cent. of arsenic. This sample is now being tested. It is usually stated that the Schneeberg and Joachimsthal miners' lung cancer is due to radium, but there appears to be no definite experimental evidence for this. Professor Löwy's experiments (1936) are negative. Döhnert's experiments (1938) with mice exposed to dust in the mines are not convincing. It is unfortunate that such definite statements have been made regarding radium as the cause; these statements are not founded on experimental fact, so far as I know. Of Döhnert's mice only two out of twenty-six had true epithelial lung tumours; there were five others with some evidence of overgrowth or tumour of mediastinal tissue or lymph tissue.

Methods of Investigation

In general the technique described in a previous paper (Campbell, 1934) was followed closely. For each experiment about 150 mice, half of them males and half females, aged about 3 months, were obtained from the Medical Research Council's farm laboratories. This is a mixed strain of mice with a tumour incidence for all organs and for mice of all ages of about 10 per cent., most of the tumours being present in the lungs. Half the mice were used as controls and the other half were exposed to the dust in a respiratory chamber of about 600 litres capacity. The experimental mice were exposed to a moderate cloud of dust inside the chamber once an hour for six hours on five days of each week for a year. The dust came into contact also with the fur and was not rubbed in or dusted off except by the mice themselves. The mice were separated into groups, usually eight or ten, all of the same sex, in boxes with perforated zinc lids (area, 28 cm. by 21 cm.),

the holes in the lid occupying approximately half of the surface. The boxes were provided with a bedding of sawdust and a supply of water. The dust clouds were produced by means of a foot-bellows connected with a bottle containing the dust, and the air also blown in ventilated the chamber sufficiently for one hour, the chamber, with seventy-five mice inside it, being of capacity large enough to prevent, in this period, any undue accumulation of carbon dioxide or undue fall in oxygen percentage. A large fan stirred up the air well and was kept running except for ten minutes every hour, when the cloud of dust was present. The dusted mice were removed every evening from the boxes in which they had been exposed to the dust and were placed in clean boxes with bedding of sawdust and a supply of water and food. The latter consisted of moistened stale brown bread, milk, cod-liver oil, oats, wheat germ, hemp, canary seed, linseed, and sprouted oats.

As before, most of the mice were allowed to fulfil their allotted span of life. A few were killed, and then only if the main result of the experiment was obviously at hand. They were examined carefully for any pathological effects, particularly as regards tumours of the lungs, and histological sections were always prepared from these for microscopical examination.

Results with Silicon Dioxide

Precipitated silica was used for the experiment. It is estimated from special collections of the silica inside the chamber that about 0.5 gramme of silica entered each mouse-box per day, and this includes the heavier particles which fall out of the cloud of dust. Each box was placed in a different position daily in rotation, as there was one position, directly under the opening for entry of the dust, which received much more than 0.5 gramme per day. Each box occupied this position only once in eight days. The particles forming the cloud and apparently inhaled by the mice were examined by microscope, and many appeared to be about 5 μ or less in diameter. As a result of the dusting the main change is nearly a trebling of the incidence of the primary lung tumours—from 7.9 to 21.3 per cent. (Tables I and VII)—in the mice living 10 months or longer. I have excluded mice younger than 10 months as lung tumours are rare among them and as the dust effect requires a longer time factor. Since the mice were about 3 months old at the start of the dusting, ninety days must be added to the durations in the tables to obtain their correct age. There was no obvious fibrosis in the lung tissue, but in the tracheo-bronchial lymph nodes more than 50 per cent. of the mice revealed fibrotic nodules in which the fibrous tissue appears softer than in the fully developed typical nodule in the lung of man in silicosis. In the case of the mice the lungs seem to have got rid of most of the silica dust through cilia action by way of the trachea and also by way of the lymphatics. It is probable that with a heavier dusting the mice might retain more silica in the lung itself, which would then show more fibrosis, but in this case it is also possible that such a degree of fibrosis might inhibit the development of primary lung tumours. Thus Saupe (1939) indicates only slight silicosis in cases of pulmonary tumour among Schneeberg miners, while many other miners with marked silicosis at the same mines escaped cancer of the lung. The presence of the typical fibrotic nodule or tumour may have inhibited the change to further malignancy. It is evident from Table I that the dusting was of such a degree as not to interfere with the rate of growth and general health of the mice. It is also obvious that the increase in incidence of primary lung tumours occurs during the last quarter of the period of existence, and that a higher proportion of the tumours were malignant in the experimental group than in the control group. An interesting point is that, although primary lung tumours are very rare before the mice are 10 months of age, one of the control mice had a malignant adenoma when it died at the age of 175 days. This mouse, however, had at the same time a general condition of lymphadenoma, the lungs and other organs containing large collections of lymphocytes. One of the experimental mice with a malignant tumour of the lung showed an extension or metastasis in the tracheo-bronchial lymph nodes; none of the batch of control mice exhibited such secondary growth.

