PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 160, 130–133 (1979)

Conversion of Progesterone to Testosterone in the Rat Leydig Cell[1] (40404)

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A very sensitive and presumably specific *in vitro* bioassay for the measurement of luteinizing hormone (LH) and chorion gonadotrophins (CG's) is based on the ability of isolated rat or mouse Leydig cells to secrete testosterone in response to such gonadotrophins (1–5). It has been reported to be a reliable method for the bioassay of LH and CG in human serum or plasma (4, 6–8), and for LH in rhesus monkey serum (5). No evidence for interfering factors in serum or plasma was found by Dufau *et al.* (4, 5) or by Romani *et al.* (7), but Lichtenberg and Pahnke (9) reported that serum factors could interfere if the serum volume was more than 5% of the total volume of the assay medium. The interference could be eliminated by extraction of the serum with either ether or charcoal (9). Our own findings show that progesterone can be an important interfering factor in this bioassay.

Materials and methods. Animals. Wistar strain rats were maintained under pathogen-free conditions, and controlled conditions of light (lights on from 0500 to 1900 hr), temperature (22°), and humidity, in the animal facility section of the Sylvius Laboratories of the University. Adult males (180 g body wt) were decapitated and the testes removed just before the setting up of a bioassay.

Pregnant rats were bred in the Sylvius Laboratories. Day 1 of pregnancy was taken as the day of insemination.

Hypophysectomy was done by the parapharyngeal approach.

Blood for "hypophysectomized rats' serum" was collected at least 4 days after hypophysectomy from only those rats that proved to have no visible remaining fragments of pituitary tissue at autopsy.

Bioassay conditions. The bioassay was used as described by Dufau *et al.* (1, 4, 5), with certain modifications, to evaluate the possible existence of a CG ("rCG") in rat serum. The basis of the assay is the radioimmunoassay (RIA) of testosterone produced by collagenase-dispersed Leydig cells of the adult rat testis in response to stimulation by LH or LH-like hormones. The decapsulated testes were incubated with collagenase (Worthington) (0.25 mg/ml) and bovine serum albumin (BSA) (1 mg/ml) in TC Medium 199 (Difco) at 37°, with shaking at 150 cycles/min, for 25–30 minutes. The tubes were then allowed to stand for 5 minutes to allow the tubules to settle, and the supernatant collected and set aside. This procedure was repeated once more, after which the pooled supernatants were centrifuged at 600*g* for 15 min. After discarding the supernatant, the packed cells were resuspended in the incubation medium (TC Medium 199) at a concentration of 42 million cells/ml. Incubations were carried out in polyethylene vials at 37°, with shaking at 150 cycles/min, in an atmosphere of 95% O_2/5% CO_2 for 3 hr. Each vial contained 0.6 ml of the cell suspension, plus the serum samples to be assayed (0.1–0.5 ml), supplemented, as needed, with hypophysectomized rats' serum to make a total incubation volume of 1.1 ml. Each serum sample volume was assayed in duplicate. After incubation the vials were centrifuged at 1500*g* for 15 min and the testosterone concentration of the supernatant was analyzed by RIA (10). The testosterone antiserum, at 50% binding, had a cross-reactivity with androstenedione of 1.0%, and with progesterone, dehydroepiandrosterone, estradiol and 17-α-hydroxy-pro-

[1] Aided in part by NIH-NICHD Program Project No. HD-07640.

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0037-9727/79/010130-04$01.00/0

gesterone of less than 0.01%. Of the 12 other androgens tested all but 5α- and 5β-DHT cross-reacted less than 0.1%; the two DHT's cross-reacted at 10–11% (11). Each supernatant from each incubation vial was assayed for testosterone in duplicate.

Radioimmunoassay of LH. Immunoreactive LH was measured by RIA using a rabbit antiserum to ovine LH as described by Welschen *et al.* (12); rat LH-RP-1 (0.03 × NIH-LH-S1) was used as a standard, and rat LH-I-3 for iodination.

