smaller than other particles, also considered to be virus, seen in the cytoplasm and at the surface of cells.

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Pulmonary Tumors in Strain A Mice Exposed to Mustard Gas. (20146)

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Sulfur mustard and nitrogen mustards have been shown to be carcinogenic when injected into mice and rats. In tests in this laboratory(1,2) both the nitrogen mustard, methyl bis (2-chloroethyl) amine hydrochloride and sulfur mustard, bis (2-chloroethyl) sulfide, when injected intravenously into strain A mice increased the incidence and average number of pulmonary tumors to an extent comparable with that Shimkin and McClelland(3) had observed from approximately the same amount of methylcholanthrene. Boyland and Horning (4) injected stock mice subcutaneously with the nitrogen mustards, methyl bis and methyl tris, and of 14 that survived 250 days, 10 had tumors of a variety of types, one of which was a spindle-cell sarcoma at the site of injection. In this laboratory(5) tumors including sarcomas that were probably fibrosarcomas, sarcomas that were neurogenic in origin, a rhabdomyosarcoma, papillomas, and a squamous-cell carcinoma were induced in mice of strains A, C3H, and $C3H_f$ at the site of subcutaneous injection of sulfur mustard and of the nitrogen mustard, methyl bis. Griffin *et al.*(6) have reported a variety of tumors in both mice and rats injected with methyl bis and with methyl tris.

From these results, particularly those in respect to the induction of pulmonary tumors, it seemed desirable to ascertain whether or not the fumes from any of these mustards would be carcinogenic. An experiment was, therefore, set up to test the fumes of sulfur mustard, bis (2-chloroethyl) sulfide.†

Methods. Mice of strain A were used. This is a highly inbred strain that because of its high genetic susceptibility to pulmonary tumors has been used extensively for testing the carcinogenic action of chemicals particularly when injected intravenously. The incidence of spontaneous pulmonary tumors in the strain is approximately 90% in animals 18 months of age and 50% in animals 12 months of age(7). One hundred sixty individually identified strain A mice (80 males and 80 females) from 2 to 3 months of age were divided equally into control and experimental groups with littermates distributed among the 2 groups. Mice of the experimental group were placed in small individual cages of $\frac{5}{8}''$ mesh wire and in lots of 8 were placed in an 8 liter desiccator. One hundredth cc sulfur mustard absorbed on a strip of filter paper to permit maximum evaporation was suspended in the desiccator which was immediately sealed. A small electric fan in the bottom of the desiccator circu-

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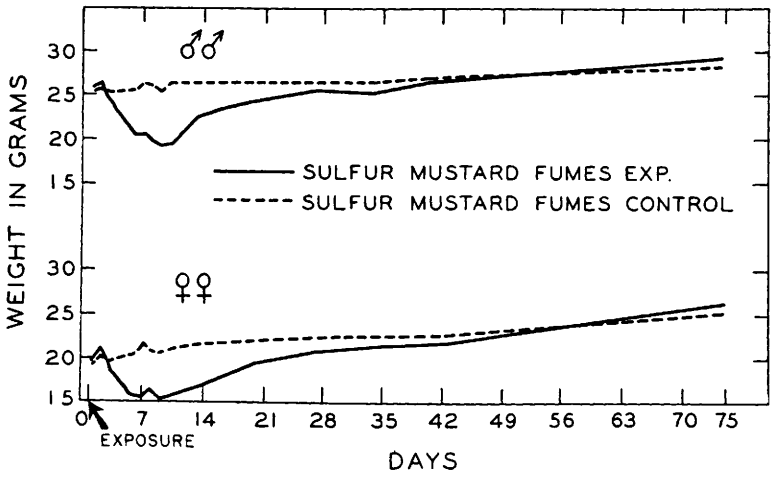

FIG. 1. Weight curves of exposed and control mice.

lated the air through the individual cages of mice during the exposure. Each lot was thus exposed for 15 minutes. Between lots the desiccator was thoroughly aired out and a new supply of sulfur mustard was suspended on a fresh filter paper. The control mice were likewise placed in individual wire cages and in lots of 8 were placed for 15 minutes in a clean sealed 8-liter desiccator. The experimental and control mice were then placed under observation in plastic cages with 8 mice to the cage and given an unlimited supply of Derwood food pellets and tap water. Sixteen males and 16 females of the experimental group and an equal number of the controls were weighed at the time of exposure, each day afterwards for 2 weeks, and subsequently at weekly intervals.