Half of the dusted mice also revealed overgrowth of the mediastinal connective tissue covering the tracheo-bronchial nodes, whereas only 10 per cent. of the control mice showed such overgrowth. In the dusted mice there was also an increase in incidence of overgrowth or hyperplasia of the tracheo-bronchial lymph nodes, 29.5 per cent. (Table VII) showing such a condition, compared with 14.3 per cent. of the controls (Control I, Table VII). There was also some increase in the number of dusted mice showing overgrowth of lymph tissue in the lung itself. Each dusting experiment is controlled by half of the available mice of the same batch and strain; thus Control I is the control for the mice dusted with silica.

Results with Iron Oxide

In my experiments the precipitated brown oxide of iron (Fe_2O_3, H_2O), British Drug Houses, was employed, and the mice were exposed to about the same quantity each day as for silica—that is, each box of eight or ten mice received usually 0.5 gramme of iron oxide. From Tables II and VII it will be seen that 32.7 per cent. of the dusted mice older than 10 months possessed tumours of the lung, compared with 9.6 per cent. of the control batch (Control IV). Further, nearly all these tumours were malignant in the dusted mice, and in two cases there were extensions or metastases in the tracheo-bronchial lymph nodes, while there were no metastases in the control group. One of the dusted mice had a fibrosarcoma of the skin of the back of the neck resembling that described by Ahlström and Andrewes (1938) for the rabbit. The cell inclusion-substance seen in the case of the mouse did not appear to

TABLE I.—*Mice Exposed to SiO_2*

Number of Days of Experiment	Number of Mice Alive		Weight (gm.) of Living Mice		Animals with Lung Tumours					
					Control			SiO_2		
	Control	SiO_2	Control	SiO_2	Total	Simple	Malignant	Total	Simple	Malignant
0	75	74	18.2	18.2	0	0	0	0	0	0
100	67	66	27.3	27.5	1	0	1	0	0	0
200	63	61	30.1	30.8	1	0	1	0	0	0
300	57	55	31.0	32.0	1	0	1	0	0	0
400	36	39	31.0	32.0	1	0	1	1	1	0
500	29	24	30.9	32.6	2	1	1	3	1	2
600	17	12	32.3	31.8	3	2	1	5	2	3
700	7	6	31.0	32.0	5	3	2	9	4	5
800	4	3	25.7	29.0	5	3	2	11	5	6
900	0	1	—	31.0	5	3	2	12	5	7
917	—	0	—	—	5	3	2	13	5	8

