HeLa cells Zn^{++} reversed inhibition of alkaline phosphatase by glycine. Low concentrations of this cation were found to reactivate dialyzed preparations of the same enzyme. Mg^{++} retarded thermal inactivation of alkaline phosphatase. Delay in heat inactivation was observed in alkaline phosphatase preparations from both prednisolone treated and control cultures of HeLa cells.

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Carcinogenic Effect of Cholesterol in Mice.* (30727)

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It was previously demonstrated that both egg white and egg yolk are carcinogenic in mice, but that their carcinogenicity is different. The white causes the development of a high incidence of lung adenocarcinoma and lymphosarcomas, while the egg yolk, in addition to these, causes the development of mammary cancer in the breeding females of the T.M. strain of mice(1). The mice of this strain on the control diet rarely develop mammary cancer. Moreover, it was found that the carcinogenic substances in egg are lipids; they are present in ether extract of whole egg and in alcohol extract of egg yolk(2). The carcinogenicity of these 2 extracts is not the same. Mice maintained on a diet supplemented with ether extract of whole egg develop a lower incidence of mammary cancer and a higher incidence of lung adenocarcinoma and lymphosarcoma than similar mice on a diet supplemented with alcohol extract of egg yolk. It is well known that some lipids are more soluble in alcohol while others are more soluble in ether. The conclusion was consequently drawn that there is a relationship between the types of malignancies and the composition of the lipids ingested by the mice. The results obtained with cholesterol alone and cholesterol and lard support the above point of view.

Methods. It was demonstrated that mice of the T.M. strain maintained on the Rockland rat diet supplemented with cholesterol and lard develop a relatively high incidence of malignancies (close to 50%). These malignancies are mainly lung adenocarcinomas and mammary cancer. Fifty per cent of the breeding females on the diet supplemented with cholesterol and lard developed breast cancer. In the present experiments mice of the T.M. strain were placed at the age of 4 weeks on the Rockland rat diet supplemented with cholesterol and lard (Group 1) or with cholesterol alone, no lard (Group 2). The cholesterol was given in the amount of 50 to 100 mg daily per group of 10 to 12 mice. For the mice of Group 1, one gram of lard and some sugar were mixed with the cholesterol. For those of Group 2, some sugar and a drop of water were mixed with the cholesterol. The mice ate the cholesterol and there was almost no waste. The mice of the control group were maintained on the Rockland rat diet only. The mice of the 3

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TABLE I. Malignancies in Breeding Mice of the T.M. Strain Maintained on Rockland Rat Diet Supplemented with Cholesterol or Cholesterol and Lard.

Diet	Total No. mice and Male	Female	Age in days	No. of mice with malignancies	Lung adenocarcinoma	Lymphosarcoma	Mammary cancer	Other malignancies
				(%)	(%)	(%)	(%)	(%)
	138							
Control†	77	61	655.6	22 (15.9)	14 (10.1)	4 (2.9)	2 (3.3)*	5 (3.6)[1]
	140							
Cholesterol† and lard	68	72	574.0	67 (48.8)	26 (18.5)	5 (3.5)	37 (51)*	6 (4.3)[2]
	118							
Control	60	58	676.3	21 (17.8)	16 (13.5)	3 (2.5)	4 (6.8)*	2 (1.7)[3]
	113							
Cholesterol and lard	52	61	575.4	77 (68.1)	28 (24.7)	2 (1.7)	40 (65.5)*	7 (6.1)[4]
	71							
Cholesterol	36	35	665.6	58 (81.6)	54 (76.0)	0	3 (8.6)*	10 (14.0)[5]

[1] Granulosa cell cancer of ovary, squamous cell carcinoma of stomach and 3 fibrosarcomas.
[2] Squamous cell carcinoma of skin, ovarian tumor, ascites tumor and 3 fibrosarcomas.
[3] Two fibrosarcomas.
[4] Two liver adenocarcinomas, 3 skin squamous cell carcinomas and 2 fibrosarcomas.
[5] Liver cancer, uterine cancer, 4 skin squamous cell carcinomas and 4 fibrosarcomas.
* Of females only.
† Published previously.

groups were bred and their offspring maintained on the same diets. Consequently each group consisted of mice of 3 or more generations. The mice were bred only twice. Males and females were then caged separately. All the mice were autopsied after death and the organs affected fixed with Bouin's fluid, sectioned and stained with hematoxylin and eosin for microscopic examination. For the pituitary gland a modification of Glenner and Lillie's staining technique was used(3).

