bursa, are felt, in part, to be the site of antibody production. Plasma cell development does not appear to be by means of the humoral substance alone.

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Changes in the Pituitary of Mice on Diets Supplemented with Egg Yolk, with Extracts of Eggs, or with Cholesterol.* (31383)

J. SZEPSENWOL, A. ROMÁN AND J. SANTIAGO
Department of Anatomy, University of Puerto Rico School of Medicine, San Juan

It was demonstrated previously that mice of the T.M. strain maintained on the Rockland rat diet supplemented with egg yolk, with alcohol extract of egg yolk, ether extract of whole egg, or with cholesterol and lard develop, in addition to other malignant tumors, a high incidence of mammary cancer. In controls (on the Rockland rat diet only) mammary cancer occurred rarely and none of the mice on the diet supplemented with hard boiled egg white developed breast adenocarcinoma(1,2). Moreover, in mice of the same strain mainstained on the Rockland rat diet supplemented with cholesterol alone, the incidence of mammary cancer was only 8.6% and there was no lymphosarcoma, while the incidence of lung cancer was 76%. In the females on the diet supplemented with cholesterol, the ovaries, which were often enlarged, had almost no follicles and no corpora lutea, while the germinal epithelium, considerably thickened, formed folds penetrating inside the gonads(3).

Cholesterol apparently stimulates growth, as indicated by the high mitotic rate in the liver, the proliferation of the germinal epithelium of the ovary and the high incidence of lung cancer, while its effect upon ovarian function is inhibitory. The hypofunction of the ovaries, which manifests itself in changes in the estrous cycle (see *Discussion*) is probably the cause of the low incidence of mam-

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mary cancer and of lymphosarcoma in the mice on the diet supplemented with cholesterol. It is known that in mice of some strains ovariectomy, at a certain age, causes a decrease in incidence of mammary cancer(4) and of lymphosarcoma(5).

On the basis of the above data it was postulated that in egg yolk and in lard there are at least 2 lipids affecting carcinogenesis. One, a sterol, like cholesterol, acts directly upon the respiratory system causing a high incidence of lung cancer. Another lipid, the nature of which is unknown, stimulates primarily the activity of the gonads and these in turn influence the development of mammary cancer and of lymphosarcoma, the incidence of which is relatively high in mice on a diet supplemented with egg yolk, or with cholesterol and lard(2,3). The question was consequently raised whether in this indirect effect of the lipids the pituitary is also involved, and, if so, in what way. The following is a study of pituitaries of mice maintained on various diets.

Materials and methods. In the present experiments mice of the T.M. strain maintained on the Rockland rat diet supplemented with egg yolk, alcohol extract of egg yolk, ether extract of whole egg, cholesterol and lard, or with cholesterol alone (as described previously, 1,2,3), were used. They were offspring of mice on the same diets. They were, consequently, on the above diets from the day of birth. The number of mice studied was as follows: 30 controls (on the Rockland rat diet only), 30 on the diet supplemented with egg yolk, 20 on the diet supplemented with alcohol extract of egg yolk, 20 on the diet supplemented with ether extract of whole egg, 20 on the diet supplemented with cholesterol and lard, and 8 on the diet supplemented with cholesterol alone. The age of the mice at the time they were killed for examination varied from 300 to 950 days. Only 1 to 4 mice per group were 800 to 950 days old. Most were not older than 600 days. Males and females were almost equally distributed in each group.

The mice were killed by decapitation. The cranial cavity was then rapidly opened, the brain removed and the base of the skull with the pituitary fixed with Bouin's fluid. The gland, trimmed of the surrounding tissue, was embedded in paraffin and sectioned serially at 5 μ. For staining, a modification(6) of Glenner and Lillie's technique(7) was used, as follows: the deparaffinized sections were stained for one hour at 60°C in a solution made up with 20 ml of distilled water, 10 ml of a citric acid-dibasic phosphate buffer of 4.5 pH, 8 ml of a 1% solution of eosin B, and 8 ml of a 1% solution of aniline blue. The sections were then washed for 30 seconds with running tap water, differentiated for 3 seconds in each of the following alcohols, 80%, 95% and 100%, cleared in several passages of xylol and mounted in permount. With this technique the granules in the acidophile cells stained brilliant red, those in the basophile cells blue black, while in the chromophobe cells there were either no granules or grey to pink granules.

Counts of the 3 types of the adenopituitary cells were made following Chalkley's method (8). Of each pituitary a minimum of 38 microscopic fields, selected at random, of 8 cells each were counted. As shown in Table I, the number of glands per experimental group studied varied from 20 to 30, except for the mice on the diet supplemented with cholesterol alone of which only 8 pituitaries were counted. In some cases an additional number of glands were studied and the number of microscopic fields counted was increased from 38 to 76, without appreciable change in the results.