TABLE II.—*Mice Exposed to Iron Oxide and to $Fe_2O_3 + SiO_2$*

Number of Days of Experiment	Number of Mice Alive			Weight (gm.) of Living Mice			Animals with Lung Tumours								
							Control			Fe_2O_3			$Fe_2O_3+SiO_2$		
	Control	Fe_2O_3	$Fe_2O_3+SiO_2$	Control	Fe_2O_3	$Fe_2O_3+SiO_2$	Total	Simple	Malignant	Total	Simple	Malignant	Total	Simple	Malignant
0	73	75	74	17.6	17.0	17.3	0	0	0	0	0	0	0	0	0
100	59	62	65	22.1	24.2	25.5	0	0	0	0	0	0	0	0	0
200	52	52	62	26.1	29.7	30.2	0	0	0	0	0	0	0	0	0
300	39	48	49	31.0	30.5	30.2	0	0	0	0	0	0	0	0	0
400	36	35	42	31.6	30.8	31.3	0	0	0	2	1	1	0	0	0
500	21	31	33	30.6	30.3	31.4	0	0	0	2	1	1	1	1	0
600	13	19	18	31.1	30.1	31.5	1	1	0	4	1	3	7	4	3
700	9	10	9	30.8	29.0	33.3	4	3	1	13	2	11	10	5	5
730	7 (K)	8	8 (K)	28.7	29.0	30.3	5	3	2	14	2	12	12	5	7
800	—	8	—	—	28.8	—	—	—	—	14	2	12	—	—	—
832	—	4 (K)	—	—	29.8	—	—	—	—	17	3	14	—	—	—

K = Killed.

stain quite so deeply as that observed in the rabbit. The lungs of this mouse exhibited both a primary malignant adenoma and extensive masses of the fibrosarcoma. It is possible that the iron oxide was a factor in the production of the fibrosarcoma both in the skin and in the lungs, but of course the fibrosarcoma in the skin may have been the primary tumour and the masses in the lung may have been metastases. There may have been some injury of the skin, permitting the entrance of some of the iron oxide. Parsons (1938a, 1938b) and Warren (1939a, 1939b) have observed that mice undergoing treatment with a carcinogenic agent show signs of blood destruction, and Warren has observed a significant increase of iron in the lymph glands. Whether this is associated with carcinogenesis cannot yet be affirmed with certainty, but similar increase in iron content of the glands has been observed in mice bearing spontaneous mammary tumours.

The mice dusted with iron oxide revealed a marked increase in the incidence of hyperplasia of the tracheo-bronchial lymph nodes, 50 per cent. of them showing such a condition, compared with 13.4 per cent. of the controls (Control IV, Table VII). The dusted group also revealed a marked increase in the incidence of hypertrophy of lymph tissue in the lungs themselves, 32.7 per cent. showing such a condition, compared with 11.5 per cent. for the controls. In some cases the lymphocytes appeared to change into a malignant type of cell, spreading and infiltrating the lung tissue of the dusted mice. There was no marked difference in the condition of the lymph tissue in other parts of the body—that is, in the "general" lymph tissue. The increase of lymphocytic tissue in the lungs and tracheo-bronchial lymph glands is apparently due to the iron oxide, but apart from this hypertrophy there was no other reaction due to the dust—that is, there was no fibrotic change in the lymph nodes, but there was an increase in fibrous tissue surrounding these nodes in one-third of the dusted mice, although only 10 per cent. of the controls showed this condition.

Turner and Grace (1938) suggested that iron might be concerned with cancer of the pancreas in man. I could find no evidence for this in the mice.

Results with Silicon Dioxide plus Iron Oxide

Precipitated silica and brown oxide of iron were well mixed together in equal quantities and the mice were dusted with the mixture. In this experiment each box of mice received about half the amount of silica and of iron oxide as in the above experiments, but together the total amount of dust was the same. The tracheo-bronchial nodes did not show any definite fibrotic nodules, so that probably the amount of silica reaching them was not sufficient to produce these nodules, although in 6 per cent. of the mice there was some increase in scattered fibrous tissue in the nodes. In about a third of the dusted mice (33 per cent.) there was an increase in the fibrous tissue surrounding these lymph nodes, whereas in only about 10 per cent. of the controls was there such an increase. There was no indication of fibrosis in the lung tissue, but the incidence of primary lung tumours (Tables II and VII) in mice living longer than 10 months was 19.3 per cent., compared with 9.6 per cent. for the controls (Control IV, Table VII). Also there were 35.5 per cent. of the dusted mice with hypertrophy of the tracheo-bronchial lymph nodes, compared with 13.4 per cent. of the controls; and an increase of lymph tissue in the lung itself was observed in 30.6 per cent. of the dusted mice, compared with 11.5 per cent. of the controls. One of the dusted mice showed a tumour of the lung with some cells with inclusion bodies somewhat resembling those of the fibrosarcoma mentioned in the case of the iron oxide; this tumour gave origin to an extension or metastasis into the tracheo-bronchial lymph nodes.