Results. The results are summarized in Table I. Of 138 mice of one control group (published previously, 2), 15.9% of the animals developed malignancies. Of these, 14 were lung adenocarcinomas. Mammary cancer occurred in only 2 of the 61 females of this group. In the other control group (run simultaneously with the group on the diet supplemented with cholesterol) the incidence of malignancies was slightly higher, (17.8%), due in part to an increase in the incidence of mammary cancer. In this group 6.8% of the females developed mammary cancer, while in the former group the incidence was only 3.3%.

On the diet supplemented with cholesterol and lard there are also 2 groups of mice. Of one group (published previously, 1) 48.8% of the mice developed malignancies which were mainly lung adenocarcinomas and mammary cancer. The latter occurred in 50% of the breeding females. In the second group, on the diet supplemented with cholesterol and lard, the incidence of malignancies was higher (68.1%) than in the previous group. This increase is due to a great extent to the higher incidence of mammary cancer—65.5% as compared with the 50% of the previous group.

The mice on the diet supplemented with cholesterol alone, lived, like the controls, to an average age of over 650 days. Of the 71 mice, 58, or 81.6%, developed malignancies. Of these, 54 were lung adenocarcinomas. Mammary cancer appeared in only 3 of the 35 females of this group, an incidence of 8.6%. Upon microscopic examination important changes were found in the liver, ovaries, and pituitary gland of the mice on the diet supplemented with cholesterol, changes not seen in the controls nor in the mice on the diet supplemented with cholesterol and lard.

In the liver, the cells vary considerably in size and their nuclei are usually hyperchromatic. But most striking is the presence of a large number of mitotic figures, which are rarely seen in the controls and in the mice on the diet supplemented with choles-

terol and lard. Another characteristic is the presence in the hepatic cell nuclei of vacuoles. In some of the mice over 50% of the liver cell nuclei have vacuoles. The latter, as previously demonstrated, are surrounded by Feulgen positive material and very often contain glycogen(4).

The ovaries of almost all the females of Group 2, often enlarged in size, contain almost no follicles. Single follicles occasionally found are usually abnormal and do not seem to reach maturity. The germinal epithelium of these ovaries is thickened considerably and forms ingrowths inside the organ. The whole ovary consists, consequently, of cavities surrounded by germinal epithelium. Only in the 3 mice that developed mammary cancer do the ovaries appear to be functional and have some follicles in the process of development and some corpora lutea. But even in these ovaries, in the spaces between the corpora lutea, the germinal epithelium is considerably thickened and forms ingrowths.

In the anterior pituitary, the most striking characteristics are the presence of giant cells 6 to 8 times larger than the normal glandular cells. These cells have large nuclei and large nucleoli; they are often binucleated and their cytoplasm is filled with basophilic granules or has no granules and is vacuolated.

Discussion. From the above results it appears that cholesterol is not an inert substance. It affects the liver cells, the germinal epithelium of the ovary and causes a high incidence of lung adenocarcinoma. The low incidence of mammary cancer in the mice on the diet supplemented with cholesterol as compared with that of the mice receiving cholesterol and lard, may be due to the poor development of the ovaries and consequently the low estrogen titer in these animals. This may also be the reason for the appearance of the giant cells in the adeno-pituitary, which resemble castration cells, and the complete absence of lymphosarcoma among the mice of this group. Cholesterol apparently stimulates growth, as was also demonstrated by Hieger(5) who succeeded in inducing the development of fibrosarcomas in mice by subcutaneous injection of cholesterol. Its effect upon differentiation of the ovary is probably inhibitory, an effect which would be counteracted by other lipids present in lard and in egg yolk, since in mice receiving cholesterol and lard or egg yolk the ovaries are well developed and the incidence of mammary cancer is relatively high.

