

Carcinogenic Effect of Egg Yolk in Mice of the C57 Bl Strain.* (31305)

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It was demonstrated previously that mice of the T.M. strain maintained on the Rockland rat diet supplemented with hens' eggs, egg yolk, or the lipids of the same develop a high incidence of neoplasms, such as lung tumors, lymphosarcomas and mammary cancer. On the control diet (Rockland rat diet only) the incidence of neoplasms is relatively low and the breeding females of this strain rarely develop mammary cancer(1). The question was consequently raised whether mice of other strains would be susceptible to the carcinogenic effect of eggs and if so whether they would develop the same kind of neoplasms as the mice of the T.M. strain. This report concerns the effect of egg yolk upon mice of the C57 Bl strain, which are known to have a low incidence of tumors(2).

Methods. Several batches of mice of the C57 Bl strain,[†] some at the age of 3 months, others of 4 weeks, were placed on the Rockland rat diet supplemented with raw egg yolk. In one set of experiments one egg yolk was given daily per group of 10 to 12 mice, while in other experiments the amount was reduced to one egg yolk daily per 20 to 30 mice. These first trials were not successful; all the mice died after one to 3 months without developing any tumors. The cause of their precocious death remained unknown, but it was thought that these mice do not tolerate egg yolk in the amount given and it was decided to try smaller quantities.

Twelve mice (6 males and 6 females) of the C57 Bl strain, 4 weeks old, were then placed on the Rockland rat diet supplemented with small quantities of raw egg yolk. The mice were bred and their offspring maintained on the same diet. The experimental group consisted, consequently, of mice of 4 genera-

TABLE I. Average Weight of C57 Bl Mice at Various Ages in Grams.

Age in days	Controls	On diet supplemented with yolk
28	11.8	12.2
125	27.8	27.7
240	30.5	30.7
365- 400	34.2	34.5
500- 600	36.0	35.9
700- 800	32.9	31.8
1000-1100	29.0	28.7

tions. The mice of each generation were bred only twice; males and females were then caged separately. Mice of several generations maintained on the Rockland rat diet only were used as controls.

Rockland rat diet and tap water were available at all times to both control and experimental mice. The latter received in addition raw egg yolk which was first given in the amount of one egg yolk daily per 50 mice and which was increased to one egg yolk daily per 30 mice when they were 6 months old. The weight of the experimental and control animals was recorded at regular intervals.

All the mice were autopsied after death and the affected organs fixed with Bouin's fluid, sectioned and stained with hematoxylin and eosin. For the pituitaries, which were also fixed with Bouin's fluid, a modification of Glenner and Lillie's technique was used (3,4). All the pituitaries were fixed immediately after the death of the animal. Glands of mice that died during the night were not used.

Results. The results are summarized in the following 4 Tables. As seen in Table I, the weight of the mice on the diet supplemented with egg yolk did not differ appreciably from that of the controls. The mortality rate of the first 3 generations of the experimental mice was relatively very low up to the age of 600 days (Table II) while that of the 4th generation was high from the age of 6 months. None of the experimental and

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TABLE II. Rate of Mortality of the C57 Bl Mice According to Age in Days.

Days	180- 200	201- 300	301- 400	401- 500	501- 600	601- 700	701- 800	801- 900	901- 1000	1001- 1199	1101- 1299	Total
Mice on diet supplemented with yolk												
1st generation	2	—	—	—	—	—	1	2	2	4	1	12
2nd "	—	1	1	4	2	8	15	16	10	10	—	67
3rd "	1	—	—	2	1	13	14	18	17	5	1	72
4th "	7	6	4	8	10	20	21	11	3	10	9	109
Total	10	7	5	14	13	41	51	47	32	29	11	260
Controls												
	2	1	2	15	14	40	49	46	28	23	8	228

control mice died before the age of 6 months. There was no appreciable difference in the average life span between experimental and control mice, while the incidence of neoplasms was almost 8 times higher in the former than in the latter. As in the mice of the T.M. strain(1), lung tumors (Fig. 1 to 4) and lymphosarcomas were the most frequent neoplasms in the mice of the C57 Bl strain on the diet supplemented with egg yolk. The age at which these neoplasms appeared varied from less than one year to about 3 years. Liver hepatoma, (parenchymal), which occurred rarely in the mice of the T.M. strain, was found in 6.5% of the experimental C57 Bl mice (Fig. 5, 6) at an average age of 769 days. The youngest mouse that died of liver hepatoma was 723 days and the oldest was 1019 days. Thirteen of these mice were males and 4 were females.

