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Relation Between P-Aminohippuric Acid Synthesis and Amino Acid Incorporation into Protein.* (23041)

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Systems carrying out synthesis of "peptidic" links, as in formation of hippuric acid, glutamine, glutathione, and pantothenic acid, are considered useful models for the study of peptide formation because of their accessibility and relative simplicity. These reactions have yielded useful information about possible steps leading to peptide bond formation and have provided a basis for a working hypothesis of protein biosynthesis(1). The 4 "model" systems have at least two things in common; namely, carboxyl activation and utilization of energy of one of the pyrophosphate linkages of ATP. It appeared worth while to determine whether a change in endocrine environment affecting peptide synthesis would influence protein formation in a qualitatively similar fashion. To answer this question it was decided to study the "PAH synthetase" system described by Cohen and McGilvery(2-4) while simultaneously studying the rate of protein synthesis. Response of each of these parameters to an evoked change in one of them provides the basis of this report. Measurement of rate of uptake of isotopically labelled amino acids into the protein fraction was chosen as the means of assessing rate of protein synthesis.

Methods. Studies were confined to liver slices from rats in the "hyperthyroid" and "hypothyroid" state. Males of the Sprague-Dawley strain were used. Maintenance on a diet containing 0.5% thyroid powder for 5 weeks was sufficient to produce hyperthyroidism in rats weighing 50-60 g at the outset. Similarly, thyroid function was suppressed significantly by a diet containing 0.05% propylthiouracil fed for 5 weeks. Degree of thyroid dysfunction was gauged by resting metabolic rate (determined as described in 5). From 60-75 mg wet weight of tissue slice was added to each incubation flask, containing 0.001 M PAB and 0.01 M glycine in phosphate-buffered Krebs-Ringer solution. Para-aminohippuric acid (PAH) was determined colorimetrically by the Bratton and Marshall procedure after differential extraction of PAB from PAH according to the method of Cohen and McGilvery(2). PAH synthetase activity is expressed as γ of PAH formed/mg dry weight of tissue/3 hours. In isotope experiments, uptake of glycine-1-C^{14} and lysine-2-C^{14} into the protein fraction of liver slices was determined as follows: Tissue slices (60-75 mg wet weight) were incubated at 38°C for 1 and 2 hours in phosphate-buffered Krebs-Ringer solution containing 0.001 M

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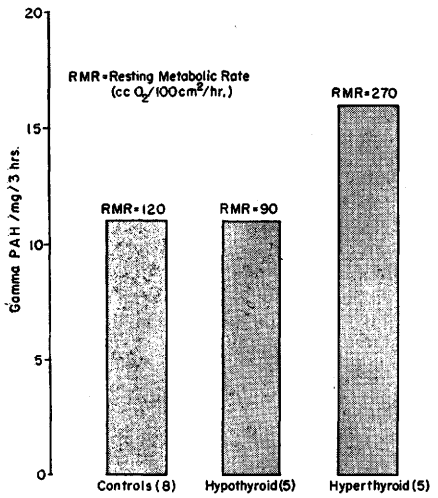

FIG. 1. Synthesis of PAH by rat liver slices. No. in parentheses indicate No. of animals in each group. Details pertaining to individual groups are given in text.

PAH and 0.01 M glycine or lysine. Duplicate flasks were used. The reaction was stopped by addition of trichloroacetic acid (TCA). The slices were then homogenized in 10% TCA solution containing unlabelled glycine or lysine. After centrifuging and washing 4 times with TCA wash, the lipids were removed by extracting twice at 50-55°C with a 3:1 alcohol-ether mixture. The preparations were plated on aluminum planchets and activity determined by counting in a windowless flow counter. Results, corrected to infinite thinness, are expressed as counts/minute/mg protein.

Results. The influence of thyroid hormone on the rate of PAH synthesis is shown in Fig. 1. Slices from control animals synthesized an average of 12 γ of PAH/mg dry weight/3 hours incubation. Propylthiouracil feeding, although depressing metabolic rate significantly, had no influence on rate of synthesis. On the other hand, hyperthyroidism, induced by feeding thyroid powder, not only increased the resting metabolism but provoked a 40% increase in rate of PAH synthesis.