Results from "Nickel" Dust

The sample of concentrated matte was obtained from the medical officer of the Mond Nickel Company. Its approximate composition as given by their chief chemist is recorded in Table III. In my first experiment with this dust it was too concentrated, with the result that the mice

TABLE III.—*"Nickel" Dust: Approximate Composition of the Concentrated Matte*

	%		%
Nickel and cobalt	40.5	Palladium	0.2
Copper	22.5	Gold	0.05
Iron	5.0	Sulphur	10.0
Lead	3.7	Silica (combined)	0.7
Arsenic	10.8	Iridium, Rhodium, Ruthenium	0.04
Silver	1.3		
Platinum	0.2		

Balance, oxygen as oxides.

died of arsenic poisoning. In my second experiment 4 c.cm. of the dust was diluted with 160 c.cm. of a sample of road dust from which the tar had been extracted by benzene. The incidence of primary lung tumours among mice living 10 months or longer was 29.8 per cent. for the mice dusted with this diluted "nickel" dust (Tables IV and VII) and 12.5 per cent. for the undusted control batch (Control III, Table VII). The diluted "nickel" dust had a somewhat adverse effect in the earlier stages of the experiment, twenty-nine dusted mice dying in the first 200 days of the dusting, compared with only eleven deaths amongst the controls. This may have been due to some undetected epidemic, but was probably connected with the arsenic in the "nickel" dust. The increase in lung tumours is significant; but the full effect of this "nickel" dust upon the lung has probably not yet been obtained, and a further experiment is contemplated with the same matte somewhat less diluted. Further, the dust used for diluting the "nickel" dust causes an increase of lung tumours in itself, so that if the "nickel" dust is very active it should increase the incidence to a greater extent

TABLE IV.—*Mice Exposed to Diluted "Nickel" Dust*

Number of Days of Experiment	Number of Mice Alive		Weight (gm.) of Living Mice		Animals with Lung Tumours					
					Control			"Nickel"		
	Control	"Nickel"	Control	"Nickel"	Total	Simple	Malignant	Total	Simple	Malignant
0	75	76	16.3	15.8	0	0	0	0	0	0
100	72	58	24.2	24.1	0	0	0	0	0	0
200	64	47	28.0	28.0	0	0	0	0	0	0
300	59	42	30.8	29.7	0	0	0	0	0	0
400	51	40	31.9	31.9	0	0	0	0	0	0
500	47	33	32.9	33.6	0	0	0	0	0	0
600	36	23	30.9	34.0	2	1	1	5	1	4
700	19	12	30.0	32.2	3	1	2	6	1	5
790	6 (K)	0	28.5	—	8	3	5	14	2	12

K = Killed.

than that obtained. The difficulty, of course, is to use a dilution which is strong enough to permit the full effect without poisoning the mice with the arsenic.

The dusted mice also revealed an increase in incidence of hypertrophy of lymph tissue both in the tracheo-bronchial nodes and in the lung tissue itself, the percentages being respectively 46.8 and 25.5 for the dusted mice and 10.9 and 7.8 for the controls (Control III, Table VII). Also in 36 per cent. of the dusted mice there was an increase in the mediastinal connective tissue surrounding the tracheo-bronchial nodes, compared with 5 per cent. for the controls. There was no evidence of fibrosis in the lung itself or in the tracheo-bronchial lymph nodes.