Mammary adenocarcinoma, which occurred in about 33% of the breeding females of the T.M. strain on a diet supplemented with egg yolk(1), was found in only 9.6% of the experimental C57 Bl breeding females, at an average age of 732 days. The youngest mouse with mammary cancer was 610 days old and the oldest 796 days. In many of these mice metastases from the mammary cancer were found in the lungs. As in the case of the mice of the T.M. strain, the C57 Bl females placed on the diet supplemented with egg yolk at the age of 4 weeks did not develop mammary cancer. The latter appeared in the females of the following generations which were on the diet from birth. Moreover, removal of the mice from the diet supplemented with egg yolk at the age of 4 weeks did not prevent the development of mammary can-

cer. In these mice, placed on the control diet at the age of 4 weeks, the incidence of mammary cancer was as high as in the females remaining throughout life on the diet supplemented with egg yolk.

Most of the lung tumors in the experimental mice were deeply situated and were continuous with the epithelial lining of the bronchi (Fig. 1 to 4). In a few of them tumorous masses projected inside the bronchi. Structurally they consisted of acini-like formations, consequently it was thought that the term adenocarcinoma is more adequate than carcinoma. These tumors were invasive and in many cases were adherent to the thoracic wall. Alveologenic lung tumors, of the type described by Steward(5) were also found in these mice, (only in 9 mice). The youngest mouse that died of lung tumor was 433 days old.

The youngest mouse on the experimental diet that died of lymphosarcoma was 6 months old. In the young mice with this neoplasm, the thymus was usually the most affected organ, while in the older ones all the viscera were heavily infiltrated with lymphocytes and the spleen was large and nodular.

It was previously demonstrated that in mice of the T.M. strain maintained on a diet supplemented with egg yolk or with the lipids of the same there is a decrease in the relative number of acidophile cells and an increase in the basophile cells in the adenopituitary, and occasionally development of tumors(6). Similar changes were found in the pituitaries of the experimental C57 Bl strain mice, except that the incidence of tumors in these mice was considerably higher than in the T.M. strain mice. No tumors of the pituitary were

found in over 100 controls examined, while 15 out of 126 mice on the diet supplemented with egg yolk developed tumors (an incidence of almost 12%). The nature of these tumors, as shown in Table IV, varies. Two were small acidophile tumors, 2 small chromophobe adenomas and 6 were larger tumors originating

from the intermediate lobe and extending into the pars distalis or the pars nervosa. The nature of the remaining 5 is hard to determine. They are very large and completely displace the anterior lobe of the pituitary, but by their relation with the latter and their structure they look like tumors of the intermediate lobe. The chromophobe and acidophile adenomas consist of round or oval cells with the nuclei more or less in the center. In the intermediate lobe tumors the cells are considerably larger than in the latter. They are tall columnar cells, with the nuclei at the base, organized into follicle-like structures.

It should be mentioned that in the controls the intermediate lobe of the pituitary consists of columnar, agranular cells often forming follicles. The nuclei in these cells are close to the base. In the mice on the diet supplemented with egg yolk, even in those that did not develop pituitary tumors, the intermediate lobe is considerably enlarged. Its cells, which are tall columnar, have the nuclei at the base and contain basophile granules in the cytoplasm. The organization of these cells into follicles becomes more generalized. The age of the mice with pituitary tumors (Table IV) varied from 449 to 994 days. Eleven of these mice were females and 4 males. There does not seem to be any relationship between the change in the pituitary and the other type of neoplasms developed by these mice. Some of these mice died of lung tumors, others of lymphosarcoma and one of mammary cancer.

