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Carcinogenic Effect of Egg White, Egg Yolk and Lipids in Mice.* (28254)

J. SZEPSENWOL
Department of Anatomy, University of Puerto Rico School of Medicine, San Juan, P. R.

It has been demonstrated that mice maintained on a Rockland rat diet supplemented with hard boiled hens' eggs develop a high incidence of malignancies(1,2). The mice eat preferably the egg yolk, but as they also consume part of the egg white, it is impossible to determine which of the 2 is carcinogenic. We have therefore carried out experiments with mice of 2 inbred strains, the "A" and the "T. M." This report concerns results obtained with the T.M. strain of mice. This strain, considered cancer resistant, was developed locally by Dr. Carmen Casas, and in 15 years of inbreeding the mice have rarely developed malignancies.

Methods. Two series of experiments were carried out. In both, the mice were divided into 4 groups and placed at the age of 4 weeks on the following diets. Group 1, the control, was maintained on the Rockland rat diet only, with no supplement. For the other 3 groups the Rockland rat diet was supplemented with hard boiled egg white (Group 2), with raw egg yolk (Group 3) or with cholesterol and lard (Group 4). One egg white, one egg yolk or 50 to 100 mg of cholesterol and 1 g of lard were given daily per cage of 10 to 12 mice. As some sugar was added to the cholesterol and lard in order to induce the mice to eat it, it was also added to the egg white and egg yolk. The egg white was boiled after removal, intact, of the egg yolk. For this an opening was made at the large extremity of the egg, the egg yolk carefully separated from the egg white and removed. The shell containing the egg white was then placed upright in a boiling water bath, avoiding penetration of water into the shell.

In one series of experiments males and females were caged separately, while in the other series the mice of the 4 groups were bred and their offspring maintained on the same diets. Each group in this series was made up, consequently, of mice of 3 to 4 generations. The mice of each generation were bred only twice; males and females were then caged separately. Some of the offspring of the mice of the 3rd and 4th generations of Groups 3 and 4 were changed to the control diet at the age of 4 to 6 weeks. They were also bred twice, but their offspring were not used in the present experiments.

All the mice of both series of experiments were autopsied after death and their affected organs fixed with Bouin's fluid, sectioned and stained for microscopic studies.

Results. The results are summarized in Tables I and II. The average life span of the control mice (Group 1) of the first series of experiments, in which the mice were virgins, was the same as in the second series of experiments in which the animals were bred. Yet the incidence of malignancies was higher in the latter than in the former. Lung adenocarcinomas, the most frequent malignancies of the control mice, were usually small tumors as compared with those found in the animals of Groups 2 and 3. Moreover, in the latter, on the diets supplemented with hard boiled egg white and raw egg yolk, respectively, lung malignancies appeared at a relatively earlier

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TABLE I. Malignancies in Virgin Mice on 4 Different Diets of First Series of Experiments.

Groups	No. of exp mice ♂	No. of exp mice ♀	Avg age, days	No. of mice with: Malignancies	More than one malignancy	Lympho-sarcoma	Lung adeno-carcinoma	Mammary cancer	Other types of malignancy
1 Control	10	10	675.3	1	0	0	1	0	0
2 Diet + egg white	7	13	438.7	18	11	17	10	0	3*
3 Diet + egg yolk	9	12	497.4	19	10	15	14	0	2†
4 Diet + cholesterol & lard	8	11	697.4	9	1	1	6	0	3‡

* Squamous cell carcinoma of stomach and 2 adrenal cortex adenocarcinomas. † Fibrosarcoma and squamous cell carcinoma of skin.
‡ Squamous cell carcinoma of skin and 2 fibrosarcomas.

TABLE II. Malignancies in Breeding Mice on 4 Different Diets of Second Series of Experiments.

Groups	No. of exp mice ♂	No. of exp mice ♀	Avg age, days	No. of mice with: Malignancies	More than one malignancy	Lympho-sarcoma	Lung adeno-carcinoma	Mammary cancer	Other types of malignancy
1 Control	50	47	677.3	16	1	2	10	0	5*
2 Diet + egg white	84	68	422.1	130	48	110	60	0	8†
3 Diet + egg yolk	70	69	354.5	121	50	98	57	23	5‡
4 Diet + cholesterol & lard	68	72	574.0	67	7	5	26	37‖	6§

* Granulosa cell cancer of ovary, squamous cell carcinoma of stomach and 3 fibrosarcomas. † Ascites tumor, fibrosarcoma, 2 squamous cell carcinomas of stomach and 4 adrenal cortex adenocarcinomas. ‡ Squamous cell carcinoma of skin, ovarian tumor and 3 ascites tumors. § Squamous cell carcinoma of skin, ovarian tumor, ascites tumor and 3 fibrosarcomas. ‖ Of 37 mice with mammary cancer, 35 were females and 2 males.

age than in the controls and in the mice of Group 4, on the diet supplemented with cholesterol and lard.

Mammary gland adenocarcinomas in the breeding females of Groups 3 and 4 were rapidly growing malignancies that metastasized usually into the lungs and mediastinum. It is interesting that the females of the first generation of the above 2 groups, those placed at the age of 4 weeks on the diets supplemented with egg yolk (Group 3) or with cholesterol and lard (Group 4), did not develop mammary cancer. Some of them developed lymphosarcoma while others were free of malignancies. Mammary tumors appeared in the mice of the 2nd, 3rd and 4th generations. Many of the mice developed lymphosarcoma in addition to mammary cancer (those of Group 3), with large spleen and thymus. The enlargement of these 2 lymphoid organs was also observed in many of the females of Group 4 in which there was no lymphosarcoma. The age at which mammary cancer developed varied from 153 to 594 days for the mice of Group 3 and from 332 to 850 days for those of Group 4.