Results from Czecho-Slovak Dust

This sample of dust is apparently not the "active" dust responsible for the lung cancer amongst the Joachimsthal miners, although it was stated to have been collected in the vicinity of the mines. The composition of the dust as found by the Government Chemist, London, is shown in Table V. This dust contains much silica, probably as silicate, but no arsenic and only small quantities of uranium and radium. The results of the dusting experiment are given in Tables VI and VII. The mice used

TABLE V.—*Composition of a Czecho-Slovak Dust*

	%		%
Silica	56.97	Sodium oxide	1.02
*Alumina	13.72	Sulphuric anhydride	3.29
Iron oxide	6.48	Copper, silver, lead, and tin oxides	0.33
Titanium oxide	0.71	Manganese, zinc, and nickel oxides	0.10
Calcium oxide	3.70	Loss on ignition (H_2O and CO_2)	8.62
Magnesium oxide	2.43		
Potassium oxide	3.07		

* Includes any uranium oxide which does not exceed 0.04 per cent. No arsenic was detected in the sample. Radium content approximately 0.1 mg. per ton.

ticular batch of controls, but not a significant increase when compared with the 11.3 per cent. average for a large number of controls. The dust appeared to be very fine, and the lungs of the dusted mice did not show much dust present, most of it being removed by way of the trachea or by the lymphatics. In 30 per cent. of the dusted mice there was some increase in the mediastinal connective tissue, compared with 5 per cent. for the controls. There was no increase in lymph tissue in the lungs themselves, but in 19.7 per cent. of the dusted mice there was some hypertrophy of the tracheo-bronchial lymph nodes, compared with 8.3 per cent. for the controls. One of the lung tumours of the dusted mice gave rise to an extension or metastasis into these nodes, but there was no extension in the case of the tumour in the control mouse.

Discussion

It is probable from Tables I, II, and IV that the experiments would have been negative if the animals had been killed much before the 600th day after starting the dusting. In the silica experiment the final ratio for the incidence of lung tumours in the dusted mice to that for the controls is three to one—a definite increase. There has been much controversy regarding the part played by silica in the development of lung cancer in man. Many observers can find no evidence that silica plays any part (Vorwald and Karr, 1938; Koletsky, 1938; Gardiner, 1940), but others find some such influence (Allen, 1934; Pancoast and Pendergrass, 1933). Anderson and Dibble (1938) conclude that a group of cases of pulmonary carcinoma exists in which the lungs contain an excess of silica and show histological evidence of silicotic fibrosis; they hold that the role of silicosis is aetiological, as does Charr (1937). As stated above, there is evidence that

TABLE VI.—*Mice Exposed to Czecho-Slovak Dust*

Number of Days of Experiment	Number of Mice Alive		Weight (gm.) of Living Mice		Animals with Lung Tumours					
					Control			Dusted		
	Control	Dusted	Control	Dusted	Total	Simple	Malignant	Total	Simple	Malignant
0	74	74	16.8	17.2	0	0	0	0	0	0
100	68	70	24.1	24.7	0	0	0	0	0	0
200	60	61	26.2	25.7	0	0	0	0	0	0
300	43	51	24.9	28.2	0	0	0	0	0	0
400	37	37	26.8	27.0	0	0	0	0	0	0
500	19	25	28.4	27.5	0	0	0	1	0	1
600	14	14	25.8	28.0	1	0	1	3	2	1
700	4	5	27.3	29.0	1	0	1	5	2	3
800	1	1	29.0	27.0	1	0	1	6	2	4
813	1 (K)	0	27.0	—	1	0	1	7	2	5

K = Killed.

were apparently very resistant to development of lung tumours, since only one mouse, or 1.6 per cent., of the control batch of sixty mice living 10 months or longer possessed a tumour (Control II, Table VII). Among the sixty-one dusted mice there were seven, or 11.4 per cent., with lung tumours—a definite increase over this par-

the full development of the silicotic nodule is inhibitory to development of malignancy and that slight fibrosis is not. Silica and iron are present in the atmosphere in the vicinity of metal-grinders, who show a relatively high incidence of carcinoma of the lung (Kennaway and Kennaway, 1936). Turner and Grace (1938) also draw

