

## A Study of the Metabolic Fate of Human Chorionic Gonadotropin in Rabbits. (30745)

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Human chorionic gonadotropin is a glucoprotein which appears in the blood and urine during normal pregnancy in two distinct phases. During one phase, the first trimester, 20,000 to 100,000 IU are present in the urine during a 24-hour period. In the second phase, the remainder of gestation, the level of HCG falls below 11,000 IU per 24 hours(1-4). The titres of HCG present in blood and in urine are parallel(5).

The biological destination of HCG is not known. Elimination from blood may be due either to metabolic destruction or to excretion by way of the biliary or urinary tract. The renal clearance of HCG in normal pregnancy has been investigated. The figures reported are 0.38 ml/min and 0.95 ml/min(4, 6). Only a small fraction of circulating HCG is cleared by the kidneys, implying that it is metabolized by the organism or excreted in the stool.

On the pretense that metabolic destruction is a possible mode of eliminating HCG, isolated organ perfusion of HCG was studied in the rabbit. The renal clearance of HCG in rabbits was also determined.

*Methods.* HCG\* was injected into the marginal ear vein of each of 20 virgin female rabbits weighing 3 to 4 lb. In 2 animals studied, a test dose of 10,000 IU was used. This dose was reduced to 1000 IU in the others. Urine was collected in closed containers to which a preservative, toluene, had been added. The urine was tested with anti-human chorionic gonadotropic serum† for HCG content at 24-hour intervals(7-11). When the elimination of HCG ceased, a fact that was determined by negative tests of 4 consecutive 24-hour urine samples, the total HCG excreted was tabulated and compared to the test dose.

\* Prepared by Upjohn.

† Prepared by Ortho Pharmaceutical Corp. (Gravindex Test).

Each 24-hour urine sample was assayed for HCG and recorded as a volume range (increments of 0.005 cc) of urine used in each test. The total 24-hour urine volume was determined as well as the dilution factor, as needed. This information along with the volume of urine needed for each test was used with the formula:

$$\frac{\text{sensitivity of test (0.08 IU)}}{\text{volume of urine used for inhibition}} = \text{IU/cc}$$

For the purpose of this study, a fixed amount (0.02 cc) of anti-serum and antigen solution was used in each test. The dilution of the urine sample as well as the amount used was varied according to the amount of HCG per urine sample. An average quantity of HCG excreted in 24 hours was computed. When excretion ceased a cumulative average per animal was calculated. The HCG recovered was compared to the amount injected.

*Results.* In 2 animals receiving 10,000 IU of HCG, 75.6% was recovered in the urine over a period of 23.5 days. When 1000 IU of HCG was injected, an average recovery of 71.8% over a period of 4.2 days was observed. In this group of 18 animals, there were 2 animals with 39% return. This was believed to be due to error in the collection of urine.

Controls were done using animals of similar weight, species, and environment. These animals received normal saline instead of HCG and samples of urine were tested with anti-HCG serum. In all instances the urine was negative for HCG.

In the follow-up study, physiological quantities of HCG were perfused over a period of 2 hours into the renal artery of 12 virgin female rabbits (Table I) by using a calibrated perfusion apparatus. In a like manner the portal vein of 32 similar animals was perfused with varying amounts of HCG

TABLE I. Renal Artery Perfusion—2 hr.

No. of animals	HCG injected	% of animals ovulating
1	50 IU	100
9	25 IU	91
2	10 IU	100

TABLE II. Portal Vein Perfusion—2 hr.

No. of animals	HCG injected	% of animals ovulating
5	50 IU	100
17	25 IU	75
6	20 IU	100
4	10 IU	100

(Table II). The failure of ovulation would have served to indicate the ability of the kidney and liver to inactivate HCG. Ovulation occurred in 86.3% of the cases, indicating that HCG remained physiologically active following renal and hepatic perfusion.

Controls were run on 20 animals by injecting 10 IU of HCG into the marginal ear vein over a period of 2 hours. Ovulation was observed in all animals.

**Discussion.** Determination of the quantity of HCG excreted by the kidneys was achieved by using anti-HCG serum. This test was chosen because of its sensitivity, specificity, and accuracy of quantitation.

When 10,000 IU of HCG were employed as the test dose, over 75% of the HCG was recovered in the urine in a period of 23.5 days. The test dose was reduced to 1000 IU of HCG. In 18 animals injected with 1000 IU of HCG an average of 71.8% of HCG was recovered in the urine in a period of 4.2 days. Subsequent studies involving the iso-

lated organ perfusion (kidney and liver) of 44 animals with varying physiologic amounts of HCG over a period of 2 hours demonstrated that little if any HCG was altered biologically by these organs. The results of this follow-up study along with the data offered in the present study suggest that a large amount of HCG is excreted unchanged by the kidney in the rabbit.

**Summary.** Approximately 75% of HCG injected intravenously into female rabbits was recovered in the urine over a period of 4 to 24 days. The duration of excretion was directly proportional to the amount of HCG administered. Perfusion of the liver and kidney, *in vivo*, with HCG did not cause biologic inactivation of the hormone.

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Received September 9, 1965. P.S.E.B.M., 1966, v121.

## Human Thymus Cell Cultures—Evidence for Two Functional Populations.\* (30746)

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Cells morphologically classified as small lymphocytes were long thought to be biologically inactive. These cells are now known

to be immunocompetent since they may initi-

\* This work was supported in part by USPHS Grants AI 04152 and 5 T1 AI13.