Inactivation of Progesterone by Fetal Mouse Tissues *in vitro*.* (27295)

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In vitro inactivation of progesterone has been achieved many times with adult hepatic tissue and recently(1,2) with adult rat renal tissue. Less attention seems to have been given to the possibility that fetal tissues may also be able to inactivate sex hormones. Brown and Meyer(3) found that homogenized livers from 16- to 20-day-old rat fetuses inactivated estradiol-17β and estrone. Pulkkinen *et al.*(4) reported the metabolism of progesterone *in vitro* by liver from newborn guinea pigs and from fetal guinea pigs of unspecified age.

The purpose of the present experiment was to test the ability of homogenates of whole mouse fetuses and of fetal mouse livers to inactivate progesterone *in vitro*.

Materials and methods. Mice of the CHI stock were mated. The day on which a vaginal plug was found was counted as day 1 of pregnancy. On the 12th to 16th day (Table I), pregnant mice were killed by separation of cervical vertebrae, and 9 to 20 whole embryos or embryonic livers were quickly removed. For each experiment 0.23-1.13 g of fresh tissue was rapidly homogenized in phosphate-saline solution in the proportion of 1 g tissue/9 ml solution. Three preparations were then made from each of 4 of the homogenates and 2 were made from the 5th. The *Tissue Blank* contained 1 ml homogenate, 1 ml phosphate-saline solution, and 1 ml sodium citrate (1 mM) in phosphate-saline. The *Control* was identical with the preceding except that 0.5 mg progesterone in 0.05 ml sesame oil was added. The *Experimental Preparation* was identical with the Control. Each Tissue Blank and each Control were mixed quickly, and 5 ml cold acetone were then added immediately to stop enzyme action. Each Experimental Preparation was incubated at 37°C for 30 min., after which the reaction was stopped by addition of 5 ml cold acetone.

Then, following a technic previously described(5), every preparation was separately extracted with acetone and ether, and the extract, containing the protein-free fraction of progestin, was taken up in sesame oil. The protein-"bound" (*i.e.*, acetone- and ether-insoluble) fraction was hydrolyzed and extracted, and the extract was separately taken up in oil. Each oily extract was then bioassayed in CHI mice for progestin(5,6). Assay results were expressed in Progesterone Equivalents (P.E.); one P.E. is defined as equal to the *activity* of 1 μg of progesterone when the latter is assayed by the method of Hooker and Forbes in the absence of estrogen and other interfering factors. Residual activity was expressed as percentage of the demonstrated(5) activity of the progesterone originally added.

Results. Table I summarizes results for the "free" fractions of progestin. No activity was detected in any Blank. Extensive inactivation occurred in each Experimental Preparation. Some loss of activity also occurred in each Control Preparation. Assay of the protein-"bound" fractions revealed no more than traces of activity.

Discussion. The results establish the ability of homogenates of whole fetal mouse embryo and of fetal mouse liver to inactivate progesterone *in vitro* during incubation for 30 minutes. The relatively small loss of theoretical activity in the extract of each Control Preparation may have been due to

TABLE I. Residual Activity of "Free" Progestin Expressed in Per Cent of Starting Activity.

	Blank	Control	Exp.
Whole embryos, 12-day	None	65%	4%
13-day	"	93	4
14-day	"	81	45
Embryo livers, 15-day	"	—	8
16-day	"	93	12

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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*.

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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

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† 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.