Results. There was a direct relation between volumes of day 12 pregnant rats' serum of between 0.1 and 0.5 ml in the incubation vial and the amount of testosterone produced by the Leydig cells. We therefore bioassayed the sera of pregnant rats, collected between day 7 and 22, for their possible "rCG" concentration, using a serum volume of 0.2 ml for each day of pregnancy tested. The findings, expressed as amounts of testosterone produced, were compared with the serum levels of LH and progesterone (13). The pattern of "rCG" strikingly resembled that of serum progesterone and was altogether different from that of serum LH (Fig. 1). [The latter, incidentally, was almost the same as that previously described by Morishige *et al.* (14)].

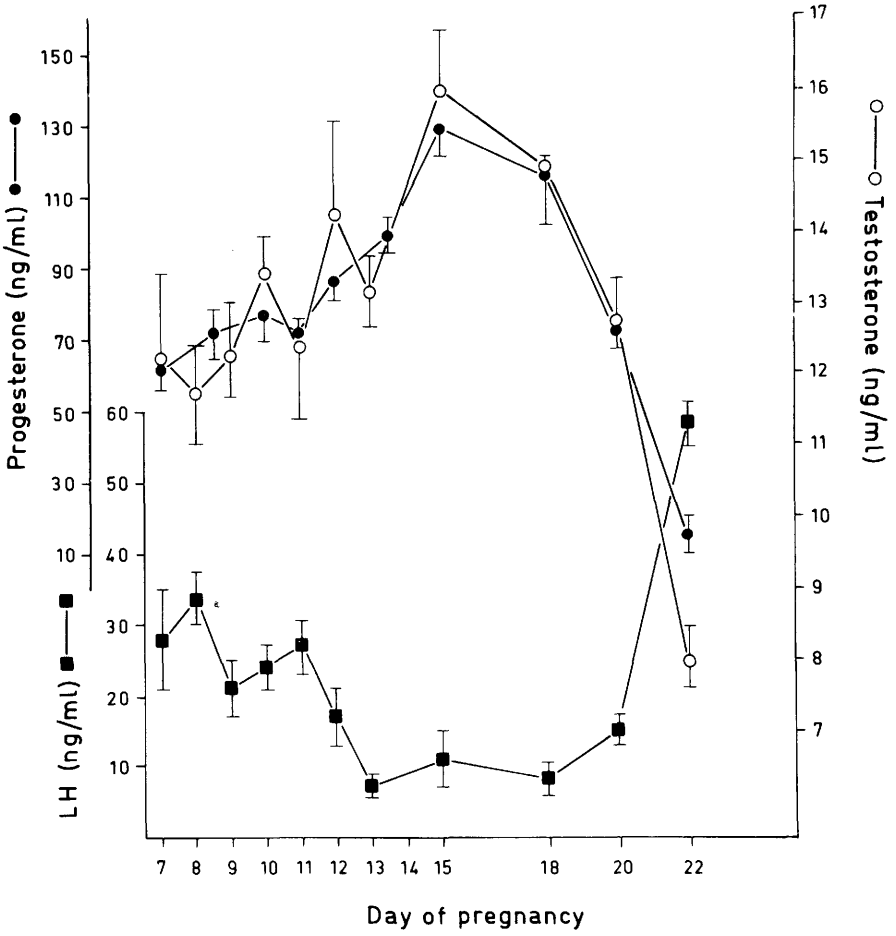

FIG. 1. The amount of testosterone produced during 3 hr of incubation of 0.2 ml of pregnant rats' serum and 0.3 ml of hypophysectomized rats' serum with 25 × 10^6 dispersed rat Leydig cells in 0.6 ml of medium 199. The testosterone values are expressed as ng/ml of the supernatants obtained after centrifugation of the incubation vials and are compared with serum levels of LH (ng/ml) assayed by RIA, and of progesterone (ng/ml). Each testosterone and LH symbol represents the mean (SEM) of four rats. The serum progesterone values were adapted from Pepe and Rothchild (13).

Because of the similarity between the patterns of "rCG" and progesterone we studied the effect of known amount of progesterone (10–200 ng) dissolved in hypophysectomized rats' serum on the testosterone produced by the Leydig cells under exactly the same bioassay conditions, except that pregnancy serum was not used. It was very obvious that the amount of testosterone produced was directly proportional to the amount of progesterone in the incubation medium (Fig. 2).