Discussion. From the above results it appears that the mice of the C57 Bl strain are as susceptible to the carcinogenic effect of egg yolk as those of the T.M. strain. The slight difference in the results may be due in part to a difference in the genetic constitution of the mice of these 2 strains and in part to the change in the diet. The C57 Bl mice were fed lower quantities of yolk than the T.M. strain mice which probably explains the slightly lower incidence of neoplasms and their later appearance in the former than in the latter. In the C57 Bl mice on the yolk diet the incidence of neoplasms was slightly over 78% and the average age of the animals at the time of death was 706 days, while in the mice of the T.M. strain on the same diet

TABLE III. Neoplasms in Mice of the C57 Bl Strain Maintained on a Diet Supplemented with Raw Egg Yolk.

Diet	No. of mice ♂ ♀	Age in days	No. of mice with tumors	Mice with more than 1 tumor	Lung tumors	Lympho- sarcoma	Liver hepatoma	Mammary cancer	Other tumors
Control	228 105 123	711.9	23 (10.0%)	0	8 (3.5%)	14 (6.1%)	0	1 (0.3%)*	0
Egg yolk	260 146 114	706.5	204 (78.4%)	38 (14.6%)	102 (39.6%)	95 (36.5%)	17 (6.5%)	11 (9.6%)*	17 (6.5%)+

* Of females only.

+ Four squamous cell carcinomas of the skin, 1 skin carcinoma of penis, 4 uterine tumors, 2 adrenal tumors, 3 ovarian tumors and 3 fibrosarcomas.