Table I contains data of an experiment testing PAH synthesis simultaneously with rate of incorporation of labelled glycine and lysine into the protein of liver slices from normal and hyperthyroid rats. *Total* radioactivity in each flask containing glycine was approximately 4 million counts/minute in total volume of 4 cc. In the case of lysine, *total* activity (associated with 1-lysine) in each flask was approximately 2 million counts/minute.

It is seen that though hyperthyroidism is associated with an increase in synthetase activity, there is not a corresponding alteration of the degree of amino acid uptake into protein.

Rate of uptake of lysine varied less from slice to slice than did uptake of glycine. After one hour incubation with lysine, average counts/minute/mg of protein were in the narrow range of 16.9 to 18.0 in slices from both normal and hyperthyroid animals.

Protein synthesis (as measured by uptake studies) and formation of the peptidic bond of PAH appear to be dissociated phenomena. This in no way detracts from the usefulness of such model reactions in studying mechanisms involved in peptide synthesis.

Summary. Activity of the enzyme system concerned with synthesis of the peptidic link in p-aminohippuric acid was studied simul-

TABLE I. Incorporation of Labelled Amino Acids into Protein of Liver Slices from Normal and Hyperthyroid Rats.

	Degree of incorporation (avg counts/min./mg protein)					
	Glycine-1-C^{14}		Lysine-2-C^{14}			
	Incubation time					
Animal	1 hr	2 hr	1 hr	2 hr	PAH synthesis/mg dry wt/3 hr	Resting metabolic rate, cc O_2/100 cm^2/hr
Normal	63.7	68.0	18.0	24.4	11.3	110
"	61.4	118.1	17.1	23.9	13.7	121
Hyperthyroid	55.6	77.9	16.9	22.9	17.1	266
"	42.0	90.4	17.1	16.2	17.0	326

taneously with rate of uptake of isotopically labelled glycine and lysine into the protein of rat liver slices. The synthetase system increased by 40% in the hyperthyroid state. Incorporation of labelled amino acids, on the other hand, was uninfluenced in the same situation.

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Restraint Hypothermia and I^{131} Uptake by Rat Thyroid.* (23042)

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Oxygen consumption in rats restrained in the cold is initially higher than in nonrestrained animals at the same environmental temperature. Soon, however, both body temperature and oxygen consumption fall in restrained animals while they remain unchanged in nonrestrained controls(1). Since not all energy expenditure in muscle activity appears as heat, the initially higher oxygen consumption in the restrained, struggling animals need not represent an increased heat production if the basal heat production was so lowered that the total heat production was inadequate for maintaining normothermia. In point of fact, cold and restraint lower liver nonprotein sulfhydryl compounds(2-4) which might indicate some defect in intermediary metabolism and a consequently lowered basal heat production. None of these data, however, clearly distinguish whether the falling metabolism is the cause or the effect of the hypothermia. Since rate of iodine uptake is believed to reflect metabolic activity of the thyroid gland, chief regulator of basal body metabolism, the present experiments on thyroid uptake of I^{131} were undertaken to explore further the effect of restraint in the cold on basal metabolism.

Methods. In studying rate of iodine uptake by the thyroid as affected by restraint and cold, 175-225 g adult male Sprague-Dawley rats were injected at zero hours with 5 μc I^{131} (as KI), immediately divided into 2 groups (one of which was restrained in loose-fitting wire mesh cylinders, flattened on the underside and closed at the ends) and put into a cold room (5° ± 2°C). Then at 1, 2 and 4 hours equal numbers of restrained and nonrestrained animals were killed with ether and their thyroids removed to glass micro slides and air dried overnight. I^{131} activity of the dried glands was determined with a scintillation counter from 10 one-minute (later one 3-minute) counts per gland pair. This was usually done on the day following death, but in any case the measurement was corrected for background (avg 255 cpm) and for decay from zero time. Six animals in each of the 6 groups were used on each of 10 days, making a total of 360 rats. In many of the 180 restrained animals colonic temperature was also recorded at intervals during the 4 hour period. To ascertain the relation between body temperature and thyroid iodine uptake in restrained and nonrestrained animals, thyroid I^{131} activity in 25 of the 4-hour restrained rats (average colonic temperature 17.8°C) was compared with that in 25 extra injected nonrestrained controls in which the

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