In the egg yolk there are apparently at least 2 lipids affecting carcinogenesis in different tissues and by a different mechanism. Some of the egg steroids would cause the development of lung cancer and probably fibrosarcoma, while other lipids, the nature of which is unknown, affect primarily the gonads and these in turn influence the development of malignancies in the mammary glands and the lymphoid system. Tannenbaum(6) found that a fat enriched diet in mice enhances the development of spontaneous mammary adenocarcinoma and induced skin cancer, while it has no effect upon spontaneous lung cancer and induced fibrosarcoma. He believes, on the basis of results obtained by others(7), that it is the fatty acid glycerides and not the non-saponifiable lipids that affect carcinogenesis. Moreover, Tannenbaum suggests that the lipids affect the genesis of tumors by two means, by a solvent action (the increased fat content of the tissue would increase the rate of transfer of the carcinogen) and by an independent effect of fat upon the developing tumor cells. Tissues, like those of the lungs, which do not store lipids, would not be affected by a fat enriched diet.

On the basis of our results it appears that the difference in reactivity to a fat enriched diet between the lungs and connective tissue on one hand, the mammary glands and the skin on the other hand, is due to a difference in the mechanism of carcinogenesis in these two groups of organs or tissues. The former would be affected by steroids (which were probably not in a high enough concentration in the diets used by Tannenbaum), while the latter are influenced by other lipids which act, not directly, but through the ovaries. The nature of these lipids, whether steroids, or fatty acid glycerides, as suggested by Tannenbaum, is being studied.

Summary. Mice of the T.M. strain maintained on a Rockland rat diet supplemented

with cholesterol and lard develop a high incidence of mammary cancer and lung adenocarcinoma. In the absence of other lipids, cholesterol alone causes the development of a high incidence of lung adenocarcinoma. The incidence of mammary cancer in the mice of this group is very low, apparently due to the poor development of the ovaries and a low estrogen titer in the females receiving cholesterol. The appearance of giant basophilc cells in the pituitary would be indicative of a low estrogen titer in these mice. The fact that cholesterol and lard cause the development of both mammary and lung cancer, while cholesterol alone causes almost exclusively the development of lung adenocarcinomas supports our point of view that there is a relationship between the nature of the malignancies in mice and the composition of the lipids in their diets.

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Bovine Growth Hormone Uptake by Epididymal Adipose Tissue.*† (30728)

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Recognition of species specificity of growth hormone has stimulated interest in the modification of bovine growth hormone in order to make it useful in man. The reasons for this species specificity are unknown, although differences in the amino acid composition of growth hormone from different species are thought to contribute to it. Since guinea pigs are also relatively resistant to growth hormone(1) we have studied them. In a previous report it was noted that bovine growth hormone disappeared from the plasma of guinea pigs after intravenous injection as rapidly as it did in rats(2). Further, it has now been found in this laboratory that the tissue distribution of human growth hormone intravenously injected into rats and guinea pigs is similar. This report presents data suggesting that guinea pig plasma retards, and rat plasma stimulates, *in vitro* uptake of bovine growth hormone into rat epididymal adipose tissue.

Methods. All experiments were performed using 5 ml Krebs-bicarbonate buffer, saturated with 95% oxygen and 5% carbon dioxide in 10 ml stoppered flasks. The order in which materials were added to the incubation flasks was found to be important. The rat was killed after the buffer was warmed to 37°C, and saturated with the oxygen and carbon dioxide mixture. Epididymal adipose tissue (200-300 mg, from male Sprague-Dawley, 100-150 g rats), albumin or serum, and finally the tritium-labeled bovine growth hormone were promptly added. The flasks were shaken (100 strokes/minute) in a metabolic shaker at 37°C during each experiment. Adipose tissue was removed from the flask, rinsed for 15 minutes in ice-cold saline, homogenized in 1 ml chloroform:methanol (1:1), and then 0.1 ml aliquots were

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