more of the various tissue (lysosomal) cathepsins(19) would appear to be more likely candidates as the protease(s) involved. The original insult responsible for initiating the release or activation of proteases is not defined in this postulation.

The able technical assistance of Miss Barbara Moyer is gratefully acknowledged. A grant from the National Foundation supported this investigation.

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Received September 2, 1964. P.S.E.B.M., 1964, v117.

Effects of Hypophysectomy, Thyroidectomy and Thyroid Hormones on Steroid Metabolism in the Rat.* (28683)

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In a preliminary study(1) we found that hypophysectomized rats eliminated accumulated C^{14}-cholesterol at a markedly slower rate than normals. Inability to eliminate absorbed sterol may be the reason that such rats accumulate cholesterol of exogenous origin more easily than normals(2,3). Since the hypophysectomized animal lacks a number of target gland hormones as well as pituitary hormones, these effects may be due to one or several of these substances. Recently Wells and Ershoff(3) have observed that thyroid hormone administration largely prevents accumulation of blood cholesterol in cholesterol-fed hypophysectomized rats.

In the present experiments, we compare rates of cholesterol-bile acid metabolism and excretion in normal, thyroidectomized, hypophysectomized, and thyroid hormone-treated-hypophysectomized rats.

Methods. Twenty, 6-month-old, female albino Sprague-Dawley rats were used in these studies: 4 were normal animals; 4 had been thyroidectomized at 3 months of age by intraperitoneal injection of 900 μc(4) of carrier-free I^{131}; and 12 had been hypophysectomized† at 3 months. Following thyroidectomy or hypophysectomy, weights and CO_2 production were checked periodically for the

* This work was supported in part by grants from Nat. Inst. Health (HE 05085-05) and Am. Heart Assn. (61 G 77).

† Hypophysectomized rats purchased from Endocrine Laboratories, Madison, Wisconsin.

3-month period intervening before the experiment. All animals were fed Rockland rat diet *ad lib*. Average weights at the beginning of the experiment were: normals, 254 g; thyroidectomized, 230 g; hypophysectomized, 185 g.

No I^{131}-activity could be detected in samples of feces excreted by thyroidectomized rats shortly before the experiment. Completeness of thyroidectomy was checked at the end of the experiment by macroscopic and microscopic examination of tissue from the region which includes the thyroid gland. No evidence of active thyroid tissue was found.

Three weeks before reaching 6 months of age, the diet of 8 of the hypophysectomized rats was supplemented with desiccated thyroid; 4 rats receiving 0.5 mg/day and 4 rats receiving 5.0 mg/day. This was continued throughout the experiment.

At the age of 6 months all animals (normals, thyroidectomized, hypophysectomized, and both groups of thyroid-treated-hypophysectomized rats) received a single intraperitoneal injection (7.219×10^6 counts/min, 2.26 mc/mM) of carrier-free dl-mevalonic acid-2-C^{14} dissolved in 1 ml of 0.9% NaCl solution. The animals were placed in metabolism cages and feces collected daily for 14 days. The rats were then killed by decapitation and samples of blood, liver, small intestine, lung and kidney removed. These tissues and the remaining carcass were assayed for cholesterol and cholesterol-C^{14} concentrations.

Serum cholesterol was determined by the method of Sperry and Webb(5); tissue cholesterol was extracted and determined as described earlier(6). Tissue β-sterol-C^{14} was precipitated as the digitonide, plated by the filtration tower technique, and counted. All C^{14}-activities were determined with a windowless gas flow counter.

Feces were dried by lyophilization immediately after collection. Fecal steroids were extracted and separated as previously described (1). $\alpha + \beta$-sterol-C^{14} and bile acid-C^{14} (contained in the "acidic fraction") were plated in cup planchets and counted.

