Relationship of Pituitary and Adrenal Cortex to Ovarian Hyperemia Reaction in the Rat.* (18736)

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Ovarian hyperemia in response to the injection of pregnancy urine described by Reiprich(1) has been found sufficiently reproducible in rats and mice to warrant its use as a standard test for pregnancy(2) and for the assay of chorionic gonadotrophin and of certain pituitary extracts. The significance of this reaction in ovarian physiology, however, is little understood for, though it apparently is an early indication of ovarian stimulation in some species, the part that it plays in ovarian function is not known.

The inability of human chorionic gonadotrophin (HCG) to produce ovarian hyperemia in the hypophysectomized animal unless a large dose of pituitary "synergist" is given has been shown(3). Further studies on the influence of the pituitary and on the role of the adrenal cortex in this phenomenon are presented in this study.

Methods. Weanling 35-45 g female rats of the Sprague-Dawley strain were used. Hypophysectomy or adrenalectomy were performed 16 to 20 hours before the injection of the test substances, which were given subcutaneously over the abdomen. When more than one injection was given, they were placed on opposite sides of the abdomen. Four hours after injection the animals were killed with chloroform and ovarian color was judged independently by two or more observers under a daylight fluorescent lamp. The ovaries were classified as positive or negative as they would be judged in the interpretation of a pregnancy test. Doubtful reactions were classified as negative. A positive response on one side combined with a doubtful response on the other was regarded as a positive test. The combination of a weakly positive and a negative reaction was considered a negative test. Intact uninjected and gonadotrophin-treated animals were included as controls in each day's experiment. The corticotropin used(4) has been found to be 25 times as active as the Armour standard La-1-A and contained no significant amounts of gonadotrophins or prolactin.

TABLE I. Effect of Various Substances on Production of Ovarian Hyperemia in Intact and Hypophysectomized Rats.

	No. of animals	
Intact	Positive	Negative
Controls (no inj.)	2	40
Cortisone acetate, 1.25 mg	0	2
HCG, 0.1 μg + cortisone acetate, 1.25 mg	0	2
HCG, .02 mg	36	8
Pituitary extr., 3.0 mg	12	0
Corticotropin, 10 mg	0	2
Hypophysectomized		
HCG, .02 mg	2	13
HCG, .5 mg	0	2
Pituitary extract, 3.0 mg	4	0
Cortisone acetate, 1.25 mg	0	3
HCG, .02 mg + cortisone acetate, 1.25 mg	15	1
HCG, .02 mg + corticotropin, 1 mg	7	1
HCG, .02 mg + corticotropin, .5 mg	2	2

Results. The minimal effective dose for ovarian hyperemia of the preparation of human chorionic gonadotrophin used was 1 μg,† and to insure maximal responses a dose of 20 μg was employed except where otherwise stated. The minimal effective dose of the anterior pituitary preparation used‡ was 0.1 mg

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† "Pranturon" generously supplied by Schering Corporation, Bloomfield, N. J.

‡ Antuitrin T, No. 099802, kindly supplied by Dr. D. A. McGinty of Parke, Davis and Co., Detroit, Mich.

TABLE II. Effect of Steroid Hormones and Gonadotrophins on Ovarian Hyperemia in Adrenalectomized Rats.

Adrenalectomized	No. of animals Positive	Negative
HCG, .02 mg	8	54
Cortisone acetate, 1.25 mg	0	2
Pituitary extract, 3.0 mg	0	9
" " 3.0 " + cortisone acetate, 1.25 mg	17	1
HCG, .02 mg + cortisone acetate, 1.25 mg	25	3
" " + cortisone acetate, 100 μg	5	1
" " + cortisone acetate, 10 μg	8	2
" " + cortisone (free alcohol), 10 μg	17	4
" " + cortisone (free alcohol), 3 μg	0	7
" " + compound F acetate, 100 μg	5	3
" " + compound F acetate, 10 μg	4	0
" " + compound F acetate, 3 μg	0	4
" " + compound A acetate, 100 μg	7	4
" " + compound A acetate, 10 μg	8	6
" " + isocortisone acetate, 10 μg	4	8
" " + Lipoadrenal Cortex, .1 cc	4	1

and a dose of 3 mg was used throughout. As shown in Table I, cortisone acetate alone in doses of 1.25 mg did not produce ovarian hyperemia in intact, adrenalectomized, or hypophysectomized animals. Corticotropin in doses as high as 10 mg did not produce ovarian hyperemia in intact animals.

No synergism between cortisone acetate and human chorionic gonadotrophin was seen in intact animals when sub-hyperemic doses of human chorionic gonadotrophin (0.1 μg) were given with large doses of cortisone acetate.