Results. The immediate response to the exposure to the mustard gas was a burning of the skin, particularly in the bare areas of the ears and nose and around the eyes. Following the first day there was also a marked reduction of weight of the exposed animals for approximately 10 days (Fig. 1). The animals then began to gain weight reaching that of the controls at approximately 8 weeks after the exposure. Thirteen mice of the experimental group died from the immediate effects of the exposure or were found dead at a later time and were too autolyzed for satisfactory examination. Three of the controls died during the course of observation and were eaten by their cage mates or were also too autolyzed for satisfactory examination.

Four months after the exposure sample lots of 30 experimental and 32 control mice were killed and examined for tumors. Nine of the experimental and 6 of the control animals had pulmonary tumors, but since the tumors of the exposed mice as well as those of the controls were small, it was decided to wait for a longer period of time before examining the rest of the animals. During the 5- to 10-month period animals were examined when they were found dead or were killed because they were moribund. At 11 months after the exposure all remaining animals of both groups were killed and examined. All tumors were fixed in Fekete's modification of Tellyesniczky's fluid,‡ sectioned and stained with hematoxylin and eosin for histologic examination. The occurrence of pulmonary tumors in the experimental and control groups is recorded in Table I. Since the 2 groups were comparable with respect to average age, incidences determined for the total of each group can be compared. Results of both sexes are combined since there was no sex difference. Of the 67 mice exposed to mustard gas 33 or 49% had pulmonary tumors as compared with 21 or 27% of the 77 control mice. This difference is highly significant: $X^2 = 7.386$;

‡ 70% ethyl alcohol, 20 parts; formalin, 2 parts; glacial acetic acid, 1 part.

TABLE I. Occurrence of Pulmonary Tumors in Strain A Mice Exposed to Mustard Gas. No. with tumors/No. examined.

	Months after exposure								% of total with tumors	Avg age, mo
	4	5	6	7	8	9	10	11		
Experimental	9/30	1/1	1/2	0/0	0/1	0/0	2/4	20/29	49	7.5
Control	6/32	0/2	0/0	0/2	0/2	2/5	3/9	10/25	27	7.5

$P < .01$. Of the 33 of the experimental group with tumors, 24 had single nodules, 7 had 2 nodules each, and 2 had 3 nodules each. All of the 21 controls with tumors had single nodules except for 3, each of which had 2 nodules. The overall average of .66 nodule per mouse in the exposed group is significantly greater than the overall average of .31 nodule in the controls; $t = 3.06$; $P < .01$.

Other neoplasms observed in the exposed animals included 3 lymphocytic leukemias. Two of these occurred in males 4 months after the exposure and one in a male 10 months after the exposure. No leukemias were observed in the controls, but since leukemia occasionally occurs in strain A mice, one cannot be certain that these 3 were induced. One experimental female had a hemangioendothelioma of the external ear at 11 months after the exposure. The only tumor other than pulmonary tumors found in the control mice was a hepatoma occurring in a female 11 months after the beginning of the experiment.

One male and 4 females of the experimental group had primary amyloidosis and the resulting nephritis that have been shown to be inherited in Strain A and probably due to a recessive gene(8,9), but usually occurring at a later age. The male also showed extensive granulocytopoiesis in the liver. Six control males and 3 control females had amyloidosis and nephritis but some of these also had dermatitis and the amyloidosis may, therefore, have been secondary.