Fecal cholesterol and coprostanol were determined by gas liquid chromatography. The petroleum ether fraction, containing fecal neutral sterols, was evaporated to dryness under nitrogen and samples acetylated with acetic anhydride and pyridine(7). After evaporation to dryness under nitrogen, the samples were diluted to 5 ml with acetone, and 5 μl injected on to the column, using a 10 μl Hamilton syringe. The 5 μl sample was preceded and followed by 1.5 μl of acetone to effect quantitative delivery and eliminate tailing due to injection. The column was a 6 ft, 1/8" I.D. glass U, packed with 1% SE-30 (General Electric) methyl silicone rubber gum, supported on 100-120 mesh Gas Chrom P. Column temperature was 218°C; detector temperature, 245°; injector temperature, 275°. The rate of argon carrier flow was 50 ml/min. An argon ionization detector was employed at a cell voltage of 1500. Peak areas were measured by planimetry. Standard curves for cholesterol and coprostanol were determined at the beginning of each day. A good separation of cholesterol from coprostanol was achieved by this method.

Results and discussion. Fig. 1 shows plots of accumulated excretions of bile acid-C^{14}, $\alpha + \beta$-sterol-C^{14} and total steroid-C^{14} over a 14-day period following injection of mevalonic acid-2-C^{14} in rats in various hormonal states. In comparison with normal rats, hypophysectomized had reduced total steroid-C^{14} and bile acid-C^{14} excretions. Excretion rate of $\alpha + \beta$-sterol-C^{14} was reduced by hypophysectomy in the early periods, but exceeded the normal rate later in the study. Cumulative $\alpha + \beta$-sterol-C^{14} excretion was not significantly different in the two groups. The upper section of Fig. 2 shows that on the 14th day of the experiment the specific activities of various tissues were much higher in hypophysectomized rats than in normals. Data taken from our previous study(1) show a similar distribution shortly after injection. If this is taken into consideration, it would appear likely that rate of sterol excretion in hypophysectomized rats is lower than in normals. This interpretation was confirmed by gas chromatographic analyses of the daily excretions of cholesterol and coprostanol (Table I). These data illustrate that curves of fecal C^{14}-excretion may be misleading

