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Metabolism of Rat Pituitary Gonadotrophin.* (29216)

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Whether or not the pituitary gonadotrophins are actually metabolized by the gonadal tissue is still debatable(1,2,3,4,5). Some evidence, however, indicates that these hormones are actively removed from the circulation by the gonads(1). As this issue may have important bearing on the maintenance of a homeostatic relationship between gonads and pituitary, a critical reexamination of the role played by the former and/or any other tissues in metabolism of gonadotrophins, seems justified. Some aspects of this study have been reported(6).

Methods. Colony bred female albino rats of the Institute weighing 100-150 g served as the pituitary donors. Immature female mice (6-8 g) of a Swiss strain maintained in the Institute, were used as the test animals for assay of pituitary gonadotrophin. All of the animals were maintained under uniform laboratory conditions throughout the experimental period.

Liver damage was produced by subcutaneous injection of carbon tetrachloride (0.1 ml every alternate day for 20 days)(7). The macroscopic and microscopic examination of the liver of such animals showed severe necrosed condition. The thyroidectomized animals were sacrificed at least 60 days after operation to ensure the inactivation of any residual thyroxine in the body(8). A batch of thyroidectomized animals was subjected to thyroxine therapy (3 μg daily subcutaneously) for 14 days (Table III). For induction of hyperthyroidism the normal rats were injected with thyroxine (3 μg daily) in a similar manner for 14 days (Table IV). The histological and histometric examination of the thyroid of the latter group of animals revealed typical hypoplastic appearance, showing hyperthyroidism.

The pituitaries were put in chilled physiological saline solution immediately after collection. They were then dried between pieces of filter paper, weighed in a Roller-Smith balance, pooled, and then homogenized in chilled physiological saline solution so that 1 ml corresponded to 4 mg of wet tissue. The homogenate was incubated at 37°C for one hour either *per se* or in combination with homogenates of the ovary, liver, spleen and the diaphragm from the same donor animals (Table I). In experiments on the effect of

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TABLE I. Gonadotrophin Inactivating Capacity of Different Tissues.

Treatment	Mean uterine wt (mg) with S.E.
(a) Saline	5.68 ± .48 (8)*
(b) Pituitary	14.45 ± .88 (11)
(c) Ovary	4.33 ± .38 (4)
(d) Liver	5.98 ± .66 (4)
(e) Spleen	5.52 ± .56 (10)
(f) Diaphragm	5.45 ± .62 (10)
(g) Thyroxine	5.06 ± .35 (4)
(h) Pituitary + ovary	13.16 ±1.05 (5)
(i) " + liver	9.00 ± .53 (9)
(j) " + spleen	15.00 ± .78 (10)
(k) " + diaphragm	14.25 ± .62 (8)
(l) " + thyroxine (*in vitro*)	17.80 ± .62 (16)
(m) " + ovary + thyroxine (*in vitro*)	17.29 ± .65 (16)
(n) " + liver + thyroxine (*in vitro*)	17.00 ± .56 (8)

* No. of animals.

carbon tetrachloride damaged liver or liver from animals with altered thyroid status (Tables II-IV), the pituitaries were taken from *normal* female rats and not from the ones which donated the liver tissue. This procedure was adopted to avoid pituitaries whose gonadotrophin content might have changed due to injection of carbon tetrachloride or disturbed thyroid status.

The homogenates of all non-pituitary tissues were prepared in such manner that 1 ml of each contained tissues 10 times the wet weight of the pituitary (in 1 ml of the homogenate). The ratio of total quantity of ovarian to pituitary tissues was found to be 10:1, and this was taken as the basis for adjustment of the ratio of other non-pituitary tissues during preparation of the homogenate.

After incubation each preparation was centrifuged and the supernatant was used for bioassay by the mouse uterine weight method (6). The test animals were injected with 0.5 ml of the supernatant by the subcutaneous route once daily for 3 days. The uterus was removed 24 hours after the final injection and weighed in a Roller-Smith balance after drying between pieces of filter paper. The dose of the supernatant was so adjusted that each animal received 1 mg of pituitary and/or 10 mg of other tissues per injection. For *in vitro* studies (Tables I and III) thyroxine was added to the incubation medium at the rate of 1 μg per 0.5 ml of the supernatant.

Results. Results are presented in Table I. Under the experimental conditions only the liver tissue inactivated gonadotrophin (b *vs* i—$P<0.001$). Neither the ovary nor other tissues (spleen and diaphragm) had any significant effect on this hormone. It was interesting that addition of thyroxine to the incubation medium prevented such inactivation of gonadotrophin by the liver tissue (i *vs* n—$P<0.001$). Further, there seemed to be a general potentiating effect of thyroxine on gonadotrophin. This was evident whether this hormone was incubated with the pituitary preparation *per se* (b *vs* l—$P<0.01$) or in combination with other tissues (b *vs* m —$P<0.02$; b *vs* n—$P<0.02$, Table I).