TABLE VII.—*Synopsis of Pathological Data*

Group of Mice	No. of Mice living 10 Months or Longer	Mice with Pulmonary Tumours	Mice with Mammary Tumours	Mice with Increase of Lymph Tissue			Mice with Broncho-pneumonia	Mice with Inclusion Bodies: Liver
				Bronchial Nodes	Lungs	"General"		
Control I	63	5 (7.9%)	3	9 (14.3%)	2 (3.2%)	2 (3.2%)	10 (15.9%)	0
Control II	60	1 (1.6%)	0	5 (8.3%)	7 (11.6%)	2 (3.3%)	6 (10.0%)	0
Control III	64	8 (12.5%)	2	7 (10.9%)	5 (7.8%)	3 (4.7%)	6 (9.4%)	3
Control IV	52	5 (9.6%)	1	7 (13.4%)	6 (11.5%)	4 (7.7%)	2 (3.8%)	0
Totals	239	19 (7.9%)	6	28 (11.7%)	20 (8.3%)	11 (4.6%)	24 (10.0%)	3
SiO_2	61	13 (21.3%)	1	18 (29.5%)	6 (9.8%)	2 (3.3%)	13 (21.3%)	0
C.S. dust	61	7 (11.4%)	1	12 (19.7%)	2 (3.3%)	0 (0.0%)	6 (9.8%)	0
"Nickel"	47	14 (29.8%)	0	22 (46.8%)	12 (25.5%)	5 (10.6%)	11 (23.4%)	2
Fe_2O_3	52	17 (32.7%)	0	26 (50.0%)	17 (32.7%)	5 (9.6%)	11 (21.1%)	1
Fe_2O_3—SiO_2	62	12 (19.3%)	0	22 (35.5%)	19 (30.6%)	3 (4.8%)	18 (29.0%)	1
Totals	283	63 (21.5%)	2	100 (35.3%)	56 (19.8%)	15 (5.3%)	59 (20.8%)	4

attention to the high mortality from cancer of the respiratory tract in engineers, foundry-workers, and grinders. My own experiments with mice have given definite positive evidence, and there is a criticism against previous negative experiments, since the animals were either not sensitive to development of lung cancer or they were not allowed to live to the cancer age.

The iron oxide dust also produced a definite increase in the incidence and malignancy of the lung tumours in mice. In man some cases of lung cancer have been recorded in which iron oxide has been regarded as the causative agent (Dreyfus, 1936). It is probably a factor in the increase in the grinders and others mentioned above.

The combined experiment in which iron oxide was mixed with the silica dust gives confirmation to both the above experiments. Other similar experiments are being conducted with various mixtures of inorganic substances, and it is felt that greater carcinogenic activity may yet be detected. The experiments support the theory of irritation without inflammation (Campbell, 1939a) as a factor in the production of lung cancer. The great increase in the incidence of hypertrophy of the lymph tissue in the lungs and tracheo-bronchial lymph nodes of the dusted mice (Table VII) is further evidence of irritation, as is the increase in the incidence of hypertrophy of mediastinal connective tissue mentioned above. In most mice, both controls and dusted, there is terminal pneumonia, but there is more evidence of thickening or pneumonitis (Maxwell, 1938) in the dusted mice. Further, the incidence of bronchopneumonia among the dusted mice is on an average double that of the control (Table VII).

As regards other tumours, there were six cases of mammary tumours among 239 controls, compared with two cases among the 283 dusted mice. Also there were further cases of mice with the recently discovered inclusion body in the liver cell (Campbell, 1939b): three of the total controls and four of the dusted mice exhibited such a condition.

