stances, phosphate, is an inorganic ion.

The inhibition of tubular transport by PAH may be more adequately explained by the hypothesis of a common energy source of limited capacity. Competition for the available energy by the activity of the mechanism for the transport of one solute would then result in diminished transport of another solute. Neither the locus nor the character of the energy limitation can be stated. Some of the possible mechanisms involved have been discussed by Selkurt[2] and by Houck.[4]

It should be emphasized that the inhibition of tubular transport of one substance by another may result in serious error when several renal functions are measured simultaneously.

Summary. The urinary excretion of phosphate is increased by the injection of sodium p-aminohippurate in man. The extent of the phosphaturia tends to vary directly with the plasma PAH level. At high PAH levels, the amount of phosphate excreted averages 26% of that filtered, a proportion comparable in magnitude to that reported after injection of parathyroid hormone.

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17180. Effect of Dietary Cholesterol on the Metabolic Rate of Hyperthyroid Rats.*

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It was recently observed that dietary cholesterol increases the survival time of rats fed toxic doses of thyroid hormone.[1,2] In an attempt to learn whether this protective action of cholesterol is correlated with a corresponding modification of the effects of thyroid on the metabolic rate, the influence of dietary cholesterol was investigated on the oxygen consumption of hyperthyroid rats.

Methods. Thirty-two male rats (USC strain), about 9 weeks of age, were distributed evenly among 4 groups which received the following diets *ad libitum*: I. This laboratory's stock diet, slightly modified to contain more vitamin B complex‡ ("N"). II. Diet I, containing, in addition, 1 to 2% cholesterol§ ("C"). III. Diet I, containing, in addition, 0.15% desiccated thyroid U.S.P.§ ("T"). IV. Diet I, containing, in addition, both 0.15% thyroid and 1 to 2% cholesterol. ("T + C"). At first, cholesterol was fed at a level of 1%, but after 3 weeks, its concentration in the diet was raised to 2%. The cholesterol was dissolved in hot cottonseed oil, replacing a corresponding amount of oil in the diet. In order to increase cholesterol absorption, 0.25% bile salt§ was added to all diets.[3]

The oxygen consumption was determined using a closed circuit apparatus provided with separate chambers for individual animals.[4] After repeated measurements during a preliminary training period of 5 weeks, feeding

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1 Marx, W., Meserve, E. R., and Deuel, H. J., Jr., PROC. SOC. EXP. BIOL. AND MED., 1948, **67**, 385.

2 Ershoff, B. H., and Marx, W., *Exp. Med. and Surg.*, 1948, **6**, 145.

‡ Whole wheat, 32.5%; oats, 32.75%; skimmed milk, 10%; alfalfa meal, 4%; yeast, Anheuser-Busch strain G, 9.5%; Wesson oil, 8%; fortified oil containing 1500 I.U. vitamin A/g and 160 I.U. vitamin D_2/g, 2%; sodium chloride, 0.5%; calcium carbonate, 0.5%; bile salt, 0.25%.

§ Cholesterin c.p., Amend Drug and Chemical Co., New York City; desiccated thyroid, U.S.P., Armour and Co., Chicago, Ill.; sodium glycocholate (pure), City Chemical Corp., New York City.

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

TABLE I.
Effects of Dietary Thyroid and Cholesterol in Male Rats.
Figures are Average Oxygen Consumption and Standard Error.

Group*	Thyroid fed	Cholesterol fed	At onset	After 3 wks	After 6 wks	After 9 wks
			ml oxygen/100 g body wt/hr			
N	—	—	123 ± 4	106 ± 3	108 ± 4	—
C	—	+	121 ± 2	106 ± 3	105 ± 3	—
T	+	—	122 ± 2	166 ± 4	162 ± 6	160 ± 6
T + C	+	+	121 ± 4	157 ± 3	144 ± 3	144 ± 4

* Each group consisted of 8 rats.

TABLE II.
Effects of Dietary Thyroid and Cholesterol in Male Rats. Average Body Weight Gain, Food Intake, and Tissue Cholesterol Content.

Group*	Thyroid fed	Cholesterol fed	Body wt gain in 9 wks and standard error, g	Food intake,† g/100 g body wt/day	Cholesterol content†	
					Plasma, mg/100 ml	Liver, %
N	—	—	122 ± 5	5.7	53	0.21
C	—	+	109 ± 7	5.9	62	0.79
T	+	—	80 ± 7	7.1	49	0.24
T + C	+	+	72 ± 8	8.2	54	0.81

* Each group consisted of 8 rats.
† No standard errors are given, since, in each group, the samples were pooled in lots of 2 or 3.

of the experimental diets was begun, and the oxygen consumption was determined every 3 weeks. Body weights were recorded every 10 days, and the food consumption was measured at 2 day intervals during the seventh and eighth week.

