the blood volume was only 15% below normal, and attributed the lower results of earlier authors to errors in the method of blood volume determination used.

Methods. This study utilized 16 male rats, ranging in weight from 150 to 180 g at the start. Thyro-parathyroidectomy was performed, under ether anesthesia, by blunt dissection and cauterization of the thyroid bed. Following this, an interval of 3 weeks elapsed, during which the rats were undisturbed and were fed upon stock diet. At the end of 3 weeks, the rats had failed to gain weight in accordance with the normal growth curve. Blood and plasma volume determinations were performed according to the hemoglobin dye method previously described.[4] Gross postmortem examination showed no regeneration of thyroid tissue at the site of operation. The heart weights were diminished in accordance with previous experience upon thyro-parathyroidectomized rats.[5]

Results. Plasma volumes were obtained in 16 rats, and blood volumes were obtained in 14 of these. There was a mean reduction of plasma volume, erythrocyte volume and total blood volume of approximately 10%, when compared with normal standards for rats of the same weight, as previously established.[4] (Table I).

4 Lippman, R. W., PROC. SOC. EXP. BIOL. AND MED., 1947, **66**, 188.

5 Addis, T., Karnofsky, D., Lew, W., and Poo, L. J., *J. Biol. Chem.*, 1938, **124**, 33.

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Protective Action of Dietary Cholesterol in Experimental Thyrotoxicosis.*

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Experiments were made to investigate further the well known effects of hyperthyroidism on cholesterol metabolism.[1] An unexpected result of these studies was the observation that a reverse relationship also existed, *i.e.* that high dietary cholesterol modified the toxic effects of high doses of thyroid hormone, in particular, as far as survival time and body weight changes were concerned. These findings are reported in the present communication.

Experimental Procedure. Normal rats were fed *ad libitum* this laboratory's stock diet, slightly modified to contain more yeast, in order to assure an adequate supply of the vitamin B complex.† In addition, thyroid powder was mixed with the diet at concentrations corresponding to 0.4-0.8% thyroid U.S.P. One group of rats (T) received the high thyroid diet as described, a second group (T + C) was fed an identical diet, except that it contained in addition, 1% cholesterol and 0.5% bile salt. The cholesterol was dissolved in cottonseed oil, replacing a corresponding amount of the oil in the diet. The bile salt was added to increase absorption of cholesterol.[2] The rats were about 9 months (Experiment 1) and 38 days (Experiment 2) old respectively at onset of the feeding periods. Litter mates were distributed evenly between the two groups. Body weights were measured once weekly, and the survival time

* Supported by a grant from the Life Insurance Medical Research Fund.

1 Peters, J. P., and van Slyke, D. D., *Quant. Clinical Chem., Interpretations I,* 2nd Edition, 1946, 497.

† Modified stock diet: 328 g whole wheat flour; 327 g ground oats; 100 g skimmed milk powder; 30 g alfalfa; 95 g yeast, Anheuser-Busch strain G; 80 g cottonseed oil; 20 g fortified cottonseed oil, containing 2500 I.U. vitamin A/g and 400 I.U. vitamin D_2/g; 5 g NaCl; 5 g $CaCO_3$.

2 Schoenheimer, R., *Biochem. Z.*, 1924, **147**, 258.

TABLE I.
Effect of High Dietary Cholesterol on Survival Time of Rats Fed Toxic Doses of Thyroid Hormone.

Exp. No.	Diet	No. of rats	Age at onset	Mean survival in days	Standard error of mean*	$\frac{D_M}{S_{D_M}}$†
1	T	6	9 mos.	23	± 4.3	13.4
	T + C	6	9 "	55+	0	
2	T	14	38 days	86	± 3.9	3.8
	T + C	7	38 "	111+	± 5.4	

* The standard error of the mean calculated from the formula $S_M = \sqrt{\frac{\Sigma d^2}{n(n-1)}}$ where "d" is the deviation from the mean, and "n" is the number of observations.

† Ratio, difference of means (D_M) to standard error of difference (S_{D_M}); results are considered significant when this ratio > 3.

$$S_{D_M} = \sqrt{\frac{\Sigma d_1^2 + \Sigma d_2^2}{(n_1 - 1)\ (n_2 - 1)} : \frac{n_1 n_2}{n_1 + n_2}}$$

was noted. In the second experiment, the food intake was also measured, the animals being kept in single cages with screen bottoms.

