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Effects of Thyroxine, Thyroidectomy and Lowered Environmental Temperature on Incorporation of Deuterium into Cholesterol.* (20290)

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The effects of thyroid- and thiouracil-feeding on the rate of incorporation of heavy hydrogen isotopes into cholesterol were studied in the rat by Karp and Stetten(1), and by Byers and coworkers(2). However, since Fleischmann and coworkers demonstrated recently that α-naphthylthiourea influences cholesterol metabolism not only by way of the thyroid gland, but also by another mechanism, completely independent of the thyroid (3), the interpretation of the above mentioned results presents some difficulties in the case of the hypothyroid groups. It was decided, therefore, to produce a hypothyroid condition in rats by means of thyroidectomy, rather than using anti-thyroid drugs, and to study the rate of incorporation of deuterium into cholesterol in these animals, and, for comparison, in hyperthyroid rats. The results are reported in the present paper.

In the course of these experiments, another difficulty in interpretation arose, *i.e.,* whether the effects observed in the hyperthyroid group were the result of a direct and specific action of the thyroid hormone, or, whether they were solely the consequence of an increase in the metabolic rate in general. Attempts were made to obtain information on this question by means of using animals in which the metabolic rate was increased independently of the thyroid gland; this was accomplished by exposing thyroidectomized rats to a lower environmental temperature in accord with observations reported by Ring(4), and Sellers and You(5). The present report includes results of experiments in which the rate of incorporation of deuterium into cholesterol was compared in thyroidectomized rats kept at 2-5°C, and at 25-30°C.

Methods. Female weanling rats, USC strain, were fed *ad libitum* a diet low in cholesterol.‡ Littermates were used as far as possible. In the first experiment which dealt with the effects of modifications of the thyroid activity, 3 groups of rats were used: One group was injected intraperitoneally with .05 mg thyroxine daily for a period of 3 weeks. Another group was thyroidectomized at an age of 4 weeks, and maintained for 2 weeks to deplete circulating thyroid hormone. Untreated animals of the same age served as

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‡ Composition of diet: 33.8% oats; 33.7% whole wheat; 15.0% non-fat milk powder; 4.5% alfalfa; 2.0% yeast (strain G, Anheuser-Busch); 8.0% cottonseed oil; 2.0% fortified oil (containing 312 U.S.P. units vitamin A and 50 U.S.P. units vit. D per g); 0.5% calcium carbonate: 0.5% sodium chloride.

TABLE I. Effects of Thyroxine and Thyroidectomy on Incorporation of Deuterium into Cholesterol in the Rat.

Group	No. of rats	Mean deuterium content of body water, atom % excess	Mean deuterium content of tissue cholesterol as % of deuterium content of body water (atom % excess)*			
			Liver	Intestine	Kidneys	Spleen
Thyroxine	15	1.51 ± .04	36.6 ± 1.2	42.2	24.4	17.5
Control	13	1.59 ± .04	27.7 ± 1.7	31.5	17.4	11.6
Thyroidectomized	5	1.79 ± .19	22.9 ± 1.0	—	—	—

* Liver samples were determined individually, samples of other organs were pooled.

