

Carcinogenic Effect of Ether Extract of Whole Egg, Alcohol Extract of Egg Yolk and Powdered Egg Free of the Ether Extractable Part in Mice.* (29473)

J. SZEPESENWOL

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

It has been demonstrated that both the white and the yolk of hen's eggs are carcinogenic in mice, but their carcinogenicity differs(1). Egg white causes the development of a high incidence of lung adenocarcinomas and lymphosarcomas, while egg yolk causes, in addition to these, a high incidence of mammary cancer in the breeding females of the T. M. strain of mice. On the Rockland rat diet only, the control mice rarely develop mammary cancer. On the basis of these results the conclusion was drawn that there are at least 2 carcinogenic substances in hen's eggs: one affecting the lymphoid and respiratory systems, present in both the egg white and egg yolk, and another causing mammary carcinogenesis, present in the egg yolk only. These substances could not be proteins, since boiling, which denatures them, does not affect the carcinogenicity of the egg. The fact that mice maintained on the Rockland rat diet supplemented with cholesterol and lard developed a relatively high incidence of lung adenocarcinomas and mammary cancer suggests that the carcinogenic substances in eggs are lipids. The question that is raised is whether, as suggested above, there is more than one carcinogenic substance in eggs, or whether a single substance, like the carcinolipin of Hradek, in different concentrations, causes the development of the different types of malignancies. Experiments have been carried out with the purpose of solving this problem. This report concerns results obtained with ether extract of whole egg, alcohol extract of egg yolk and powdered egg from which the ether extractable lipids were removed.

Methods. Mice of the T. M. strain 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 ether extract of whole powdered egg (Group 2), with alcohol extract of raw egg yolk (Group 3), or with whole powdered egg from which the ether extractable part was removed (Group 4). Both the ether extract of whole egg and the alcohol extract of egg yolk were prepared by the distillation method with the use of the Soxhlet apparatus. They were given daily in the amount of 150 to 200 mg per mouse. Some sugar was added to each diet so that the mice would consume it. The powdered egg, from which the ether extractable part was removed (egg proteins) was given in the amount of 200 to 300 mg daily per mouse of group 4. The mice of the 4 groups were bred and their offspring maintained on the same diets. Each group consisted, consequently, of mice of 5 to 6 generations. The mice of each generation were bred twice; males and females were then caged separately. Some of the mice of the second and third generations were changed to the control diet at the age of 4 weeks.

All the mice of the 4 groups were autopsied after death and their affected organs fixed with Bouin's fluid, sectioned and stained for microscopic studies.

Results are summarized in Table I. As previously reported, the control animals lived considerably longer than the mice on diets supplemented with eggs or, as in the present experiments, with egg lipids. In the case of Group 4, on the diet supplemented with powdered egg from which the ether extractable part was removed (egg proteins), the unextracted lipids are probably the ones that caused carcinogenesis, since it is known that ether extraction is incomplete unless the egg substance is previously hydrolyzed with acid.

Lung adenocarcinomas, in 10% of the control animals, were relatively small tumors as

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TABLE I. Malignancies in Breeding Mice of T. M. Strain Maintained on Rockland Rat Diet Supplemented with Alcohol Extract of Egg Yolk, Ether Extract of Whole Egg or with Egg Proteins (After Removal of Ether Extractable Part).

Diet	Total No. mice and		Age in days	No. of mice with malignancies	Lung adenocarcinomas	Lymphosarcomas	Mammary cancer	Other malignancies
	♂	♀						
Control	138			%	%	%	%	%
	77	61	655.6	22 (15.9)	14 (10.1)	4 (2.9)	2 (3.3)*	5 (3.6) ^(a)
Ether extract	200							
	104	96	485.1	156 (78)	77 (37.5)	38 (19)	61 (63.5)*	13 (6.5) ^(a)
Alcohol extract	225							
	107	118	384.4	179 (79.5)	63 (28)	35 (12.5)	99 (83.9)*	3 (1.3) ^(a)
Egg proteins	112							
	42	75	358.2	81 (74.3)	14 (11.9)	10 (8.5)	63 (84)*	3 (2.5) ^(a)

^(a) Granulosa cell cancer of ovary, squamous cell carcinoma of stomach and 3 fibrosarcomas.

^(b) Squamous cell carcinoma of stomach, liver hepatoma, 2 fibrosarcomas, 5 skin squamous cell carcinomas, 2 adrenal adenocarcinomas, ovarian adenocarcinoma and uterine leiomyosarcoma.