After an experimental period of 9 weeks, the animals were autopsied, and weights and total cholesterol contents of various tissues were measured, the latter according to a modified Schoenheimer-Sperry-Chaney procedure.[5]

Results. The data on the determination of the oxygen consumption indicate that the administration of thyroid raised the metabolic rate by about 60% (Table I). The addition of cholesterol to the high thyroid diet caused a reduction of this value by about 10%, the difference being statistically significant ($P < 0.01$ for the 6 week period; $P =$ approximately 0.025 for the 9 week period). The metabolic rate of normal rats was not significantly modified by the addition of cholesterol to the diet.

Thyroid administration depressed the body weight gain and stimulated the animal's appetite, as was to be expected (Table II). The addition of cholesterol to the diet did not modify the food intake significantly, neither in normal nor in hyperthyroid rats. It is obvious, therefore, that the depressing effect of dietary cholesterol on the metabolic rate can not be attributed to a decrease in food intake.

Liver weights were found increased as a consequence of both thyroid and cholesterol administration, group T + C showing the highest values. Thyroid hormone, but not cholesterol, caused significant enlargement of the kidneys and adrenals also. These changes were to be expected.

Although total cholesterol concentrations of the plasma were not much modified, the level of liver cholesterol was markedly increased by cholesterol feeding, regardless of the thyroid content of the diet (Table II).

Discussion. Although cholesterol feeding significantly reduced the oxygen consumption of the hyperthyroid rats, the decrease amounted to only about 10% and was not sufficient for the metabolic rate to approach normal levels. The recently reported protective action of cholesterol as regards the survival time of thyrotoxic rats is much more pronounced;[1,2] it is questionable, therefore, whether this effect on the survival time is based on a mechanism related to the meta-

[4] Mason, G. D., and Winzler, R. J., to be published. The authors are much obliged to Dr. Winzler for the privilege of using this apparatus.

[5] To be published.

bolic rate.

The results reported here may be related, however, to observations of Saegesser,[6] who reported a depression by cholesterol of thyroxine effects on the rate of metamorphosis of tadpoles, and on the oxygen requirement of rats in the oxygen deficiency test ("Sauerstoffmangelversuch") of Asher and Streuli.[7]

[6] Saegesser, M., *Klin. Wochenschr.*, 1933, **12**, 672.

Summary. Dietary cholesterol significantly decreased the oxygen consumption of thyroid-fed rats, but the reduction was not sufficient for the metabolic rate to approach normal levels. This effect of cholesterol cannot be attributed to a depression of the food intake.

[7] Asher, L., and Streuli, H., *Biochem. Z.*, 1918, **87**, 359.

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17181. Calcification of Cell Cultures in the Presence of Embryo Juice and Mammalian Sera.

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Upon undertaking to learn if leprosy bacilli would multiply in cells cultured from leprous lesions, the mistake was made of trying to use the serum and chick embryo juice in proportions suitable for the cultivation of chick tissues. Following the first or second renewal of a liquid phase composed of human serum 25% and chick embryo juice 15%, the central explants or transplants became opaque to transmitted light and a brownish precipitate accumulated in the surrounding plasma. Once this happened a further renewal of the same type of medium caused a dense precipitate to appear among the growing cells, inhibiting growth and usually resulting in loss of the cultures. In the meantime the central fragments have acquired, by reflected light, a whitish color due to the accumulation of bone salts, and could be heard to crunch when cut with a knife. Though the phenomenon described may have been an extreme example of rapid calcification in cell cultures, similar difficulties have plagued other investigators. Earle[1] reported trouble with opaque plasma (in this case in areas removed from the colony sites) during the cultivation of Walker's rat carcinoma in horse serum and chick embryo juice. Gey and Gey[2] reported unfavorably on the use of chick embryo juice with human cord serum and later stated[3] that the difficulty was associated with turbidity in the plasma. Parker[4] reports that "these precipitates have been encountered in many laboratories and in every sort of culture. In Fischer's laboratory, in Berlin-Dahlem, where there was a lot of it at one time, the general feeling developed that it was a calcium compound and related in some way to the Tyrode's solution that was being used."

Since chick embryos were the only source of tissue extract available at Culion, the factors causing calcification were studied and means developed for avoiding or minimizing these difficulties in the presence of mammalian sera.

Methods. Cultures were prepared as previously described,[5] except that 2% embryo juice was used to transfer the 1.5 mm explants of human skin, leproma, or divided tissue culture to the 13 mm tubes containing one drop of chicken plasma 50%. After adding 0.5 ml of liquid phase and equilibration under 30 mm (4%) CO_2, the tubes were incubated in a

* The greater part of the work was done in the Leonard Wood Memorial Laboratory, Culion, Philippines, 1941-43.

[1] Earle, W. R., *Am. J. Cancer*, 1931, **24**, 566.

[2] Gey, G. O., and Gey, M. K., *Am. J. Cancer*, 1936, **27**, 45.

[3] Gey, G. O., personal communication.

[4] Parker, R. C., personal communication.

[5] Hanks, J. H., *J. Cell. and Comp. Physiol.*, 1948, **31**, 235.