controls. Deuterium oxide was administered to all groups during the last 4 days of the experimental period as follows: One initial injection of 25% v/v deuterium oxide was given intraperitoneally at a level of 3.9 ml per 100 g body weight, to bring the deuterium content of the body fluids to 1.5 atom % excess; for the remaining 4 days, deuterium oxide was furnished with the drinking water at a concentration of 2.5% (v/v). In the second experiment which concerned the effects of a lowering of the environmental temperature, 2 groups of female rats were thyroidectomized at 4 weeks of age and then maintained for 2 weeks without any treatment, as mentioned before. Deuterium oxide was then administered as described above, except that the period of administration was extended to 6 days. During this period, one of the groups was kept in the cold room, at a temperature of 2-5°C. The other group remained at room temperature which varied between 25 and 30°C. No thyroxine was applied to these animals. In all experiments, the animals were approximately 7 weeks old, when sacrificed. At that time, the livers, and, in some of the experiments, the intestines, kidneys, spleens and lungs were excised. In the case of the thyroidectomized animals, the region of the neck was carefully checked for completeness of the operation. All tissues were minced and extracted first with ethylalcohol, then with ethylether, using Soxhlet extractors. The solvents of the extracts were removed by evaporation, and the lipid residues were hydrolyzed by refluxing for 30 minutes in 50 ml acetone-alcohol 1:1, containing 6 ml of 33% w/v KOH. After neutralization, the total cholesterol content was determined in most of the samples in a small aliquot, using a modified Schoenheimer-Sperry procedure(6). The remaining cholesterol was precipitated as the digitonide, the precipitate washed with water and ethylether, and then dried in a vacuum desiccator. The cholesterol digitonide was combusted, and the collected water was reduced over zinc at about 400°C to a gas mixture containing hydrogen and deuterium as reported recently from this laboratory(7). The deuterium content of the gas mixture was determined using a Nier-type isotope ratio mass spectrometer (Consolidated Engineering Corp.). The body water samples, obtained from the carcasses by vacuum distillation, were purified by distillation over alkaline potassium permanganate. The deuterium content of these samples was measured as described above.

Results and discussion. The rats treated with thyroxine exhibited typical symptoms of hyperthyroidism such as an increased physical activity, tachycardia, an increased respiratory rate, and an increased food and water intake and fecal excretion; the thyroxine level was not high enough, however, to influence significantly the body weight gain. The thyroidectomized rats showed somewhat less pronounced changes in the opposite direction. The total cholesterol content of the organs examined was not significantly altered in the experimental groups, in agreement with observations by Karp and Stetten(1).

The results of the first experiment which concerned the effects of thyroxine and thyroidectomy on the incorporation of deuterium into cholesterol, are summarized in Table I. The data show that the deuterium content of the liver cholesterol was significantly higher in the hyperthyroid group, and significantly lower in the thyroidectomized animals, than in the controls. The same trend was observed for the intestine, kidneys and spleen. The

values for the lungs showed practically no difference (not included in Table I). Computations of standard errors were not possible in the case of the latter organs, as the determinations were made on pooled samples. Since the deuterium content of the body fluids was practically the same in the 3 groups, within the limits of error of the procedure, the data were interpreted to indicate that the thyroid hormone stimulated the incorporation of deuterium into cholesterol in the liver, intestine, kidneys and spleen. These findings are essentially in agreement with observations made by Karp and Stetten on liver and carcass cholesterol(1), and by Byers and co-workers on cholesterol extracted from pooled viscera(2). These authors used different technics, however, to alter the thyroid activities of their experimental animals, *i.e.*, feeding of desiccated thyroid and thiouracil, respectively. As mentioned above, difficulties in interpretation arise, if data concerning cholesterol metabolism are obtained from rats treated with anti-thyroid drugs, since Fleischmann and co-workers showed that α-naphthylthiourea influences the metabolism of the sterol even in the absence of the thyroid gland(3). In the present work, these difficulties were avoided, since no sulfa drugs were used, and the observations made on the hypothyroid group can be related unequivocally to a thyroid deficiency.

A further difference in experimental conditions concerns the food and water intake of the experimental animals. In the experiments presented above, the rats had free access to food and water during the entire experimental period. Byers and coworkers, on the other hand, observed the incorporation of tritium into cholesterol under fasting conditions (both food and water were withheld).

The question, whether these results can be interpreted to indicate that thyroid hormone stimulates cholesterol synthesis or turnover, cannot be answered unequivocally until some other possibilities are ruled out. It is not known, *e.g.*, whether a hyperthyroid condition makes possible an exchange reaction between carbon-bound hydrogen and deuterium,§ or, whether thyroid causes primarily an increase in the deuterium content of smaller molecules which serve as precursors of cholesterol(1). However, Byers and coworkers concluded that thyroid stimulated the rate of cholesterol synthesis, not only on the basis of the reported observations on the incorporation of tritium into cholesterol(2), but also, because additional evidence along these lines was obtained from experiments on the relation of thyroid to biliary cholesterol excretion(9). Thus, the results presented above, when considered in conjunction with these reports, might be interpreted to indicate that thyroid stimulated the rate of synthesis or turnover of cholesterol.