Discussion. It is impossible to make an accurate comparison between the carcinogenic potency of mustard gas as indicated in this experiment with that of other carcinogens as shown in other tests. In Hartwell's(10) survey of compounds which have been tested, it is noted that few of the well-known carcinogens have been tested by inhalation. Campbell(11) reported somewhat higher incidences of pulmonary tumors in mice that had inhaled chimney soot, exhaust soot, cigarette smoke or road dust than was observed in control mice. There was no indication, however, that genetically homogeneous mice were used, and, thus, it is impossible to estimate the significance of the differences observed. Seelig and Benignus(12) observed an 8% incidence of pulmonary tumors in mice of the old Buffalo strain that had been subjected to an atmosphere of soot dust as contrasted with an incidence of 2% in the control mice. Later (13) they obtained no pulmonary tumors in genetically very resistant mice of strain C57BL that inhaled a 10% mixture of carcinogenic tar in lampblack. Rajewsky *et al.* (14) reported 11 pulmonary tumors in 33 white mice that had inhaled radon, but here also failure to use an inbred strain with a known incidence of pulmonary tumors precludes an estimate of the significance of their results or a comparison between them and those reported herein.

Pulmonary tumor response to many carcinogens, including sulfur mustard, when injected in strain A mice have generally been much greater than that reported here. This, however, is probably due to difference in dosage. While in this experiment it was not possible to estimate the dosage accurately, in view of the fact that only .01 cc of the sulfur mustard was placed in the 8-liter desiccator and that not all of it evaporated, and since the mice were in the desiccator for only 15 minutes, it must be assumed that the amount of sulfur mustard that came into contact with the lungs of each animal was extremely small.

The pulmonary tumor response to mustard gas in this experiment approximates that reported by Lorenz *et al.*(15) in strain A mice that received 2500 r gamma irradiation given at the rate of 8.8 r per 8 hours per day.

Summary. Strain A mice exposed for 15 minutes to vapors from .01 cc sulfur mustard in an 8-liter desiccator had a significantly

higher incidence of pulmonary tumors with a significantly higher average number of nodules than did their littermates kept as controls.

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Age Changes in the Fine Structure of Anterior Pituitary of the Mouse.* (20147)

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Morphologic changes which have been described in the cells of aging animals include loss of regularity of nuclear and cellular outline(1-3): increased occurrence of cells with giant nuclei(4) and multiple nuclei(5,6); increased amounts of cytoplasmic pigments (1,7,8), and vacuolization of the cytoplasm (3). Recently Payne(9-11) has made extensive observations on cellular changes in the anterior pituitary, adrenal, and thyroid glands of the aging cock. His most striking findings involved swelling and vacuolization of the mitochondria, in some cases so extensive that they destroyed the cell. Jayne(8) has corroborated this work in the adrenal and pituitary of the rat.

To amplify the studies of Payne and others on the morphological changes in cell structure with age, a study of the anterior pituitary gland of the Swiss Albino mouse has been made, using the greater resolving power now available with the electron microscope. In addition to swelling and disruption of the mitochondria, irregularity of the nuclear outline, a disruption of the fine structure of the endoplasmic reticulum, and loss of the highly organized appearance of the young cell have been observed.

Materials and methods. Five Swiss albino mice 1-2 months of age were compared to 6 old individuals ranging in age from 12 to 14 months. The animals were killed by beheading and bleeding, and the pituitaries were dissected out within 5 minutes of the time of death. The procedures used for fixation and embedding closely followed those which had been outlined by Palade(12). The gland was fixed for 4 hours in 1% osmic acid in Ringer's solution buffered with acetate veronal to a pH of 7.5. The material was then washed 3 times in distilled water and dehydrated by successive passage through 50%, 70%, 95%, and absolute ethanol. The tissue was transferred from absolute ethanol to a half and half mixture of absolute ethanol and methacrylate, where it remained for an hour. The methacrylate solution was made up of three parts butyl methacrylate to one part methyl methacrylate. After three washings in 100% methacrylate solution, the tissue was trans-

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