Some Hormonal Effects on Phosphorylation in the Liver of Rats.* (20555)

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The ratio of phospholipide and nucleoprotein synthesis in the liver of rats appears to be rather constant in animals maintained on various experimental diets, irrespective of the fat or protein content(1). There appears to be a constant relationship of the synthesis of phospholipides to that of the nucleoproteins, whether or not the liver is fatty, fibrosed or normal(1). This constant rate of synthesis of liver phospholipides appears changed very little by dietary intake, but may be influenced by metabolic requirements of the body. The thyroid gland plays a definite role in phospholipide metabolism(2). In experimental animals, thyroxine increases the rate of synthesis in the liver which reflects a similar increase in the plasma(3). Thiouracil and thiourea diminish the phospholipide turnover(3,4). The growth hormone produces an increase in the turnover of liver phospholipides in normal or in hypophysectomized rats(5). In view of the above findings, experiments were undertaken to ascertain the effects of various hormones on phospholipide and nucleoprotein synthesis in the liver.

Methods. Male, albino rats (100-110 g) were divided into 3 groups, with a control for each group maintained on Diet 29(6). *Group 1.* A total of 37 animals received Diet 29(6) for 3 weeks. The diet for 16 of these was supplemented with 1% thiourea. *Group 2.* Eleven animals were maintained on Diet 29(6), fasted 24 hours and received an intraperitoneal injection of 5 mg of "beef thyrotropin" in saline (fat mobilization fraction of the pituitary)† and 13 controls received physiological saline. *Group 3.* Four animals were maintained on Diet 29(6) and received daily intraperitoneal injections of 0.20 mg of pure growth hormone for 10 days and 3 controls received physiological saline. Radioactive phosphorus was administered intraperitoneally as inorganic phosphate, containing 2-4 μc of P^{32} and after 6 hours the animals were killed by decapitation. The liver was removed, rapidly weighed and, in most cases, divided into equal portions for analyses. The acid-soluble phosphorus was removed by extracting with 10% trichloroacetic acid (TCA) containing 0.4 M $MgCl_2$(8). The lipides were extracted from the TCA insoluble residue with alcohol and alcohol-ether and purified with chloroform(9). On aliquots of the chloroform solution, the weight of the lipides, the radioactivity(9), and phosphorus(10) were determined. After the solvent extraction (TCA, alcohol-ether), the liver residue was digested for 3 hours at 90°C in one normal NaOH to determine total protein-bound phosphorus (11). This fraction contained both the nucleoprotein and phosphoprotein(11,12). Phosphoprotein is only a minor component of animal tissue(12). On aliquots, the total phosphorus(10), nitrogen(13), and radioactivity were determined. From these results and the radioactivity measurements, the relative specific activities‡ of the phospholipides and nucleoproteins, and the ratio of phospholipide phosphorus to nitrogen were calculated.

Under the conditions of our experiment, an increase in the formation of phospholipides and nucleoproteins may reasonably be

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† This fat mobilization fraction was assayed using the 7 hr mouse method of Payne(7). Twelve mice receiving saline, 0.125 mg and 0.500 mg of beef thyrotropin showed respectively 4.6, 5.6 and 11.8% of fat in the liver. (Courtesy of Dr. S. L. Steelman, Armour Laboratories, Chicago).

‡ The relative specific activity is the ratio of the specific activities of the phospholipide P or nucleoprotein P to that of the acid-soluble P.

TABLE I. Effect of Thiourea, Thyrotropin (Pituitary Fat Mobilization Factor) and Growth Hormone on the Phospholipide and "Nucleoprotein" Synthesis in the Liver of Rats.

Exp. condition		No. of Samples	Total lipides, g	R.S.A.* Phospholipide P (Per g of fat free tissue)		R.S.A.* Nucleoprotein P	Phospholipide P / Nitrogen	R.S.A.* phospholipide P / R.S.A. nucleoprotein P
Thiourea								
Controls		22		.147 ±.024	—	.062 ±.013	.037 ±.004	2.454 ±.519
Thiourea		31		.090 ±.019	—	.051 ±.010	.034 ±.007	1.764 ±.276
Difference between means	*t*			9.605	—	3.549	1.764	6.269
	P			P <.01		P <.01	P >.05	P <.01
Thyrotropin								
Controls		25	.55 ±.10	.212 ±.020	.038 ±.006	.073 ±.012	.033 ±.003	2.954 ±.402
Thyrotropin		22	.83 ±.20	.205 ±.028	.036 ±.006	.084 ±.017	.036 ±.003	2.484 ±.289
Difference between means	*t*		6.01	.962	1.148	2.602	3.440	4.551
	P		P <.01	P >.05	P >.05	P <.05	P <.01	P <.01
Growth hormone								
Controls		6	.58 ±.06	.126 ±.012	.022 ±.002	.060 ±.008	.031 ±.003	2.127 ±.202
Growth hormone		8	.56 ±.07	.165 ±.016	.027 ±.003	.075 ±.007	.027 ±.002	2.217 ±.158
Difference between means	*t*		.58	4.824	4.142	3.747	3.314	.938
	P		P >.05	P <.01	P <.01	P <.01	P <.01	P >.05