^(c) Squamous cell carcinoma of stomach, uterine cervical cancer and fibrosarcoma.

^(d) Ovarian tumor, uterine tumor and fibrosarcoma.

* Of females only.

compared with those found in the mice of Groups 2, 3 and 4. Moreover, they appeared at a more advanced age than in the latter. In 2 of the mice of Group 2 metastases from lung cancer were found in the wall of the upper part of the abdominal aorta (1 case) and in the diaphragm (another case).

Mammary gland adenocarcinomas, that occurred in only two of the control females, were the most frequent malignancies in the 3 experimental groups. The incidence was higher in Groups 3 and 4 than in Group 2. The mammary tumors were rapidly growing malignancies that metastasized into the lungs and mediastinum. It is interesting that the 9 females of the first generation of Group 3 and the 3 females of Group 4, those placed at the age of 4 weeks on the diet supplemented with alcohol extract of egg yolk, or with the egg proteins, did not develop mammary cancer. Some were free of malignancies while others died of lung adenocarcinoma or lymphosarcoma. Mammary cancer appeared in the mice of the second and the following generations. In Group 2, of the 10 females of the first generation 7 developed mammary cancer. Yet the incidence of the malignancy in the following generations was considerably lower than in those of Groups 3 and 4.

Lymphosarcoma, the incidence of which was highest in Group 2, involved usually the thymus, spleen and most of the viscera

that were invaded by lymphocytes. It occurred very often in mice that developed mammary cancer (many of the experimental mice had 2 or more primary malignancies). In most of the mice with mammary adenocarcinoma, even in those that did not have lymphosarcoma, the thymus and spleen were considerably enlarged.

The offspring of the mice of the second and third generations of Groups 2 and 3, which were removed from the diet supplemented with ether extract of whole egg or with alcohol extract of egg yolk and placed at the age of 4 weeks on the control diet, developed as high an incidence of the same types of malignancies as their littermates that were maintained throughout life on the above experimental diets. This question is still being studied.

Discussion. From the results it appears that the lipids of the egg are as carcinogenic as the whole egg. Like the egg yolk they cause development of lymphosarcomas, lung adenocarcinomas and mammary cancer. In the case of Group 4, it is believed, as mentioned previously, that the carcinogenicity of the supplement is not due to the proteins, but to the unextracted lipids. The possibility that the substance in the egg lipids responsible for mammary carcinogenesis is estrogen is unlikely, since the seminiferous tubules in the males, on the same diets, are well de-

veloped and very active. Moreover, the mice of the T. M. strain do not seem to have the milk factor; on the Rockland rat diet alone they rarely develop mammary cancer. Recently, in another series of experiments, newborn mice of the T. M. strain, offspring of mice on the diet supplemented with egg yolk that developed mammary cancer, were foster nursed by lactating females of the C57 Bl strain, some of which were maintained on the Rockland rat diet only with no supplement, while others were kept on the Rockland rat diet supplemented with raw egg yolk. The mice of the 2 groups were bred and their offspring maintained on the same diets. Of the females of the first group (foster nursed by mice on the control diet) none developed mammary cancer, while of those of the second group (foster nursed by mice on the diet supplemented with yolk and maintained on the same diet) many developed mammary cancer. Moreover, new-born mice of the C57 Bl strain were foster nursed by lactating females of the T. M. strain (on the diet supplemented with egg yolk) and so far none has developed mammary cancer.

The mice of the T. M. strain are apparently affected by the carcinogenic lipids of the egg very early in life. Their removal from the diet supplemented with egg lipids at the age of 4 weeks does not prevent the development of a high incidence of malignancies. These results also indicate that the lipids do not cause carcinogenesis simply by increasing the caloric intake of the animal. The mice in this case were maintained on the control diet from the age of 4 weeks, yet developed a very high incidence of malignancies.

The total incidence of malignancies in these 3 experimental groups is slightly lower than in the mice on the Rockland rat diet supplemented with whole egg yolk (Szepsenwol(1)). Moreover, there are slight differences in incidence of malignancies between the 3 groups: highest in Group 3 (79.5%) and lowest in Group 4 (74.3%). In Group 2 the incidence of malignancies is close to that of Group 3 (78%). These differences in total incidence of malignancies, however, are too small to be of significance. What

