incomplete extraction or to limited inactivation in the few seconds after progesterone was added and before cold acetone was added to stop the process. On the basis of *in vitro* results, it appears likely that inactivation of progesterone may also occur *in vivo* in the fetal mouse and that the liver may play a role in the process. In this connection, it is interesting to recall that blood returned from the placenta *via* the umbilical vein passes first to the liver.

Summary. Homogenates of whole fetal mouse of the 12th, 13th, and 14th days of pregnancy and of fetal mouse liver of the 15th and 16th days inactivate progesterone *in vitro*.

1. Forbes, T. R., Coulombre, A. J., Coulombre, J., *1st Internat. Cong. Endocrin.*, 1960, 715.
2. ———, *Endocrinology*, 1961, v68, 858.
3. Brown, F. U., Meyer, R. K., *Am. J. Physiol.*, 1958, v195, 179.
4. Pulkkinen, M., Lehtinen, A., Hartiala, K. J., *Ann. Med. Exp. et Biol. Fenniae*, 1961, v39, 57.
5. Hooker, C. W., Forbes, T. R., *Endocrinology*, 1947, v41, 158.
6. Forbes, T. R., *Brook Lodge Symposium on Progesterone*, 1961, 71.

Received December 18, 1961. P.S.E.B.M., 1962, v109.

Effects of Cholesterol Feeding on Plasma and Liver Cholesterol Levels in the Hypophysectomized Rat.* (27296)

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It is well established that hypothyroidism induced by thyroidectomy or administration of antithyroid drugs or I^{131} raises serum cholesterol levels(1-6) and results in a marked increment in serum cholesterol level over that of the euthyroid animal following cholesterol feeding(3,7-9). Since hypophysectomy removes the source of thyrotropin secretion, resulting thereby in hypofunctioning of the thyroid gland, comparable results might also be anticipated in the hypophysectomized animal. In the present communication data are presented on the effects of hypophysectomy on plasma and liver cholesterol levels of rats fed cholesterol-free and cholesterol-supplemented diets and the effects of desiccated thyroid feeding thereon.

Procedure. Eighty-eight hypophysectomized male rats (Sprague-Dawley strain) ranging from 93 to 134 g in body weight, and 42 intact male rats, which ranged from 123 to 143 g in body weight and were litter mates of the above, were selected for the present experiment. Both intact and hypophysectomized rats were divided into 4 comparable groups and were fed the following diets: Group I received a highly purified ration consisting of sucrose, 61%; casein,† 24%; cottonseed oil, 10%; salt mixture,‡ 5%; and the following vitamins/kg of diet: thiamine hydrochloride, 20 mg; riboflavin, 20 mg; pyridoxine hydrochloride, 20 mg; calcium pantothenate, 60 mg; nicotinic acid, 100 mg; ascorbic acid, 200 mg; biotin, 4 mg; folic acid, 10 mg; para-aminobenzoic acid, 400 mg; inositol, 800 mg; 2-methyl-1,4 naphthoquinone, 5 mg; Vit. B_{12}, 150 μg; choline chloride, 2 g; Vit. A, 5000 U.S.P. units; Vit. D_2, 500 U.S.P. units; and alpha-tocopheryl acetate, 100 mg. Group II was fed a diet similar to the above but supplemented with 1% cholesterol. Groups III and IV were fed diets similar respectively to those of Groups I and II but containing in addition 0.05% desiccated thyroid.§ The vitamins, cholesterol and desiccated thyroid were added in place of an equal amount of sucrose. The rats were placed in metal cages with raised screen bottoms (3

* This investigation was supported in part by a research grant from Nat. Heart Inst., P.H.S., N.I.H.

† Vitamin-Free Test Casein, General Biochemicals, Inc., Chagrin Falls, Ohio.

‡ Hubbell, Mendel and Wakeman Salt Mixture, General Biochemicals, Inc., Chagrin Falls, Ohio.

§ Thyroid Powder, U.S.P., Armour Laboratories, Chicago, Ill.