There has been some controversy regarding the histology of the mouse lung tumour, and at one time these were all called simple non-metastatic tumours. More recently this idea had to be abandoned. In my experiments I have now sixteen cases of primary lung tumours exhibiting extensions or metastases into the tracheo-bronchial lymph nodes or into other organs. In two cases metastases have been observed in the kidney, in one case in the spleen, and in one case in the heart. Certainly some of the mouse lung tumours are simple papillomas or adenomas resembling somewhat the condition known as jaagsiekte in sheep (De Kock, 1929; Dungal, 1938). It is possible that these simple neoplasms in sheep are also due to dust of certain kinds, although Dungal suspects the presence of a virus. Naeslund (1940) found that dusting guinea-pigs with certain dusts tends to cause adenoma-like growths in the lung. Among my own mice I have observed both simple and malignant tumours. In most cases there are mixtures of the two types, while the tumour often seems to originate as a simple tumour near the pleura and to increase in malignancy with irritation or age. There is no doubt as to the malignancy of some of the tumours, the cells resembling those of adenocarcinoma seen in the lung of man, and in some cases there is a tendency to formation of spindle cells which infiltrate rapidly. This increase in malignancy is common among the specially dusted mice but rare among the controls. There have been only two of my control mice with primary malignant lung tumours showing extensions into the tracheo-bronchial nodes, but in both of these primary tumours there was much fibrosis—that is, evidence of some previous irritation in the lung. It must be remembered that in the lungs of the control or not specially dusted mice there is often much dust which has been inhaled from the atmosphere or content of the boxes. This dust may be responsible for the lung tumours in these controls. The highest incidence for lung tumours in the batches of fifty to sixty control mice has been 20 per cent., but in the corresponding batch of specially dusted mice it was 45 per cent. In all, of 593 control mice 11.3 per cent. possessed lung tumours, compared with 31.5 per cent. in 727 experimental or dusted mice. The mechanism of irritation in the cell responsible for the increase in the latter is not known; it may, however, be concerned with cell respiration or oxidation (Campbell, 1940), at any rate so far as iron is concerned.

Summary

Dusting with precipitated silica or with brown oxide of iron trebles the incidence of primary lung tumours in mice living 10 months or longer. There is a definite increase in malignancy with the iron oxide. The amount of silica used did not cause formation of fibrotic nodules in the lung tissue of the mice, and it is suggested that the fully formed fibrotic nodule of silicosis inhibits malignancy. The tracheo-bronchial lymph nodes of the mice exhibited some degree of fibrotic nodule formation due to silica. The dusting also caused an increase in the number of mice showing hypertrophy of the lymph tissue in the lungs and in the tracheo-bronchial lymph nodes. The connective tissue surrounding these nodes was also increased, as was the incidence of bronchopneumonia. The above results with silica and iron give experimental support to the statistical evidence that there is a relatively high incidence of carcinoma of the lung in metal-grinders, engineers, and foundry-workers.

Preliminary experiments with a "nickel" dust have also been concluded.

It is pointed out that previous negative results in animal experiments with silica and other dusts are probably due

to the fact that the animals were not permitted to live to the age for production of lung tumours or the animals employed were not sensitive to the development of lung tumours.

Animal experiment has not yet established the cause of lung cancer in the Schneeberg and Joachimsthal miners.

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RAPID DEATH IN BILE PERITONITIS

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Bile peritonitis following operations on the biliary tract is fortunately not a common condition in the experience of most surgeons. It is, however, of some considerable gravity, attended as it is by a mortality of from 30% (Ricketts, 1905) to 50% (Manson and Eginton, 1938). There are, of course, many other causes of bile peritonitis, and it may be of interest to recapitulate the classification given by James (1938): (1) rupture of an infected gall-bladder; (2) traumatic rupture of the biliary passages; (3) spontaneous rupture of a common bile-duct cyst; (4) leakage from unruptured but infected biliary passages, the so-called "biliary dew" of Leriche.

Our purpose here is to present a case of diffuse bile peritonitis following operation on the biliary passages in which the outstanding feature was the rapidity of death.