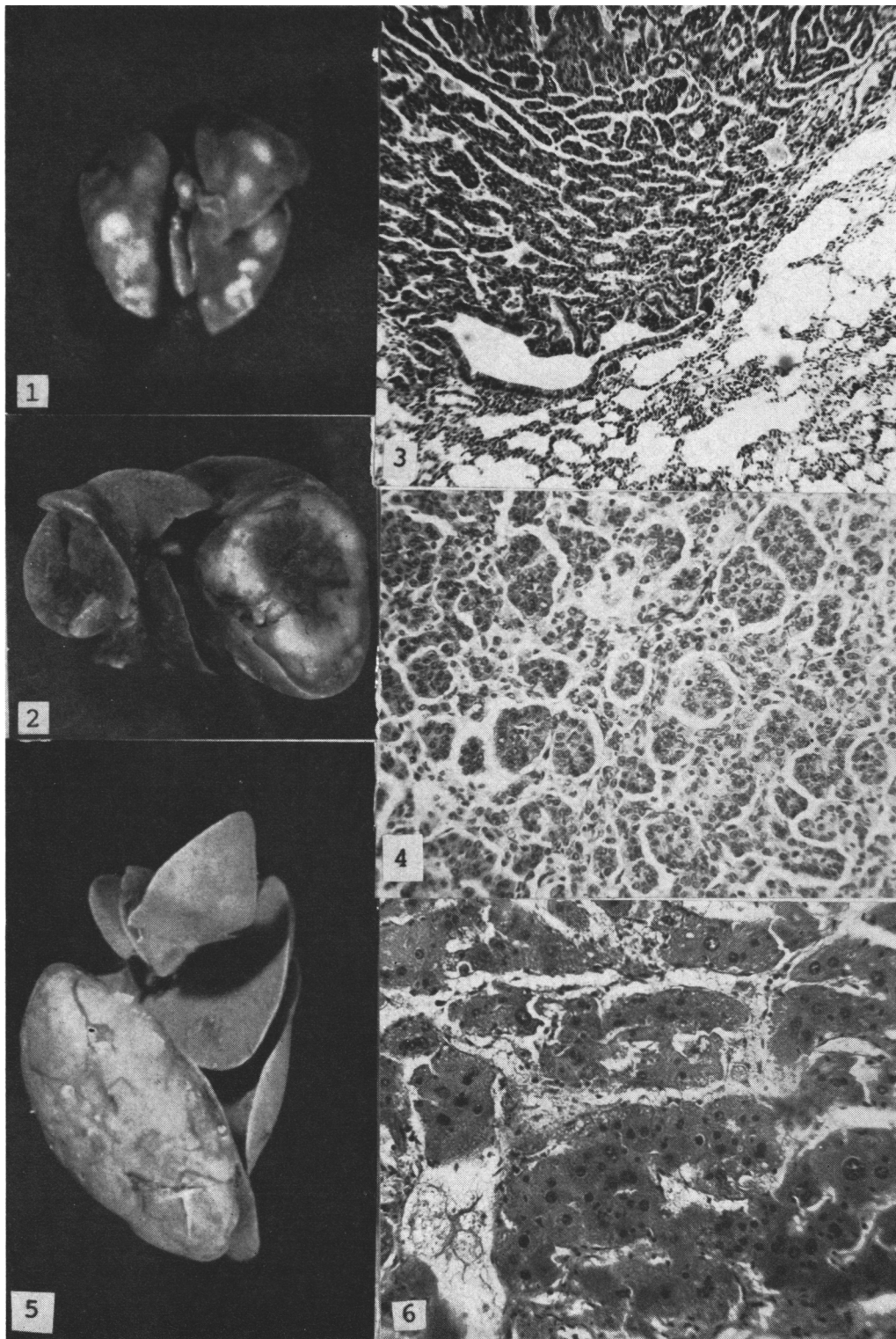


FIG. 1. Multiple lung tumors of a C57 Bl mouse 696 days old, maintained on the diet supplemented with yolk from day of birth.

FIG. 2. Extensive lung tumor of a C57 Bl mouse, 703 days old, maintained on the diet supplemented with egg yolk from day of birth.

FIG. 3. Section of lung tumor of Fig. 1, showing its bronchial origin. Hematoxylin and eosin; $\times 125$.

FIG. 4. Section of lung tumor of Fig. 2, showing its acini-like structure. Hematoxylin and eosin; $\times 250$.

FIG. 5. Liver tumor of a C57 Bl mouse, 763 days old, maintained on the diet supplemented with yolk from day of birth.

FIG. 6. Section of the above liver showing that it is a parenchymal hepatoma. Hematoxylin and eosin: $\times 250$.

the incidence was 87% and their average life span only 354 days(1).

Lymphosarcoma and lung tumors appear to be the most frequent neoplasms in the C57 Bl mice as well as in the T.M. mice. No difference could be found in the extension of these two types of neoplasms between the mice of the two strains. In both of them the lymphosarcomas were usually generalized, affecting the thymus, the spleen and many of the other viscera; occasionally they were limited either to the thorax or to the abdomen. The lung tumors involved most frequently the greatest part of the respiratory organ.

Liver hepatoma occurred rarely in the mice of the T.M. strain, while in those of the C57 Bl strain 6.5% of the mice on the diet supplemented with egg yolk developed such tumors, probably due to the fact that the latter lived twice as long as the former. The

youngest mouse of the C57 Bl strain that died of liver hepatoma was 723 days old, while of the T.M. strain very few on the diet supplemented with yolk lived to the age of 2 years. On the diet supplemented with ether extract of whole egg, upon which the mice of the T.M. strain lived a little longer, one of them developed liver hepatoma(7).

Contrary to the above, the incidence of mammary cancer was higher in the T.M. breeding females on the diet supplemented with yolk (33%) than in those of the C57 Bl mice (9.6%). In this case a difference in the genetic constitution of the mice of these two strains is probably the cause, since it is known that the mice of the C57 Bl strain are very resistant to mammary carcinogenesis and even after the introduction of the milk factor (by foster nursing) their incidence of breast cancer is only 10%(8).

TABLE IV. Pituitary Tumors in Mice of the C57 Bl Strain Maintained on the Rockland Diet Supplemented with Egg Yolk.

