as a factor in tumor physiology is apparent from the work of Warburg on glycolytic metabolism of tumors. The present work does not, however, afford sufficient basis for excluding the possibility of other factors being operative, nor for further elaboration of hypotheses as to the interrelationship between the effects of methylene blue, redox potentials, type and amount of oxidation, and tumor physiology.

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Effect of Cholesterol Feeding under Varying Conditions upon Lipids of Rat Livers.

RUTH OKEY.

From the Laboratory of Household Science, University of California, Berkeley.

Our interest has been drawn during the past few years to the question of the effect of various dietary deficiencies or excesses upon the deposition of cholesterol and cholesterol ester in tissues; manifested pathologically as fatty livers, arteriosclerotic arteries and gall stones. The following is a preliminary report of the first of a series of studies of tissues of rats fed cholesterol with diets of known but varying fat content, vitamin intake, etc.

The litters of rats used were cut to 8, 4 males and 4 females, shortly after birth. At 21 days of age, 2 males and 2 females from each litter were placed upon the diets containing cholesterol, while the littermate controls were placed upon the same diets without the cholesterol. After a feeding period of approximately 60 days the rats from both groups were killed by cutting the spinal cord at the base of the brain. The tissues were separated as quickly as possible and samples weighed for determination of moisture and of lipid content and in some cases for histological examination. The samples for lipid determinations were ground with sand and extracted to exhaustion with alcohol-ether.

The present report is limited to the composition of the livers of 2 groups of 16 animals each fed 1% cholesterol with basal diets consisting of 20% baked and extracted casein, 4% Osborne-Mendel salt mixture, 4% agar. In the first series this was supplemented with 15% Crisco and 47% starch, while in the second series 10% Crisco and 62% starch were used. For the experimental

groups the cholesterol was dissolved in the melted Crisco and thoroughly mixed with the rest of the diet, so that cholesterol intake was strictly proportional to food intake.

Northwestern yeast was used as a source of vitamin B complex for the first 40 days of each experiment after which a fully adequate amount of Harris yeast extract was substituted. We had planned to use carotene as a source of vitamin A but the only preparation available proved unsatisfactory. Hence we were compelled to use cod liver oil, which was given at the rate of one drop per day. Otherwise the control diets were sufficiently low in sterol that the alcohol ether solubles material from 1 gm. diet gave no color with the Liebermann Burchard reagent. Yeast and cod liver oil were therefore the chief sources of sterol in the control diets.

The food intake of the animals was such that the total cholesterol fed per day was, at a very rough approximation, 1 mg. per gram rat.

The sterol fed animals showed not only a marked increase in the fatty acid content of the livers, but also an almost phenomenal increase in sterol as ester. The liver fatty acids for the control rats on the diets containing 15% Crisco were approximately 12% as determined by oxidation, for the cholesterol fed animals, 23%. The corresponding figures for sterol as ester were 0.19 and 7.25% respectively.

Free sterol was very nearly the same for the 2 groups, *i. e.*, about 0.25%.

Sterol ester percentages for the animals given only 10% fat in the diet were only slightly lower. In this group, however, the livers of the female animals fed cholesterol seemed to enlarge less than those of the males. Hence, while the actual weight of sterol stored was slightly greater in the males, the percentage concentration was higher in the females. Fatty acids were actually higher in the livers of the females. The number of animals used so far is probably too small to justify conclusions as to the influence of sex on storage of lipid in the liver, but the results are at least suggestive of the necessity for more work. Unfortunately in our series of determinations on the 15% fat intake each of our samples was made up of the livers from 2 males and 2 females mixed together for extraction.

In one group of animals from each series the vitamin B complex (as Harris yeast extract) was removed from the diet 10 days before killing. The livers from the cholesterol-fed animals in these groups showed a distinct increase in moisture content and a marked de-

crease in both fatty acid and sterol as ester. Littermates fed vitamin B complex + cholesterol at the 10% fat level showed 16.6% fatty acid and 6.0% cholesterol as ester for the males, 23.2% fatty acid and 6.6% cholesterol as ester for females. Those deprived of the yeast extract showed 11.9% fatty acid for males, 13.9% for females and 4.5% for males and 2.5% for females cholesterol as ester, respectively. However the figures for the animals receiving no cholesterol showed about the same proportionate decrease in fatty acids, 8.5% to 5.4% for males and 10.5 to 5.6% for females. Cholesterol as ester in both was negligible in amount.

Further data will be necessary to determine whether this is a result of lowered food absorption alone, but some as yet incomplete studies indicate that the effect is specific for vitamin deficiency.

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On the State of Calcium and Phosphate of the Blood.

DAVID M. GREENBERG. (With the assistance of W. E. Cohn and E. V. Tufts.)

From the Division of Biochemistry, University of California Medical School, Berkeley.

The report by Smith and Sternberger[1] that in many of their ultrafiltration experiments the sum of the ultrafiltrate and concentrate calcium fell very considerably short of the serum calcium and that the difference was due to an adsorption of calcium by the collodion membranes, led us to undertake an investigation of the phenomenon. This loss is important in relation to the differentiation of the blood calcium into a diffusible and non-diffusible fraction by procedures such as the one of Greenberg and Gunther.[2]

It was found that the loss from serum with only normal amounts of calcium is negligible and only when the serum calcium is very considerably augmented, either by *"in vitro"* or *"in vivo"* measures, does the amount of calcium taken up by the membrane become of importance. The significance of diffusible and non-diffusible calcium determined under the usual circumstances on fresh blood serum is in no way vitiated by this finding.

This is borne out by the experiment given in Table I, which is representative of our results. The table shows the changes produced

[1] Smith, R. G., and Sternberger, H. R., *J. Biol. Chem.*, 1932, **96**, 245.

[2] Greenberg, D. M., and Gunther, L., *J. Biol. Chem.*, 1929, **85**, 491.