Another difficulty in interpretation concerns the nature of the causative mechanism responsible for the observed changes in the rate of deuterium incorporation, *i.e.*, the question, whether the effects found were the result of a specific and direct action of the thyroid hormone, or whether they were solely the consequence of an increase in the metabolic rate in general. In order to obtain some information on this problem, it was decided to alter the metabolic rate independently of the thyroid gland, and to study the effects of this alteration on the incorporation of deuterium into cholesterol. Ring(4), and Sellers and You(5) have demonstrated that the metabolic rate is significantly increased, when an animal is exposed to a lower environmental temperature, even in the absence of the thyroid gland. Therefore, the incorporation of deuterium into cholesterol was compared, in the following experiment, in thyroidectomized rats exposed to an environmental temperature of about 2-5°C, and in those kept at about 25-30°C. The results summarized in Table II indicate that,

TABLE II. Effect of Environmental Temperature on Incorporation of Deuterium into Liver Cholesterol in Thyroidectomized Rats.

Environmental temp., °C	No. of rats	Mean deuterium content of body water, atom % excess	Mean deuterium content of liver cholesterol as % of deuterium content of body water (atom % excess)
2– 5	9	1.52 ± .14	29.3 ± 3.1
25–30	6	1.48 ± .12	29.1 ± 1.9

§ According to Schoenheimer and Rittenberg, carbon-bound hydrogen is "stably bound", excepting those hydrogen atoms which are attached to a carbon atom adjacent to a keto group(8).

in the absence of the thyroid, a lowering of the environmental temperature did not modify the deuterium content of the liver cholesterol under the conditions of this experiment. These data were interpreted to indicate that an increase in the metabolic rate alone did not influence the incorporation of deuterium into cholesterol in the liver. It was concluded, therefore, that the phenomenon observed in the hyperthyroid rats as reported above was similarly not mediated primarily by an increase in the metabolic rate, but that it was very likely a direct consequence of the increased thyroid activity.

Summary. 1. The incorporation of deuterium into cholesterol was studied in rats injected with thyroxine, in normal rats and in thyroidectomized rats. 2. It was observed that thyroxine stimulated, and thyroidectomy depressed, the incorporation of deuterium into cholesterol in the liver, intestine, kidneys and spleen, but not in the lungs. 3. In thyroidectomized rats, a lowering of the environmental temperature by approximately 20-28°C did not alter the rate of incorporation of deuterium into cholesterol in the liver.

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Response of Dogs to Intravenous Administration of Polyvinylpyrrolidone and Its Monomer.* (20291)

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Hecht and Weese(1) first reported the effective use of polyvinylpyrrolidone (PVP), a fractionated polymer of N-vinylpyrrolidone synthesized from acetylene and formaldehyde, as a plasma volume expander in clinical shock. At that time they also showed that the dog exhibits a sensitivity to PVP; this observation has been confirmed by several other investigators(2,3,4). Untoward reactions to PVP, used as a therapeutic agent in shock, have not been encountered either in man or other species of laboratory animals(1,4-8). During a survey of the more recent colloidal solutions recommended for use in clinical shock, the authors found that PVP was inferior to certain other colloids tested in dogs subjected to hemorrhagic shock; undoubtedly the peculiar canine reaction, in which hypotension and hemoconcentration are the primary manifestations, contributed to these results. Attempts to render the dog less sensitive to infusions of PVP by prior small injections(1,2) failed in our hands.

It was considered of interest to attempt to delineate the cause of the "canine reaction". In view of the use of cortisone in certain hypersensitive conditions and its positive effect in the rat after injection of dextran(9, 10) and human globin(10), this steroid was used to determine its effects on the reaction of the dog to PVP. Thus, the purpose of this investigation was two-fold: 1) to trace down the component of PVP solutions responsible

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