The offspring of the mice of the 3rd and 4th generations of Group 3, which were removed from the diet supplemented with raw egg yolk and placed at the age of 4 to 6 weeks on the control diet, died at an average age of 469.1 days. Eight of the 9 mice of this group (7 females and 2 males) developed malignancies. Of these 6 were mammary adenocarcinomas. The 35 mice (25 females and 10 males) which were removed from the diet supplemented with cholesterol and lard and placed at the age of 6 weeks on the control diet, died at the average age of 606.4 days. Twenty-two of them developed malignancies; of these 14 were mammary cancer.

Discussion. From these results it appears that both egg yolk and egg white are carcinogenic in mice. The incidence of lymphosarcoma and lung adenocarcinoma is as high in the mice receiving egg white as in those receiving egg yolk. Moreover, there is no appreciable difference between the animals of Groups 2 and 3 in time of appearance of malignancies nor in rapidity of their growth. In the animals of both groups the lymphoid system of the abdominal cavity is the first to be affected. Occasionally, the thymus is the main focus of the malignancy. The adenocarcinomas of the lungs, whether caused by egg white or by egg yolk, are very extensive, frequently destroying a whole lung.

The carcinogenic effect of the 2 components of the egg, however, is not entirely of the same nature. The egg yolk causes in addition to lymphosarcoma and lung adenocarcinoma, the development of mammary gland adenocarcinoma. Of Group 3, on the diet supplemented with egg yolk, 23 of the 69 breeding females (33%) developed mammary cancer, while of the 68 breeding females of Group 2, on the diet supplemented with egg white, none developed mammary gland malignancies. On the other hand, cancer of the adrenal cortex and of the non-functional part of the stomach has occurred, so far, in mice of Group 2, but not in those of Group 3. The number of these malignancies was, however, too small to be significant.

The mammary gland carcinogen in the egg yolk is probably a lipid. It is present in an alcohol or ether extract of eggs (as appears from other experiments), and in the mixture of cholesterol and lard, while the egg white, poor in fatty substances, does not contain it. The mice of Group 4, on the diet supplemented with cholesterol and lard, had a higher incidence of mammary cancer than those of Group 3, but this is probably due to the fact that they had a lower incidence of the other types of malignancies and lived longer than the animals on the diet supplemented with egg yolk. Many of them developed mammary cancer at the age of over 2 years.

The possibility that the substance in egg yolk responsible for mammary carcinogenesis is estrogen is unlikely. The mice developing mammary cancer do not lose weight, except at the final stage of the disease; the spleen and thymus are not atrophic, but are usually enlarged, and the seminiferous tubules in the males, on the same diet, are well developed and very active. Enlargement of the thymus was observed even in the mice with mam-

mary cancer of Group 4 (on the diet supplemented with cholesterol and lard), which rarely developed lymphosarcomas.

In 15 years of inbreeding, mammary cancer rarely occurred in mice of the T.M. strain, and in our experiments none of the 47 breeding females of Group 1 (control) developed this malignancy. Consequently, the presence of the milk factor in the mice of this strain is unlikely. This question however, is being studied on mice of this strain, foster nursed by lactating females of the C57Bl strain.

It appears thus that there is more than one carcinogenic substance in eggs. One substance responsible for the development of lymphosarcoma and lung adenocarcinoma is present in both egg yolk and egg white, and the other substance stimulating mammary carcinogenesis would be present in the yolk only.

Summary. Mice of the T. M. strain were maintained from the age of 4 weeks on the Rockland rat diet (Group 1) supplemented with hard boiled egg white (Group 2), raw egg yolk (Group 3) or with cholesterol and lard (Group 4). In one series of experiments males and females were caged separately, while in the other one they were bred and their offspring maintained on the same diets. Each of the groups of the second series of experiments consisted of mice from 3 to 4 successive generations. The results were: The mice of Groups 2 and 3 developed a very high incidence of lymphosarcoma and lung adenocarcinoma. The mice of Group 3 developed, also, a relatively high incidence of mammary cancer (33% of the breeding females) which did not appear in the animals of Group 2 nor in the controls. Incidence of mammary cancer was particularly high in the mice of Group 4. From these results it appears that both egg white and egg yolk are carcinogenic, but that their carcinogenicity differs. A carcinogenic substance causing the development of lymphosarcomas and lung adenocarcinomas, would be present in both, while a mammary carcinogen, lipid in nature, is present in the yolk only.

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Blood Flow Rates in Small Vessels of the Hamster Cheek Pouch. (28255)

ROBERT F. SLECHTA AND GEORGE P. FULTON
Department of Biology, Boston University, Boston, Mass.

Changes in blood flow rates have been used traditionally as criteria of small blood vessel activity, but methods of estimation have been largely subjective or indirect. Recently developed microcinematographic techniques, however, now provide the basis for direct quantitative determination of blood flow velocity and volume in individual microscopic vessels(1,2).

Materials and methods. A modification of the method of Hugues(1) was used in quantitative studies of blood flow in vessels of the cheek pouch of the golden hamster. Animals of 100 to 135 g body weight were anesthetized with 0.2 ml/100 g of sodium pentobarbitol (50 mg/ml). The cheek pouch was prepared for observation and immersed in mammalian Ringer's solution according to the technique of Lutz and Fulton(3). Single vessels, 14 to 80 μ in diameter, were viewed through a monocular microscope equipped with a water immersion objective at a magnification of 500X. A zirconium arc light source was used. A beam-splitter, attached to the body tube, projected the light-masked image of a straight segment of a vessel into a Grass 35 mm recording camera. The image was focused so that the direction of blood flow was