Results. The experimental results are summarized in Table I and Fig. 1. In the first experiment, in which older rats and a desiccated hog thyroid preparation containing 0.65% total I and 0.31% thyroxine were used, the high dietary cholesterol had striking effects, both appreciably prolonging the length of survival time and protecting against the loss in body weight. All rats of group T died within 5-36 days, the average survival time being 23 days. In group T + C, all animals were alive at 55 days, at which time the animals were killed for autopsy. Fig. 1 indicates that the excessive weight loss caused by thyroid hormone (group T) also was prevented or diminished by the addition of cholesterol + bile salt (group T + C).

In Experiment 2 in which young rats and a different hormone preparation‡ were used, the same trend was observed, but the protective action of cholesterol was less marked. The difference in the mean survival times between the groups T and T + C was still significant. The body weight results were equivocal, however; only in the case of the male rats was a similar trend observed as in Experiment 1.

The possibility was considered that the animals on the high cholesterol diet (T + C) ate less due to the presence of bitter-tasting bile salt in their food. In this case the amount of thyroid hormone consumed by this group would have been correspondingly smaller. In order to investigate this possibility, the food consumption was measured in Experiment 2. The results indicate that the animals ate practically the same amount of food in both groups. The average intake per day was 17 g in group T, and 16 g in group T + C. It is highly improbable that such an insignificant difference in the amount of food and thyroid hormone consumed was responsible for the marked differences in survival time and body weight observed. The latter differences have to be attributed, therefore, to an action of the cholesterol protecting from the toxic effects of thyroid hormone.

This conclusion was confirmed by experiments made recently in collaboration with Dr. B. Ershoff.[3] In those experiments, conditions were essentially the same,§ except that bile salt was entirely omitted from the diet. It can be assumed that under these con-

‡ Thyroid powder, U.S.P. Armour.

[3] Ershoff, B. H., and Marx, W., to be published.

§ A different strain of rats and a different stock diet were used; the cholesterol was mixed with the diet as a dry powder (for group T + C).

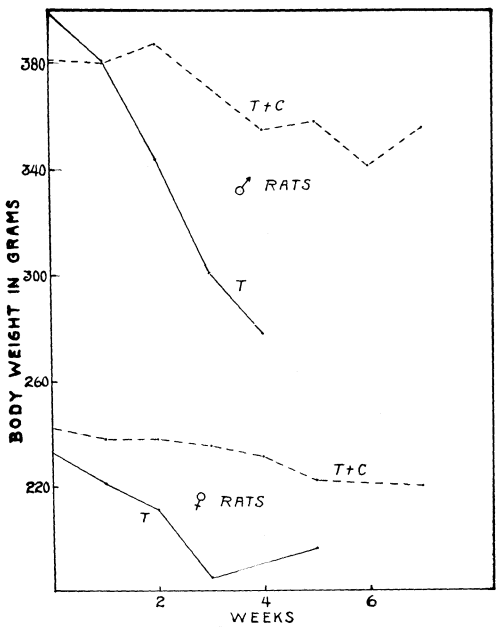

FIG. 1.

Effect of dietary cholesterol on the body weight of rats fed toxic doses of thyroid hormone. T = thyroid alone; T + C = thyroid + cholesterol.

ditions both groups T and T + C consumed the same amount of diet, and therefore, of thyroid hormone. It is interesting that, in this experiment, the survival time was again significantly prolonged as a consequence of the high dietary cholesterol, in spite of the fact that the absorption of the latter was very likely smaller than in the experiments discussed above.

Discussion. The authors are not aware of any report in the literature referring to experimental observations on a protective effect of high dietary cholesterol in case of thyrotoxicosis. The findings are in agreement, however, with a hypothesis recently proposed by Hoffmann and Hoffmann.[4] According to this theory, thyroid hormone stimulates the enzymatic breakdown of lecithin to lysolecithin. Lysolecithin in turn, is believed to affect the central nervous system, to influence cell permeability, and to exert, at higher concentrations, "toxic effects in various organs." These are the effects which cholesterol is supposed to counteract, or to neutralize. It might be added, in connection with this concept, that an antagonism between phospholipids and cholesterol was postulated by various investigators, and many instances can be quoted where such an antagonism was demonstrated experimentally.[5]

Summary. High dietary cholesterol prolonged significantly the survival time of rats fed toxic doses of thyroid hormone; in some, but not in all groups the animals' weight loss was also reduced.

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[4] Hoffmann, F., and Hoffmann, E. J. de, *Public. del Institut. Fisiol.*, Universidad de Chile, 1943; quoted by Foldes, F. F., and Murphy, A. J., PROC. SOC. EXP. BIOL. AND MED., 1946, **62**, 218.

[5] Bills, C. E., *Physiol. Rev.*, 1935, **15**, 1.