To examine the specificity of this gonadotrophin inactivating effect, liver tissue damaged by carbon tetrachloride was incubated with the pituitary preparation. Such liver tissue did not inactivate gonadtrophin (Table II).

As a sequel to thyroxine effect, the gonadotrophin inactivating capacity of the liver of hypo- and hyperthyroid rats was investigated in detail. It was observed that the liver tissue

TABLE II. Gonadotrophin Inactivating Capacity of Liver Damaged by Carbon Tetrachloride.

Treatment	Mean uterine wt (mg) with S.E.
(a) Saline	3.33 ± .16 (5)*
(b) Pituitary	16.33 ±1.30 (5)
(c) Damaged liver	3.70 ± .44 (4)
(d) " " + pituitary	15.18 ±3.34 (8)

* No. of animals.

TABLE III. Gonadotrophin Inactivating Capacity of Hypothyroid Rat Liver.

Treatment	Mean uterine wt (mg) with S.E.
(a) Saline	3.70 ± .07 (12)*
(b) Pituitary	14.70 ±2.00 (14)
(c) Liver (normal) + pituitary	9.00 ± .53 (9) †
(d) Hypothyroid liver	3.09 ± .25 (7)
(e) *Idem* + pituitary	6.74 ± .34 (19)
(f) " + thyroxine (*in vitro*)	5.15 ± .54 (12)
(g) " + thyroxine (*in vitro*) + pituitary	10.33 ± .84 (11)
(h) " + thyroxine (*in vivo*)	3.00 ±1.70 (4)
(i) " + thyroxine (*in vivo*) + pituitary	13.85 ± .88 (8)

* No. of animals. † Data from Table I.

TABLE IV. Gonadotrophin Inactivating Capacity of Hyperthyroid Rat Liver.

Treatment	Mean uterine wt (mg) with S.E.
(a) Saline	3.60 ± .38 (10)*
(b) Pituitary	18.91 ± 1.66 (15)
(c) Hyperthyroid liver	4.53 ± 1.18 (15)
(d) *Idem* + pituitary	17.97 ± 1.55 (21)

* No. of animals.

of hypothyroid animals caused more effective inactivation of gonadotrophin (c *vs* e—$P<0.001$, Table III). Addition of thyroxine to the incubation medium seemed to reduce such potent gonadotrophin inactivating capacity of the hypothyroid rat liver (e *vs* g—$P<0.001$). However, the liver tissue from hypothyroid rats subjected to thyroxine therapy (*in vivo*) did not inactivate gonadotrophin (Table III). This was also the case with the liver tissue of the hyperthyroid animals (Table IV).

Discussion. Our results show that under *in vitro* conditions, the liver, but not the ovary(4,5), of rats inactivates homologous pituitary gonadotrophin. This property seems to be specific to liver and is not shared by other non-genital tissues like the spleen and the diaphragm. The failure of liver tissue damaged by carbon tetrachloride to inactivate gonadotrophin, adds further to this specificity.

It is interesting that addition of thyroxine to the incubation medium prevents such inactivation of gonadotrophin by the liver. The results of similar experiments with the pituitary preparation alone or in combination with the ovarian tissue, show that under *in vitro* conditions thyroxine tends to exert a potentiating effect on gonadotrophin. This seems to be in contrast to the reported *in vivo* inhibitory action of this hormone on gonadotrophin(9). However, the situation is somewhat different with the liver tissue of hypo- and hyperthyroid rats. The thyroid hormone seems to reduce the potent gonadotrophin inactivating capacity of the hypothyroid rat liver both *in vitro* and *in vivo*. The liver tissue of hyperthyroid animals loses this property altogether. These facts, therefore, suggest that such effectiveness of thyroxine under *in vitro* conditions may be due partly to its general potentiating influence on the gonadotrophin preparation; the analogous *in vivo* activity, on the other hand, may be the outcome of a primary action on the liver tissue itself.

It is possible that some enzymatic mechanism in the liver causes such inactivation of gonadotrophin by an attack on one or other of the bonds (sugar, amino-acids, sialic acid) known to be critical for biological activity of this hormone(10,11). Further, from the results of experiments with induced hypo- and hyperthyroidism it is evident that the normal euthyroid status is essential for optimal inactivation of gonadotrophin by the liver tissue. Such an association between thyroid and liver may be an important peripheral mechanism for maintenance of a homeostatic relationship between gonads and pituitary.

Summary. The liver, but not the ovary, spleen or diaphragm inactivates homologous rat pituitary gonadotrophin *in vitro*. Liver tissue damaged by carbon tetrachloride loses this property altogether. Thyroxine prevents such inactivation of gonadotrophin when added to the incubation medium. Liver tissue from thyroidectomized animals causes more potent inactivation of gonadotrophin, which could be prevented by thyroxine both *in vitro* and *in vivo*. Liver tissue of intact thyroxine-treated animals does not inactivate gonadotrophin. The possible mechanism of such inactivation is discussed.

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