TABLE I. Effects of Cholesterol Feeding on Plasma and Liver Cholesterol and Liver Total Lipid Levels in Intact and Hypophysectomized Male Rats.

Dietary group	No. of animals	Body wt at sacrifice, g	Plasma cholesterol, mg/100 ml Free*	Plasma cholesterol, mg/100 ml Total*	Liver total lipid, %*	Liver cholesterol, mg/g Free*	Liver cholesterol, mg/g Total*
Intact rats—sacrificed after 15 days							
Basal ration	6	218.0	16.0 ± .7	74 ± 2	3.4 ±.2	1.51 ±.07	1.83 ± .06
" + 1% cholesterol	8	213.6	15.9 ± .9	105 ± 4	5.9 ±.3	2.22 ±.05	9.2 ±1.0
Hypophysectomized rats—sacrificed after 15 days							
Basal ration	10	111.5	37.1 ± 2.1	135 ± 3	3.5 ±.1	1.83 ±.10	2.27 ± .13
" + 1% cholesterol	10	108.4	115.0 ± 6.0	436 ±20	8.2 ±.3	3.31 ±.15	23.4 ±1.2
Intact rats—sacrificed after 30 days							
Basal ration	6	276.5	13.6 ± .7	75 ± 5	4.4 ±.1	1.62 ±.09	2.13 ± .03
" + 1% cholesterol	9	268.7	17.8 ± 1.7	98 ± 5	6.4 ±.3	2.33 ±.18	9.7 ±1.0
" + .05% thyroid	6	277.8	17.8 ± 1.1	88 ± 4	3.4 ±.1	1.69 ±.09	2.14 ± .08
" + 1% cholesterol + .05% thyroid	6	273.2	23.3 ± 2.9	116 ± 9	6.7 ±.7	2.29 ±.14	9.3 ±2.6
Hypophysectomized rats—sacrificed after 30 days							
Basal ration	15	115.5	35.4 ± 2.6	127 ± 4	4.7 ±.2	1.95 ±.12	2.34 ± .09
" + 1% cholesterol	15	117.2	126.0 ±11.0	507 ±36	11.3 ±.4	4.55 ±.21	39.5 ±2.1
" + .05% thyroid	11	124.5	25.3 ± 1.9	116 ± 6	3.6 ±.1	2.04 ±.06	2.56 ± .06
" + 1% cholesterol + .05% thyroid	14	124.9	37.6 ± 1.8	147 ± 3	7.8 ±.5	3.24 ±.14	23.8 ±2.8

* Including stand. error of mean.

animals per cage) and were provided the test diets and water *ad libitum*. Animals were fed on alternate days and all food not consumed 48 hours after feeding was discarded. Body weights were recorded weekly. Hypophysectomized rats that failed to plateau in body weight were discarded and not included in the experiment. Representative animals in Groups I and II in both the intact and hypophysectomized series were sacrificed after 15 days and all remaining animals in the experiment after 30 days of feeding.‖ At time of sacrifice, the rats were anesthetized with sodium pentobarbital, and blood was withdrawn from the aorta into a heparinized syringe. Livers were excised, blotted to remove excess blood, weighed and stored in a freezer until analyzed. Lipid was extracted from the livers by the method of Thompson *et al.*(10), and cholesterol was determined on liver and plasma by the method of Nieft and Deuel(11). Total lipids were determined gravimetrically on an aliquot of the liver extract.

Results. Intact rats fed cholesterol exhibited a mild increment in plasma cholesterol levels, a small but statistically significant increment in liver total lipid and a marked increment in total liver cholesterol (primarily in the esterified fraction). Hypophysectomized rats fed the cholesterol-free diet (Group I) did not differ significantly from intact rats on the same ration in respect to values for liver total lipid and total liver cholesterol but did exhibit a moderate increment in plasma cholesterol over that of intact rats. Hypophysectomized rats fed cholesterol (Group II), however, exhibited a highly significant increment in plasma cholesterol and a greater increment in liver total lipid and total liver cholesterol levels than occurred in intact rats fed the same diet. Similar findings were obtained in both the 15-day and 30-day series. Desiccated thyroid when fed at a 0.05% level had little, if any, effect on plasma and liver cholesterol and total liver lipid values in either intact rats on the cholesterol-free or cholesterol-containing diet or

‖ Ten hypophysectomized rats died during the experimental period and 3 were discarded due to gain in body weight. The remainder were sacrificed as indicated in Table I.

hypophysectomized rats on the cholesterol-free diet. It largely counteracted, however, the increment in plasma cholesterol and partially counteracted the increment in liver total lipid and liver cholesterol induced by cholesterol feeding in the hypophysectomized rat.