Results. Grossly, the pituitaries of the experimental mice appeared to be larger than those of the controls. They were not weighed because, when trimmed before embedding in paraffin, they were not completely isolated from the surrounding tissue. This was done in order to be able to section all the glands in the same horizontal direction. The results are summarized in Table I and II.

The relative number of acidophile cells in the adenopituitary decreased considerably in the mice of all the experimental groups, as compared with that of the controls, and this decrease is statistically significant (Table I). The greatest decrease was observed in the mice on the diet supplemented with choles-

TABLE I. Averages of Acidophile, Basophile, Chromophobe and Other Cells in Pituitaries of Mice on Different Diets.

	Control	Yolk	Alc ext	Eth ext	Chol & lard	Chol
			Acidophile cells			
n	30	30	20	20	20	8
Mean	34.48	29.60	28.88	27.66	27.45	22.85
X_a-X_b	—	—4.88	—5.60	—6.82	—7.03	—11.63
S_x	9.72	5.92	5.40	6.53	7.64	2.16
t	—	2.36	2.35	3.17	2.55	3.36
			Basophile cells			
n	30	30	20	20	20	8
Mean	3.13	6.88	3.63	3.37	6.58	6.65
X_a-X_b	—	+3.75	+ .50	+ .24	+3.45	+3.52
S_x	1.48	1.87	2.90	1.12	4.18	1.73
t	—	8.72	.81	.62	5.00	5.87
			Chromophobe cells			
Mean	44.76	44.10	50.25	51.81	44.61	41.59
			Other cells			
Mean	17.63	19.42	17.24	17.16	21.36	28.91

terol alone. On the contrary, the relative number of the basophile cells in the pituitaries of the experimental mice increased as compared with the controls, but this increase is statistically significant only in the case of the mice on the diet supplemented with egg yolk, with cholesterol and lard or with cholesterol alone. In the mice on the diet supplemented with alcohol extract of egg yolk or with ether extract of whole egg the increase is slight and statistically not significant. As mentioned previously, the greatest change in the pituitary occurred in the mice on the diet supplemented with cholesterol alone, in which, in addition to the decrease in the relative number of acidophile cells and the increase in the relative number of basophile cells, a considerable number of giant cells were found. These large cells had either basophile granules or were vacuolated(3).

It should be mentioned that in 4 of the experimental mice (2 on the diet supplemented with egg yolk, 1 on the diet supplemented with alcohol extract of egg yolk and 1 on the diet supplemented with ether extract of whole egg) tumors of pituitary were found. They consisted of chromophobe and a few isolated basophile cells. Their origin could not be determined. They were large and the anterior lobe of the pituitary was reduced to a narrow band at the periphery of the tumor. Structurally, they resemble intermediate lobe adenomas, found recently in mice of the C57 Bl. strain maintained on a diet supplemented with egg yolk(9).

Discussion. The above results clearly

TABLE II. Malignant Tumors in Mice (on the Various Diets) of Which the Pituitaries Were Studied.

	Total No. of mice	No. of mice: 300-600 days old	601-700 days old	701-950 days old	Avg age (days)	Mice with: Lung neoplasmas*	Lymphosarcoma	Mammary adenocarcinoma	Other tumors
Controls	30	24	2	4	615	4	0	1	0
Yolk	30	25	4	1	405	19	0	4	1[1]
Alc ext	20	18	2	0	407	7	0	10	1[2]
Ether ext	20	18	1	1	475	15	6†	4	2[3]
Chol & lard	20	18	1	1	521	8	0	8	2[4]
Cholesterol	8	5	2	1	615	8	0	0	2[5]

[1] Skin carcinoma.
[2] Fibrosarcoma.
[3] 2 fibrosarcomas.
[4] 2 skin carcinomas.
[5] 2 fibrosarcomas.
Many mice had 2 or more primary tumors.
* Adenomas or adenocarcinomas. One of these tumors, in a mouse on the diet supplemented with ether extract of whole egg metastasized to the wall of the aorta.
† Five thymic and one nonthymic type of lymphosarcomas.

demonstrate that in mice of the T.M. strain maintained on the Rockland rat diet supplemented with egg yolk, extracts of eggs, cholesterol and lard, or cholesterol alone, there is a decrease in the relative number of the acidophile cells and increase in the basophile cells of the adenopituitary. On the other hand, as demonstrated previously(1,2,3) the mice maintained on the above diets develop a high incidence of malignant tumors of various kinds. The incidence of neoplasms in the experimental animals is 4 to 5 times higher than in the controls (Table II). Similar results have been obtained recently with mice of the C57 Bl. strain (a well known cancer resistant strain). The mice of this strain maintained on the Rockland rat diet had an incidence of neoplasms of only 10%, while on the diet supplemented with egg yolk 78.4% of the animals developed tumors(10). At the same time the acidophile cells in the anterior pituitary decreased from 57.9% to 45.7% and the basophile cells increased from 2.9 to 3.4%. Moreover, 12% of the mice on the diet supplemented with egg yolk developed pituitary tumors, most of which originated from the intermediate lobe(7).