Discussion. These data show that if this *in vitro* bioassay is used to measure LH-like hormones in the presence of relatively high amounts of progesterone, the results will not necessarily represent only the concentration of the gonadotrophin, since progesterone, even in the absence of gonadotrophins, can be converted to testosterone. It should, however, be emphasized that we used relatively large amount of serum (200 μl) with a high progesterone concentration (60–130 ng/ml), while Dufau *et al.* used 100 μl or less of serum with a much lower progesterone concentration (1, 4, 5). This may explain why they found no evidence for interference by serum factors. Our data probably explain, at least in part, those of Lichtenberg and Pahnke (9), and suggest that other precursors of testosterone (e.g., androstenedione or dehydroepiandrosterone) may also interfere in this bioassay.

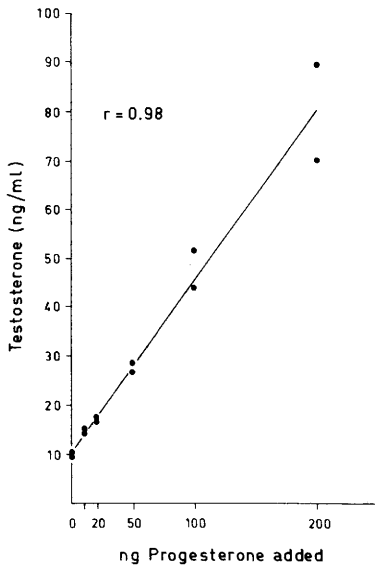

FIG. 2. Effect of different amounts of progesterone dissolved in 0.5 ml of hypophysectomized rats' serum on testosterone production, during 3 hr of incubation with 25×10^6 dispersed rat Leydig cells in 0.6 ml of medium 199. The testosterone produced is expressed as ng/ml of the supernatants obtained after centrifugation of the incubation vials.

Thus, if this bioassay is to be used to measure LH, CG's, or related gonadotrophins, the determination of the content of progesterone or of other steroid precursors of testosterone in, and, if necessary, their elimination from, the material to be assayed, may be an essential requirement for the method to retain its specificity.

It is also obvious that the pattern of serum "rCG" concentration during pregnancy in the rat (Fig. 1) was entirely due to the quantitative conversion of progesterone to testosterone. In fact, since the two curves were so obviously the same, one may conclude that, if an "rCG" exists (15), its concentration in the serum is either constant during the course of pregnancy (which seems highly improbable), or is too low to be measured with this bioassay as we have used it, or it does not stimulate testosterone production by rat Leydig cells *in vitro*.

Summary. The bioassay for LH-like hormones, based on the amount of testosterone produced *in vitro* by collagenase-dispersed rat Leydig cells in response to the hormone to be tested, was used to see whether a chorionic gonadotrophin (CG)-like hormone could be detected in the serum of pregnant rats. The curve of the serum "CG" concentration (i.e. *in vitro* testosterone produced) during the course of pregnancy was different from that of serum LH, determined by RIA, but was very similar to that of serum progesterone. This similarity proved to be due entirely to the conversion of progesterone to testosterone by the Leydig cells, since known amounts of progesterone (10–200 ng) dissolved in hypophysectomized rats' serum were converted quantitatively to testosterone when added to the cells under the same conditions used to test the pregnancy serum. The bioassay may thus be used as a specific measure of LH-like hormones only under conditions in which it is certain that steroids which can be converted to testosterone are either removed, or are present in amounts too small to have a significant effect on the values obtained.

We wish to express our thanks to the NIAMDD, Bethesda, M.D., for the gift of rat LH-RP-1 and rat LH-I-3, to Drs. J. Dullaart and J.Th.J. Uilenbroek, Erasmus University of Rotterdam, for the gift of the antiserum to ovine LH, to Dr. M. Frölich, Department of Chemical Pathology, University Hospital, Leiden, for the gift of the antiserum to testosterone, and to Organon, B. V., Oss, Netherlands, for the gift of crystalline progesterone.

We also thank Dr. Jack Kostyo, Editor-in-Chief of Endocrinology for permission to reproduce the curve of progesterone values shown in Figure 1.

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Received September 1, 1978. P.S.E.B.M. 1979, Vol. 160.