Effects of Liver Residue and Chorionic Gonadotropin on Ovarian Development in the Hyperthyroid Rat.* (17654)

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It has been demonstrated that massive doses of thyroid inhibit ovarian development in the immature rat, ovaries remaining infantile both in weight and microscopic appearance(1).

This effect may be completely counteracted by the oral administration of desiccated whole liver(2). The protective factor in liver is retained in its water-insoluble fraction and is apparently distinct from any of the known nutrients(3,4). In the present communication data are presented indicating that chorionic gonadotropin is also effective in counteracting ovarian inhibition in the immature hyperthyroid rat.

Procedure and Results. Three experimental rations were employed in the present experiment: diets A, B and C (Table I). Diet A was a purified ration containing the B vitamins in synthetic form only. Diet B was similar in composition but contained in addition U.S.P. desiccated thyroid, added at a level of 0.5% of the ration. Diet C was similar to diet B but was supplemented with 10% liver residue powder,† added in place of an equal amount of sucrose. Previous findings indicate that this material is a potent source of one or more factors effective in counteracting the ovarian inhibition of immature rats fed massive doses of thyroid(4). Sixty female rats of the Long-Evans strain were selected at 21 to 23 days of age and an average weight of 44.2 g for the present experiment. Animals were placed in metal cages with raised screen bottoms to prevent access to feces and were fed *ad lib* the diets listed above. Ten rats each were fed diets A and C; the remaining 40 animals were placed on diet B.

Thirty-one of the 40 rats on diet B survived the 36th day of feeding. These animals were then divided into 2 groups consisting of 12 rats each. Seven animals were discarded due to infection, poor condition or atypical growth. Each rat in Group I received 80 International Units of chorionic gonadotropin‡ over a 72 hour period, consisting of 4 daily intraperitoneal injections of 20 International

TABLE I.
Composition of Experimental Diets.

Dietary component	Diet A	Diet B	Diet C
Thyroid*		0.5	0.5
Liver residue powder†			10.0
Casein‡	22.0	22.0	22.0
Salt mixture§	4.5	4.5	4.5
Sucrose	73.5	73.0	63.0

To each kg of the above diets were added the following synthetic vitamins: thiamine hydrochloride, 72 mg; riboflavin, 9 mg; pyridoxine hydrochloride, 15 mg; calcium pantothenate, 67.2 mg; nicotinic acid, 60 mg; 2-methyl-naphthoquinone, 5 mg; and choline chloride, 1.2 g.‖ Each rat also received 3 times weekly the following supplement: cottonseed oil (Wesson) 500 mg, alpha-tocopherol acetate 1.5 mg and a vitamin A-D concentrate containing 50 U.S.P. units of vitamin A and 5 U.S.P. units of vitamin D.¶

* Thyroid Powder, U.S.P., Armour and Co., Chicago, Ill.

† Extracted Liver Residue, Wilson Laboratories, Chicago, Ill.

‡ Vit. Test Casein, General Biochemicals, Inc., Chagrin Falls, Ohio.

§ Sure's Salt Mixture No. 1(5).

‖ In view of the increased requirements for thiamine, pyridoxine, and pantothenic acid in the hyperthyroid rat(6), the B vitamins in the present experiment were administered in excessive amounts in order to assure an adequacy of these factors in the diet.

¶ Nopco Fish Oil Concentrate, assaying 800,000 U.S.P. units of vitamin A and 80,000 U.S.P. units of vitamin D per gram.

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† This liver fraction consists of the coagulated, water-insoluble material remaining after removal of the extractable water-soluble substances.

TABLE II.
Effects of Liver Residue and Chorionic Gonadotropin on the Ovarian Weight of Immature Rats Fed Massive Doses of Thyroid.

Dietary group	No. of animals	Final body wt,* g	Ovarian wt, * mg
A	10	157.8 ± 6.9	45.6 ± 3.9
B—Saline series	12	118.6 ± 5.1	23.2 ± 2.1
B—Gonadotropic series	12	117.8 ± 4.0	53.8 ± 4.4
C	10	173.2 ± 5.9	49.7 ± 5.2

* Including standard error of the mean calculated as follows: $\sqrt{\frac{\epsilon d^2}{n}} / \sqrt{n}$ where "d" is the deviation from the mean and "n" is the number of observations.

Units each. Group II was similarly treated except that saline solution was employed. Both groups were continued on diet B for the duration of the experiment. All animals were autopsied after the 40th day of feeding (96 hours after the 1st injection); ovaries were weighed, fixed in 10% formol, and sections prepared and stained with hematoxylin and eosin.

In agreement with earlier findings a marked retardation in growth was observed in all thyroid-fed rats on the synthetic dietary regime (diet B). This effect was completely counteracted by the administration of liver residue powder (diet C). A similar situation was observed in respect to ovarian weight (Table II). Ovaries appeared immature both in weight and microscopic appearance in 11 of the 12 rats receiving saline (diet B). With one exception not a single rat in this group showed mature follicles or corpora lutea. In contrast to the above mature follicles and corpora lutea were present in 9 of the 10 rats fed diet C and 8 of the 10 animals on diet A. In addition, ovarian weight in these animals averaged approximately twice that of saline injected rats (diet B). Ovarian weight was similarly increased in animals receiving the chorionic gonadotropin. In addition, mature follicles and corpora lutea were present in 100% of the rats in this series.

‡ Chorionic Gonadotropin (Lyophilized), Armour and Co., Chicago, Ill. This material was dissolved in saline solution and diluted to a concentration of 20 International Units per cc.

Findings indicate that the ovarian inhibition of immature rats fed massive doses of thyroid is not due to an inability on the part of ovarian tissue to respond to gonadotropic stimulation. It would appear, therefore, that the failure of gonadal development in the immature hyperthyroid rat is due either to (1) an impaired formation or release of pituitary gonadotropins or (2) an increased ovarian threshold to gonadotropic stimulation. Since no inhibition of ovarian development was observed in thyroid-fed rats receiving liver residue, and since this liver fraction did not counteract other manifestations of hyperthyroidism such as an increased B.M.R., a reduction in ventricular creatine concentration, or an increase in ventricular, kidney and adrenal weight(3,4), it would seem that liver residue contains one or more factors which either (1) promote the formation or release of pituitary gonadotropins or (2) lower the ovarian threshold to gonadotropic stimulation. A third possibility is that liver residue contains an orally active gonadotropin. No data are available to indicate which of these alternatives, if any, is correct.

Summary. Ovarian development was inhibited in young rats fed massive doses of desiccated thyroid, ovaries remaining immature both in weight and microscopic appearance. This effect was completely counteracted by the administration of either liver residue or chorionic gonadotropin.

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