appears significant to us is the difference between the 3 groups in incidence of the various types of malignancies, and particularly in that of mammary cancer on the one hand, and all other types of malignancies on the other. In Group 2, on the diet supplemented with ether extract of whole powdered egg, only 63.5% of the breeding females developed mammary adenocarcinomas, while in Groups 3 and 4, 84% of the females were affected by mammary cancer. But the incidence of all other malignancies, was higher in Group 2 (63%) than in Group 3 (41.8%) and Group 4 (22.9%). This difference in incidence of the various malignancies in the 3 groups could not be explained except on the basis of a difference in composition of the lipids in the diets, since it is known that some lipids are more soluble in alcohol and others more soluble in ether. Recently in another series of experiments it was found that mice of the T. M. strain maintained on the Rockland rat diet supplemented with cholesterol and lard developed a high incidence of mammary adenocarcinomas and lung cancer, while on a diet supplemented with cholesterol alone, they developed almost exclusively lung adenocarcinoma, and rarely mammary cancer. These results would indicate that the carcinogenicity of eggs is not due to a single lipid but to many different lipids, the combination of which is necessary for production of certain types of malignancies like those of the mammary glands.

Summary. Mice of the T. M. strain were maintained from the age of 4 weeks on the Rockland rat diet (Group 1) supplemented with ether extract of whole powdered egg (Group 2), alcohol extract of egg yolk (Group 3) or with whole powdered egg from which the ether extractable part was removed (Group 4). The mice of the 4 groups were bred and their offspring maintained on the same diets. Each of the groups, consequently, consisted of mice of 5 to 6 generations. The results were: The mice of all 3 experimental groups developed a high incidence of malignancies, varying from 74.3% to 79.5%, as compared with the 15.9% of Group 1, the control. The incidence of the different malig-

nancies was not the same in the mice on the diet supplemented with ether extract of whole egg as in those receiving alcohol extract of egg yolk or powdered egg from which the ether extractable part was removed. In the latter 2 groups the incidence of mammary cancer was considerably higher and that of the other types of malignancies (lung adenocarcinoma, lymphosarcomas, etc.) lower than in Group 2. This difference in incidence of the above two groups of malignancies is probably due to a difference in composition of the extracts used, since it is known that some lipids are more soluble in alcohol and

others more in ether. In the case of Group 4, it is believed that the carcinogenicity is not due to the egg proteins, but to the unextracted lipids. These and other results indicate that there is more than one carcinogenic lipid in eggs.

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Effects of 5-Fluorouracil and Adrenocortical Hormones on Tumors and Adrenals in Rats Bearing Transplanted Tumors.* (29474)

A. CANTAROW, J. J. RUPP AND J. W. GODDARD

Departments of Biochemistry and Medicine, Jefferson Medical College, Philadelphia, Pa.

We have found that 5-fluorouracil (5-FU) inhibits fetal growth, liver regeneration, testosterone-induced growth of the seminal vesicles, and somatotropin-induced growth of the epiphyseal cartilage(1). The high toxicity of 5-FU for hypophysectomized rats interfered with similar studies of its effect on ACTH-stimulation of adrenal growth. However, the results suggested that this process might not be inhibited by 5-FU. The present study was undertaken for the purpose of investigating this matter further. Observations were extended to tumor-bearing rats because of the possibility that the hypophyseal-adrenocortical mechanism may respond differently in the presence of a tumor than in the normal animal.

Materials and methods. Male rats (Barkbridge Farms, N. J.), weighing about 150 g, were fed laboratory chow and had free access to water. Tumors were implanted subcutaneously in the groin by trocar technic, the Walker 256 tumor and the Murphy-Sturm lymphosarcoma in Wistar and the Morris hepatoma 3924C in A X C rats.

(1) In the studies of effects on adrenal weight, treatment with hormones and 5-FU was begun when tumor growth was well established. Cortisone acetate was injected subcutaneously, 5 mg daily for 10 days, ACTH gel subcutaneously, 5 units twice daily for 5 days, and 5-FU intraperitoneally, 25 mg/kg/day for 5 days. In the combination treatment schedules, ACTH or/and 5-FU was/were given on the last 5 days of cortisone administration.

(2) In the studies of effects on tumors, these were allowed to grow for 10 days. The adrenocortical steroids were administered daily from day of implantation, and 5-FU was given daily for the last 5 days. The several dosage schedules are indicated in Table IV.

All animals were sacrificed by exsanguination under light ether anesthesia 24 hours after the last injection.

Results and discussion. *Effect of 5-FU on adrenals of normal rats (Table I).* Administration of 5-FU resulted in significant increase (49%) of adrenal weight in normal Wistar rats. Body weight was maintained and there was no other evidence of drug toxicity in these animals. Increased adrenal

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