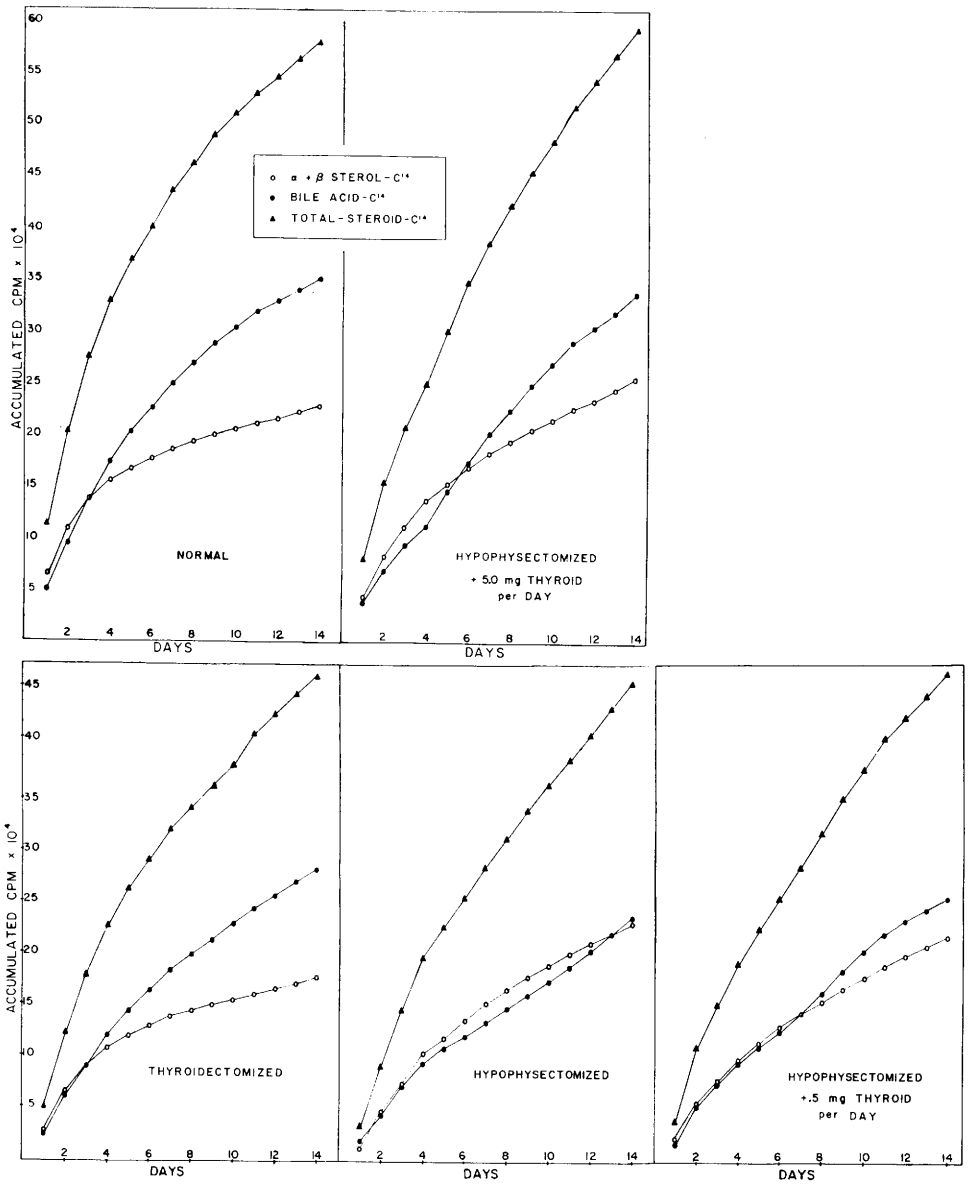

FIG. 1. Cumulative excretion of $\alpha + \beta$-sterol-C^{14}, bile acid-C^{14}, and total steroid-C^{14} in normal, hypophysectomized, thyroidectomized, and hypophysectomized-thyroid-treated rats, following injection of mevalonic acid-2-C^{14}.

when interpreted in terms of steroid excretion without further information as to tissue specific activities.

The specific activity of the β-sterol pool of liver, from which bile acids are synthesized, was higher in hypophysectomized than in normal rats (Fig. 2). Since the excretion rate of bile acid-C^{14} in hypophysectomized rats was considerably less than in normals, it follows that bile acid excretion must be strongly inhibited in hypophysectomized rats. Experiments are in progress to establish this point.

The total steroid-C^{14} synthesized in normals was greater than in hypophysectomized animals as determined by the sum of total steroid-C^{14} excreted plus total β-sterol-C^{14} remaining in tissues at the end of 14 days (Table I). In spite of this, more β-sterol-C^{14}

TABLE I. Sterol Synthesis and Excretion.

Group	Total steroid-C^{14} synthesized, counts/min	Feces excreted (dry wt), g/24 hr	Total sterol excretion, mg/24 hr	Coprostanol excreted, mg/24 hr	Cholesterol excreted, mg/24 hr	Coprostanol/ cholesterol ratio
Normal	6.00×10^6	$3.71 \pm .63$	9.95	$4.79 \pm .74$	$5.16 \pm .78$	.966
Thyroidectomized	4.47×10^6	$3.15 \pm .24$	7.21	$3.29 \pm .69$	$3.92 \pm .55$	.780
Hypophysectomized	4.45×10^6	$2.32 \pm .31$	5.53	$2.48 \pm .58$	$3.05 \pm .48$	.828
Hypophysectomized + .5 mg thyroid	4.53×10^6	$2.35 \pm .16$	4.89	$2.22 \pm .32$	$2.67 \pm .13$	.857
Hypophysectomized + 5 mg thyroid	5.64×10^6	$2.80 \pm .35$	5.96	$2.96 \pm .97$	$3.00 \pm .73$	1.12

remained in the tissues of hypophysectomized than normal rats after 14 days (Fig. 2) due to the lower rate of excretion in the fecal steroid fractions.

The effects of thyroidectomy on steroid metabolism differed from those of hypophysectomy. There was an equal reduction in output of $\alpha + \beta$-sterol-C^{14} and bile acid-C^{14} (Fig. 1) resulting in a pattern similar to the normal but reduced in magnitude. Fecal cholesterol and coprostanol excretion were reduced from normal (Table I) but not to the extent observed in hypophysectomized rats. Thyroidectomized animals incorporated less mevalonic acid-2-C^{14} (Table I) into tissue steroids than normal animals. The specific activity of the tissue β-sterol after 14 days (Fig. 2) was somewhat higher than in normal rats, due to the slower steroid-C^{14} excretion rate.