Removal of the pituitary blocked ovarian hyperemia in response to chorionic gonadotrophin in doses as high as 0.5 mg but did not abolish hyperemia brought about by the anterior pituitary preparation. Cortisone acetate and corticotropin restored the response to chorionic gonadotrophin in hypophysectomized animals.

Table II shows that adrenalectomy abolished the hyperemia response both to human chorionic gonadotrophin and to the anterior pituitary extract. Cortisone acetate restored this response to both substances. Doses of cortisone acetate, free cortisone, and compound F acetate as small as 10 μg were found to be effective. Iso-cortisone acetate and compound A acetate were effective in slightly larger doses. Lipoadrenal Cortex (Upjohn) was effective in a dose of 0.1 cc, the smallest dose tested.

Progesterone in oil in a dose of 1 mg produced ovarian hyperemia in adrenalectomized chorionic gonadotrophic treated animals as did desoxycorticosterone at a dose of 2.5 mg (Table III). Testosterone propionate in oil, methyl androstenediol in oil, stilbestrol in oil, estradiol in oil, and pregnenolone as an aqueous suspension were not effective in this regard in the doses tested.

The data presented indicate that secretions of the adrenal cortex are necessary for the production of ovarian hyperemia in the rat in response to both human chorionic gonadotrophin and pituitary extract. Also, the effect of corticotropin in restoring the ovarian hyperemia response appears to be mediated through the adrenal cortex.

Summary. Adrenalectomy and hypophy-

TABLE III. Effect of Other Steroids on the Production of Ovarian Hyperemia in Adrenalectomized Immature Rats.

Adrenalectomized	No. of animals Positive	Negative
HCG, .02 mg + progesterone in oil, 2.5 mg	2	0
" " + progesterone in oil, 1.0 mg	5	2
" " + progesterone in oil, .1 mg	0	5
" " + progesterone in oil, .01 mg	1	5
" " + desoxycorticosterone acetate in oil, 2.5 mg	2	0
" " + desoxycorticosterone acetate in oil, 1 mg	0	5
" " + testosterone propionate in oil, 2.5 mg	1	4
" " + testosterone propionate, in oil, 1.0 mg	1	3
" " + methyl androstenediol in oil, 1 mg	1	4
" " + stilbestrol in oil, 2.5 mg	0	3
" " + estradiol in oil, 1 mg	1	4
" " + pregnenolone, 2.5 mg	0	2

sectomy were found to abolish the ovarian hyperemia response to human chorionic gonadotrophin. Corticotropin and cortisone acetate restored this effect in hypophysectomized rats, and cortisone acetate, as well as other steroids, in the adrenalectomized rats. Adrenalectomy but not hypophysectomy prevented this response to an anterior pituitary preparation. Cortisone acetate reinstituted the effect in the adrenalectomized anterior pituitary injected animals.

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Heme Synthesis and Erythrocyte Life Span in the Cat.* (18737)

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The interpretation of the hematologic syndromes resulting from exposure to ionizing radiations depends in large measure on the life span of the formed elements in the circulating blood(1,2). The rate of utilization of leukocytes(3) and thrombocytes(4) has already been determined for the cat. In other experiments(5,6) the functional regeneration of myeloid and erythroid elements in the cat was compared. In order to interpret this work a measure of the life span of the red cell in this species was needed.

While a variety of methods have been used for determining erythrocyte life span, the method of isotope tagging of the porphyrin ring is probably the least objectionable and was the one chosen.

Material and methods. Five apparently healthy adult cats from the stock colony, immune to infectious feline agranulocytosis, were used. They were maintained on a diet of canned commercial dog food, a small weekly allotment of fresh horse meat and an admixture of ground Rockland Rat Chow. Each was given 100 mg/kg of glycine-N^{15} containing 63.3 atoms % excess of N^{15}. The isotopic glycine divided into 15 doses was fed over a period of 3 days. The animals weighed 2.7 to 3.1 kg with a mean of 2.9 kg. Erythrocyte counts, white blood cell counts and hemoglobin values were determined weekly. Blood samples for analysis were obtained at about 2 week intervals by placing the animals under light ether anesthesia and cutting down on the femoral artery with the usual aseptic precautions. The blood samples were drawn in oxalate, the packed red cells re-suspended in physiological saline, re-centrifuged and finally lysed with distilled water. Hemin was obtained from the lysed cells by routine procedures(7,8). The hemin N^{15} concentrations were determined on a Consolidated-Nier Isotope Ratio Mass Spectrometer. The blood volume of each cat was determined at the end of the experiment by the method of Evans Blue dilution.

Results. During the course of the work it became apparent that individual cats gave somewhat different hemin N^{15} concentrations at a time when their red cell heme was maxi-

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