Sex	Age in days	Time on diet in days	Type of pituitary tumor	Other neoplasms in the same mouse
♀	578	578	Intermediate lobe tumor	Lymphosarcoma
♀	710	710	" " "	" "
♂	778	778	Basophile tumor*	Lung tumor
♀	785	785	Chromophobe of p. distalis	" "
♂	788	788	Intermediate lobe tumor	" "
♀	794	794	Chromophobe tumor*	Lymphosarcoma
♂	813	813	Intermediate lobe tumor	Lung tumor
♂	835	835	Chromophobe of p. distalis	" "
♀	913	913	Intermediate lobe tumor	" "
♀	921	921	Acidophile of p. distalis	Lymphosarcoma
♂	931	931	Intermediate lobe tumor & chromophobe of p. distalis	Lung tumor
♀	954	954	Chromophobe tumor*	" " & lymphosarcoma
♀	994	994	Acidophile of p. distalis	" " & uterine tumor (cervix)
♀	449	28†	Chromophobe tumor*	Mammary adenocarcinoma
♀	644	28†	Basophile tumor*	Lung tumor

* These tumors were large and their origin was hard to determine. Structurally they resemble the intermediate lobe tumors.

† These mice were removed from the diet supplemented with yolk and placed on the control diet at age of 4 weeks.

Finally the mice of these two strains differ in incidence of pituitary tumors; it is higher in the mice of the C57 Bl strain than in those of the T.M. strain, which could be attributed in part, to the longer life span of the former and to the difference in genetic constitution. The age of most of the mice of the C57 Bl strain which developed pituitary tumors varied between 700 to close to 1000 days. Only 3 mice with such tumors were below that age. Gardner and Strong(9) found that of 7 different strains, only the mice of the C57 Bl strain developed pituitary tumors after prolonged treatment with large doses of estrogens, indicating that these mice are genetically predisposed to the development of such tumors. According to the above investigator all the tumors caused by the estrogen treatment were chromophobe adenomas. In the present experiments, under the effect of the diet supplemented with egg yolk, most of the tumors were of the intermediate lobe. It is possible that the estrogen induced tumors (9) are different from the tumors caused by the dietary lipids (as in the present experiments) although it was found that when the tumor is very large, as in the case of those described by Gardner and Strong(9), an adenoma of the intermediate lobe can easily be mistaken for one of the anterior lobe, particularly if the latter is invaded and displaced by the growing mass. In the present experiments tumors of the pituitary were found in various stages of development, hence it was easy to see that most of them originated from the intermediate lobe.

It is not entirely new that tumors of the pituitary should originate from the intermediate lobe. In the hamster the usual reaction to prolonged estrogen treatment is the development of such tumors(10,11). Similarly, it was found that in a few cases rats treated with estrogen developed tumors of the intermediate lobe of the pituitary(12). The mechanism of action in all of these cases is hard to understand, particularly as the function of the intermediate lobe of the pituitary is unknown. One wonders whether, in addition to intermedin (which regulates melanophore activity in lower vertebrates) it plays some part in growth regulation. In the present ex-

periments the development of tumors of the intermediate lobe of the pituitary may be due to the disturbance in the regulatory mechanism of growth and the development of a high incidence of neoplasms.

Summary. Mice of the C57 Bl strain maintained on a diet supplemented with raw egg yolk, like those of the T.M. strain, developed a high incidence of neoplasms. The most frequent neoplasms were lung tumors and lymphosarcomas. Liver hepatoma, which occurred rarely in mice of the T.M. strain, was found in 6.5% of the experimental C57 Bl strain mice. The incidence of mammary cancer in these mice was only 9.6%, as compared with the 33% found among the breeding females of the T.M. mice on the diet supplemented with yolk; a difference which may probably be due to a difference in genetic constitution of these two strains of mice. Finally, many of the mice of the C57 Bl strain on the diet supplemented with egg yolk developed pituitary tumors, the majority of which appeared to originate from the intermediate lobe.

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