Since the effects of thyroidectomy differed significantly from those of hypophysectomy, it was of interest to study the effects of different levels of thyroid hormone on steroid metabolism in hypophysectomized rats. When 0.5 mg/day of desiccated thyroid was fed to hypophysectomized rats, there was a slight decrease in cholesterol plus coprostanol excretion (Table I). Bile acid-C^{14} excretion was slightly increased (Fig. 1), and after 14 days the specific activity of tissue β-sterol was somewhat reduced (Fig. 2). Thyroid at the 5.0 mg/day concentration slightly increased cholesterol plus coprostanol output (Table I). Total steroid-C^{14} excretion returned to near normal rates (Fig. 1). This resulted from the higher specific activity of tissue β-sterol (Fig. 2) and an increase in rate of bile acid-C^{14} excretion (Fig. 1). The higher specific activity resulted from an increased total steroid-C^{14} synthesis (Table I).

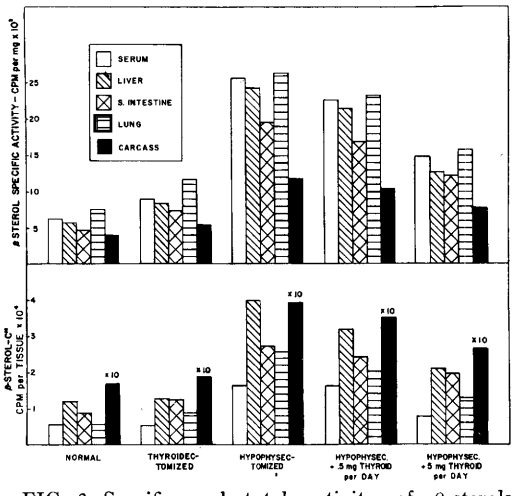

FIG. 2. Specific and total activity of β-sterols (cholesterol) in rat tissues 14 days after injection of mevalonic acid-2-C^{14}.

At both the 0.5 and 5.0 mg/day thyroid concentrations, more β-sterol-C^{14} remained in the tissues at 14 days than in normals (Fig. 2). It would seem that, while the lack of the thyroid hormone in the hypophysectomized state is important with respect to the changes in steroid metabolism in the rat, it is only one of the factors involved. It is possible that increasing the thyroid intake might have eliminated all differences from normal noted above. However, the value of using high thyroid intake levels in such studies is limited by development of thyrotoxicosis(8).

One of our more interesting findings is the large increase in rate of metabolism of β-sterol-C^{14} to bile acid-C^{14} effected by the thyroid hormone in the hypophysectomized rat (Fig. 1). Such an increase does not occur in normal rats treated similarly(9,10,11).

The differences in rate of β-sterol metabolism to bile acids in hypophysectomized as compared with normal rats could arise from changes in: (A) the feedback pathways; (B) the bile acid spectrum; (C) the efficiency of the bile acid-cholesterol feedback, which depends upon the rate at which the bile acids are absorbed and/or passed on to the large intestine. On the other hand, one of the primary effects of hypophysectomy on steroid metabolism might be reduction of the rate of β-sterol metabolism to bile acids. If any of these factors were restored to normal when hypophysectomized rats are treated with thyroid hormones, the observed increase in cholesterol metabolism could result. The rate of passage of the intestinal contents may be of considerable importance since the amount of feces excreted per day appears to be related to sterol turnover in these animals. The dry weights of feces excreted are shown in Table I. It should be noted however that the addition of 5.0 mg/day of desiccated thyroid did not restore the weight of feces excreted per day to normal.

Preliminary studies (unpub.) show that the rate of injected cholic acid-C^{14} excretion is retarded in hypophysectomized rats. This, combined with normal feedback, could be the primary factor limiting synthesis of bile acid-C^{14} from β-sterol-C^{14} in these rats. If these findings are confirmed, the understanding of the importance of cholesterol-bile acid feedback in regulating cholesterol metabolism in the rat will be enhanced.