* Relative specific activity.

Figures preceded by the ± sign indicate stand. dev. *t* is the test of significance applied to difference between the means. P is probability for chance occurrence of this difference.

assumed when higher values for the relative specific activities are found. Various factors, such as differences in the rates of absorption and in the equilibrium between intra- and extra-cellular specific activities of the inorganic phosphorus, may conceivably produce changes in the specific activity of the inorganic fraction. Comparisons of the relative specific activities should provide a means of reducing these causes of error.

Results and discussion. The data obtained from the present investigation concerning some hormonal factors which influence phospholipide and nucleoprotein metabolism, phosphorus, and nitrogen distribution in the 3 groups of animals are reported in Table I. To evaluate the statistical significance of the results, the *t* test of significance(14) was applied to the difference between the means of the results from each group.

On feeding thiourea, statistically significant decreases between fed animals and controls were noted when the relative specific activities (ratio of specific activity of phospholipide P or nucleoprotein P, respectively, to specific activity of acid-soluble P) of phospholipide or nucleoprotein fractions of the 2 groups were compared. However, if the ratio of relative specific activity of the phospholipide to relative specific activity of nucleoprotein is compared in the thiourea-fed animals as against the controls, it turns out that the ratio is lower in the thiourea-fed animals. This would indicate that phospholipide synthesis in the liver of animals fed thiourea, as evidenced by the P^{32} incorporation, is inhibited more than is nucleoprotein synthesis. If the synthesis of both fractions were inhibited equally, although P^{32} incorporation in the experimental animals would be reduced, the ratios of relative specific activity of phospholipides to relative specific activity of nucleoproteins would be the same in the 2 groups of animals.

It is of interest that this ratio of synthesis of phospholipides to nucleoproteins is constant in the liver, irrespective of dietary fat or protein content and is not influenced by the presence or absence of fat in the liver(1). Campbell and Kosterlitz(15) have demonstrated in the rat that the ratio of liver protein nitrogen to phospholipides remains approximately constant on various levels of dietary proteins. In the present studies, it has been found that with administration of the pituitary fat mobilization hormone (beef thyrotropin) there was a 51% increase of mobilization of the fat in the liver in 6 hours. This increase in total lipides was statistically significant ($P < 0.01$). However, there was no change in the phospholipide synthesis. Pituitary fat mobilization hormone apparently does effect nucleoprotein synthesis, for there was an increase in the relative specific activities of the nucleoproteins.

The growth hormone, as shown in Table I, fails to affect the total lipide concentration in the liver, but stimulates both phospholipide and nucleoprotein synthesis ($P < 0.01$). This effect of growth hormone on lipide phosphorylation is in agreement with that of Geschwind *et al.*(5).

Summary. 1. Thiourea diminished phospholipide and nucleoprotein synthesis in the liver of rats. 2. The fat mobilization fraction of the pituitary gave a 51% increase in total lipides in the liver of rats in 6 hours. However, there was no change in the synthesis of phospholipides, but a statistical increase in the synthesis of nucleoproteins. 3. The Growth Hormone increased both phospholipide and nucleoprotein synthesis in the liver of rats.

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Further Studies of Metabolic Removal of Alkyl Groups from Nitrogen in Barbituric Acid Derivatives.* (20556)

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In an earlier study(1) of the metabolic fate of N-substituted derivatives of barbital, it was found that when N-methyl barbital or N-ethyl barbital is administered to dogs, a considerable amount of barbital is excreted in the urine. A trace of pure barbital was recovered from the urine of one dog receiving N-*n*-propyl barbital; from another one none could be isolated. The isopropyl, allyl, *n*-butyl, and phenyl derivatives of barbital

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