Case Report

A man aged 51 was admitted to Hammersmith Hospital with a history of having had cholecystectomy performed seven years previously, following which he was perfectly well for eighteen months. He was then suddenly stricken with a stabbing type of pain in the epigastrium which was of such severity that he had to be taken to hospital and kept under observation for four days, during which time he was very nauseated but did not vomit. There was no shivering and no jaundice, but the stools were pale and he suffered from chronic constipation. Thereafter he had many similar attacks, and just prior to admission the dyspepsia which was present before his original operation returned and he was losing some weight. The attacks kept him in bed for two or three days, and between times he had to be very careful with his food, especially avoiding fats, fish, and condiments.

On examination the patient was a healthy-looking man who never smoked or drank alcohol; temperature and pulse were normal. There was a slight icteric tinge to the sclerae. The abdomen showed the old right supra-umbilical paramedian incision. There was an area of tenderness sand rigidity beneath the ninth right costal cartilage. The liver was not enlarged. The examination was otherwise negative. Radiographs did not disclose any evidence of an opaque biliary calculus, and a barium meal revealed no abnormality.

Eight days after admission an exploration of the common bile duct was carried out by G. G. T. The abdomen was opened through the original incision, and by following the scarred area over the gall-bladder fossa the duct was identified without much difficulty. It was somewhat dilated, but not to any great extent, and would not, for instance, admit the tip of the little finger. There was no external evidence of inflammation, nor could calculi be palpated from outside. On incision the escaping bile did not look abnormal and was without odour; unfortunately it was not sent for bacteriological examination. Five small pigment calculi were removed from its lower end by means of the Desjardins forceps, but *in situ* these were stuck together, forming a single conglomerate concretion. No calculi were found in the hepatic ducts. The patency of the lower end of the common duct was established by passing a 9/12 Lister bougie through into the duodenum. With the intention of carrying out cholangiography during convalescence a No. 10 soft rubber catheter, with an additional hole cut in its side, was inserted into the common duct directed towards the duodenum, the incision in the duct being closed round about it by suture. The abdomen was closed in layers without further drainage. From the time the patient recovered from the anaesthetic he had more pain than usual, and twenty-four hours after operation he complained very much of colicky spasms on the right side of the abdomen. There was no vomiting and no distension, and the abdomen was soft on palpation. The pain increased in severity to the point where the patient cried out, and there was no relief from the passage of flatus. Under the influence of morphine he remained comfortable for twelve hours, and then awakened complaining of more severe pain in the upper right quadrant of the abdomen, which was extremely tender but soft. The tongue was moist and there was no vomiting. One ounce of mucus was aspirated from the stomach. The rectum was empty. The wound was dry and there was only a trace of bile from the tube. Nothing abnormal was found in the chest. The pulse rate was 140 and of poor volume, and the respiratory rate 40; the temperature was normal.

The patient rapidly passed into a state of profound shock. Two hours later some rigidity, together with extreme tenderness and dullness, developed in the flanks and intraperitoneal bile leakage was suspected. As a diagnostic procedure an exploratory needle was inserted in the left flank and 75 c.cm. of clear viscid bile removed. The shock was treated by appropriate measures, including blood transfusion. For three hours there was a good response, but while preparations were being completed for an operation to provide drainage the patient died.

It may be useful to recapitulate that the operation was concluded about midday on a Thursday. Except for the unexplained pain the condition was not considered unsatisfactory at midday on Friday. After that time deterioration was rapid, and by midnight the condition was one of profound shock. In spite of an apparent improvement death occurred about eight o'clock on Saturday morning. It was only possible to get permission to open up the abdominal incision, and that not until forty-eight hours after death. There was a low form of plastic peritonitis near the operation area. The pelvis and the left flank contained about a pint each of fluid which was mostly bile; it was slightly brownish rather than yellow, but without offensive odour. The liver was a dirty brown colour, crepitant and friable, and the cut surface looked like a sponge; the appearance suggested infection with a gas-forming organism, and this was confirmed on histological examination. There was no obvious gas in the peritoneum and no sign of gross infection in the wound.