Little is known concerning the control of the rates of excretion of fecal neutral sterols. These substances apparently arise from bacterial metabolism of sterols excreted via the bile and secreted by the wall of the small intestine(12,13). A number of factors, including dietary fat(14), dietary cholesterol (15), plant sterols(16), and body weight(17) affect the rate of neutral sterol excretion in the rat. Whether any of these is involved in the decreased rate of excretion seen in the hypophysectomized rat is uncertain. It has been suggested that the ratio of cholesterol to coprostanol in the excreted sterol might have an influence on the rate of sterol excretion, since coprostanol is less readily absorbed than cholesterol. This ratio was not significantly altered in the various groups (Table I). It is doubtful whether an altered ratio would affect excretion, since sterol absorption takes place in the proximal end of the small intestine, while coprostanol is synthesized by bacteria in the caecum and colon. Since hypophysectomy is known to decrease the rate of cholesterol synthesis from acetate(18) in the rat, the decreased rate of fecal sterol excretion may be directly related to this factor.

Summary. Steroid turnover in normal (N), hypophysectomized (H), I^{131}-thyroidectomized (T), and hypophysectomized-thyroid-treated (HT) rats was investigated. Following injection of dl-mevalonic acid-2-C^{14}, feces were collected for a 14-day period. When compared with normals, cumulative fecal bile acid-C^{14} excretion was reduced about 20% in H and T; while in HT, bile acid-C^{14} excretion approached normal rate. Cumulative fecal α + β-sterol-C^{14} excretion was reduced 28% in T, but was similar to normal in H and HT. Total fecal cholesterol plus coprostanol excretion was reduced in both H (—45%) and T (—25%). Thyroid hormone treatment of hypophysectomized rats resulted in a small increase in fecal sterol excretion, but the daily rate was still below normal (—30%). The total steroid-C^{14} synthesized from mevalonic acid-2-C^{14} was reduced from normal in both T and H. HT synthesized almost normal amounts of total steroid-C^{14}. It was concluded that lack of the thyroid hormone in hypophysectomized rats accounts for some of the effects of hypophysectomy on steroid metabolism. However other hormones must also be important, since the effects of thyroidectomy were not the same as those of hypophysectomy and thyroid administration did not restore some aspects of steroid metabolism to normal.

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Received September 2, 1964. P.S.E.B.M., 1964, v117.

Quantitative Assay of Endogenous Pyrogen in Serum.* (29684)

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Fever has long been used as a bioassay for endotoxin. To quantitate this procedure more precisely, Keene *et al* introduced the concept of the *minimum pyrogenic dose* (mpd) as a point of reference(1). These investigators observed that the amount of endotoxin tested correlated most closely with a fever index determined on the basis of the entire febrile response from time of injection to time of defervescence . Recent studies by Bornstein *et al*(2) demonstrated that when fever was produced with pyrogen derived from leukocytes, both the 1 and 2 hour fever indices, corresponding to the area beneath the curve during the first 1 or 2 hours after injection, correlated well with the number of cells from which the pyrogen was extracted. Kaiser and Wood(3) as well as Bornstein(2) found that the response of rabbits to graded doses of LP was relatively uniform and dose-dependent for individual recipients, allowing the derivation of a dose-response curve which was initially steep and linear, began to fall off with fever approximately 1°C in magnitude, and eventually reached a hyperthermic plateau at somewhat higher levels. Although this graded response was fairly uniform for individual animals, considerable variation was noted between different recipients.

The endogenous pyrogen (EP) found in the serum of febrile animals following a variety of stimuli is thought to be derived from injured leukocytes and to be biologically similar to LP(4). Preliminary experiments from this laboratory showed that the dose response curve for serum EP was similar to that described for leukocytic pyrogen(5). The studies to be described deal with the validity of the quantitative assay of EP in serum.

Materials and methods. All needles, glassware and instruments were sterilized in hot air ovens at 170°C for 3 hours. All serum and other injectables were cultured in thioglycollate broth for 72 hours and discarded unless sterile.

Male white rabbits of mixed breed weighing 1.4 to 4.0 kg were obtained from a single supplier and were housed in air-conditioned rooms. Rabbits used for pyrogen assay were of uniform weight and age, were handled gently and pre-trained in the assay situation for several days prior to use. All injections were made into the marginal ear vein which was shaved and cleansed immediately prior to injection. A minimum of 2 hours was allowed for acclimatization; animals whose temperatures fluctuated more than 0.2°C from the baseline were not used. Recipients

* Supported by grants from Nat. Inst. of Allergy and Infect. Dis., U.S.P.H.S.