The simultaneous changes in the pituitary and in the carcinogenic susceptibility of mice maintained on various diets raises the question whether there is any causal relationship between these two phenomena. One wonders whether the change in the relative proportion of the two functional types of cells in the adenopituitary is a determining factor in the change of susceptibility of mice to carcinogenesis.

Many investigators who studied the effect of prolonged administration of estrogen upon mammary carcinogenesis in mice found changes in the adenopituitary. These changes were an increase in size of the gland, a marked decrease in number of chromophile cells (both acidophile and basophile) and an increase in chromophobe cells(11,12). Some of these mice developed tumors of the anterior lobe which were all described as chromophobe adenomas(11,12). Similar changes were found in rats treated for long periods with large doses of estrogens(13). In such animals the acidophile cells of the adenopituitary decreased from 34.2% to 14.1% and the basophile cells from 3% to 0.1% after 80 days of treatment. Both of these cells completely disappeared in rats treated for longer periods, and in many cases chromophobe adenomas developed.

It is unlikely that in egg yolk and in lard the substance responsible for the changes in the pituitary is estrogen. The seminiferous tubules in the males on the various diets are well developed and active. No sign of atrophy could be found. Moreover, the changes in the pituitary in our experimental mice were different from those caused by large doses of estrogen. The relative number of basophile cells increased rather than decreased.

The increase in the relative number of basophile cells and the decrease in acidophile cells are not inversely related. The decrease in acidophile cells occurred in the mice of the T.M. strain of all the experimental groups and in the mice of the C57 Bl. strain on the diet supplemented with egg yolk, while a significant increase of the basophile cells took place only in the mice of 3 experimental groups, (those receiving egg yolk, cholesterol and lard, and cholesterol alone). In the mice on the diet supplemented with alcohol extract of egg yolk, or ether extract of whole egg, as well as in the mice of the C57 Bl. strain receiving egg yolk, the change in the basophile cells was not significant. One consequently wonders whether the changes in the relative number of the 2 types of cells are not independent phenomena caused by 2 different mechanisms.

The increase in the relative number of basophile cells in the pituitaries of the mice on the various experimental diets may not be due to the same mechanism. In the mice on the diet supplemented with cholesterol alone, with no other lipids, the ovaries were almost inactive. They had no follicles nor corpora lutea(3). It was consequently not surprising to find in these mice an increase in the relative number of basophile cells and the presence of giant cells which are like castration cells. The ovarian activity of some of the females on the diet supplemented with cholesterol has been studied by way of daily vaginal smears, for periods of 30 to 50 con-

secutive days. At the age of 4 to 5 months the estrous cycle in these mice is irregular, as compared with that of the controls (mice of the same age maintained on the Rockland rat diet only). In the latter the period of estrus (characterized by cornified epithelium) lasts one to two days and is followed by a period of diestrus. In the mice on the diet supplemented with cholesterol the estrous period lasts very often 6 to 8 days and is followed by a period of proestrus (characterized by nucleated epithelium). A diestrous smear, characterized by the presence of lymphocytes, is seldom seen. The hypofunction of the gonads would in this case be the cause of the increase in the relative number of basophile cells and the presence of giant cells in the pituitary. The mechanism by which cholesterol inhibits ovarian activity, whether it acts directly upon the gonads or indirectly through the pituitary, is unknown. In female rats, cholesterol administered immediately after birth has a masculinizing effect(14).

Summary. The adenopituitaries of mice of the T.M. strain maintained on the Rockland rat diet supplemented with egg yolk, alcohol extract of egg yolk, ether extract of whole egg, cholesterol and lard, or cholesterol alone have been studied. It was found that in the mice of all the above groups there is a relative decrease in acidophile cells and an increase in basophille cells, as compared with the controls (mice on the Rockland rat diet only). The decrease in acidophile cells is statistically significant for all the experimental groups, while the increase in basophile cells is significant only in the case of the mice receiving egg yolk, cholesterol and lard, or cholesterol alone. In the mice on the diet supplemented with alcohol extract of egg yolk or with ether extract of whole egg the increase in basophile cells is not significant. The changes in the two types of cells are not inversely related and it is thought that they are due to different mechanisms. The decrease in the relative number of acidophile cells is probably caused directly by some of the steroids of the diet. The increase in basophile cells of the adenopituitary, in the case of the mice on the diet supplemented with cholesterol alone, would be due to hypofunction of the gonads, while in the mice of the other groups it is probably influenced by some of the dietary lipids, the nature of which is unknown.

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