Discussion. Findings indicate that the elevation in plasma cholesterol levels induced by feeding a diet containing 1% cholesterol was significantly greater in hypophysectomized than intact rats. These findings are similar to those in the hypothyroid rat(8,9). Since hypophysectomy removed the source of thyrotropin secretion resulting thereby in a hypothyroid state, and since desiccated thyroid when administered at the small dosage of 0.05% of the diet largely counteracted the increment in plasma cholesterol levels induced by cholesterol feeding in the hypophysectomized rat, it would appear that the impaired cholesterol metabolism of the hypophysectomized rat was largely if not entirely due to the resulting thyroid insufficiency. Desiccated thyroid under conditions of the present experiment, however, did not reduce plasma cholesterol levels of hypophysectomized rats to that of their intact counterparts, suggesting that the absence of pituitary hormones other than thyrotropin may have contributed to the observed results, although the possibility that larger doses of thyroid may have been more effective in this regard was not eliminated. It would appear from present findings that the hypophysectomized rat might prove particularly useful for studying the effects of various agents on cholesterol metabolism, their interrelationships with one another, and the *modus operandi* of such effects. In the intact animal it is difficult to determine if the effects noted following administration of a test substance are a direct result of the material administered or whether they are mediated through the pituitary. This difficulty would obviously be circumvented by use of the hypophysectomized rat.

Summary. Intact and hypophysectomized male rats were fed a cholesterol-free and cholesterol-supplemented diet for 30 days and the effects of these rations determined on plasma and liver cholesterol and liver total lipid levels. On the cholesterol-free diet plasma cholesterol levels were moderately higher in hypophysectomized rats than in intact rats; no significant differences were observed between the 2 groups in liver cholesterol and liver total lipid values. On the cholesterol-supplemented diet, hypophysectomized rats exhibited a significantly higher increment in plasma and liver cholesterol and liver total lipids than occurred in intact rats. Desiccated thyroid when fed at a 0.05% level in the diet largely counteracted the increment in plasma cholesterol and partially counteracted the increment in liver cholesterol and liver total lipid induced by cholesterol feeding in the hypophysectomized rat.

1. Parhon, C. I., Derevici, H., *Compt. rend. soc. biol.*, 1926, v95, 787.

2. Hurxthal, L. M., *A.M.A. Arch. Int. Med.*, 1934, v53, 762.

3. Turner, K. B., Present, C. H., Bidwell, E. H., *J. Exp. Med.*, 1938, v67, 111.

4. Chaikoff, I. L., Entenman, C., Changus, G. W., Reichert, F. L., *Endocrinology*, 1941, v28, 797.

5. Fleischmann, W., Schumacker, H. B., *Bull. Johns Hopkins Hosp.*, 1942, v71, 195.

6. Boyd, G. S., Chapter in *Hormones and Atherosclerosis*, edited by G. Pincus, Academic Press, Inc., New York, 1959, p49.

7. Steiner, A., Kendall, F. E., *Arch. Path.*, 1946, v42, 433.

8. Horlick, L., Havel, L., *J. Lab. and Clin. Med.*, 1948, v33, 1029.

9. Page, I. H., Brown, H. B., *Circulation*, 1952, v6, 681.

10. Thompson, S. Y., Ganguly, J., Kon, S. K., *Brit. J. Nutrition*, 1949, v3, 50.

11. Nieft, M. L., Deuel, H. J., Jr., *J. Biol. Chem.*, 1949, v177, 143.

Received December 18, 1961. P.S.E.B.M., 1962, v109.