Immunologic Test for Chorionic Gonadotropin in Serum of the Pregnant Monkey (*Macaca mulatta*)[1] (34814)

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Chorionic gonadotropin is found in the blood and urine of the pregnant monkey (1–3). Biologic assays of this hormone (MCG) are time consuming and more difficult to perform than the immunologic tests currently utilized for measurement of human chorionic gonadotropin (HCG) in pregnant women. Because of the immunologic cross reaction between MCG and HCG (4), Kinzey was able to use a commercial human pregnancy test to dignose pregnancy in monkeys. He found measurable concentrations of MCG between Day 11 and Day 46 of pregnancy. Another report concluded, however, that immunologic pregnancy tests were unreliable in monkeys (6). The object of the present study was to assess the accuracy of a commercial human pregnancy test in diagnosing monkey pregnancy.

Methods. Blood was taken from a leg vein of female rhesus monkeys (*Macaca mulatta*) of the Yale Obstetrics Colony. One milliliter of serum in a test tube was acidified with 0.17 ml of 0.5 normal HCL. Four milliliters of acetone were pipetted into the serum. After thorough mixing the resulting suspension was poured through Whatman No. 42 filter paper. Two additional milliliters of acetone were used to rinse the test tube and this, too, was poured through the filter paper. The precipitate on the filter paper was allowed to dry for approximately 1 to 2 hr. It was then suspended in 1.0 ml of cold isotonic saline. After vigorous stirring with a glass rod and centrifugation the supernatant fluid was used for testing.

A commercial UCG titration test[2] contains antiserum to HCG and red blood cells treated with HCG. Addition of test material containing HCG or MCG causes inhibition of hemagglutination and the formation of a ring at the bottom of a test tube (positive test). A negative result is represented by a homogenous layer at the bottom of the tube. Control tubes containing only buffer, treated red blood cells, and test serum should invariably show ring formation because no antisera are present for the agglutination reaction. The results can be read within 2 hr. The test is sufficiently sensitive to detect concentrations of HCG as low as 500 IU per liter. In the great majority of our tests the results were read without prior knowledge of the pregnancy status of the monkey. A total of 37 monkeys were tested over a 2-year period. During that time an individual animal might be tested both in the pregnant and nonpregnant state, but the total number of tests from one monkey never exceeded four. Pregnancy was diagnosed by rectal palpation. The length of pregnancy was determined from mating records.

Results. In 17 monkeys who were between 20 and 33 days of pregnancy, 17 of 20 tests (85%) were positive. One of the false negative tests occurred on Day 30 in an animal that had been positive on Day 23. Two monkeys had false negative tests on Day 21 and Day 24, respectively. The latter test had been preceded by a negative result on Day 9.

Outside of the 20- to 33-day limits, there were false negative tests on Days 9, 16, 18, 37, 54, 61, and 80. The monkey which gave a negative test on Day 37 had had a positive

[1] Supported in part by grants from the Lalor Foundation and the Ford Foundation.

[2] UCG Titration Test, Wampole Laboratories, Stamford, Connecticut.

result on Days 22 and 29 of the same pregnancy. Five of the seven false negative tests (Day 16, 18, 54, 61, and 80) occurred in animals which were tested only that one time during their pregnancy.

Pregnancy tests were negative in 42 of 48 samples (87.5%) taken from nonpregnant monkeys. There were six false positives.

In nine tests control rings did not form, and these tests could not be interpreted as being either positive or negative.

Discussion. Using the mouse uterine weight assay Tullner and Hertz (2) were able to detect MCG in serum of monkeys 15–34 days pregnant. From Day 15 through Day 28 the tests were 100% accurate, while from Day 29 through 34 the accuracy was 69%. One test was positive at Day 40, but 32 other tests performed between Days 35 and 48 of pregnancy were negative. The peak amount of MCG in urine occurs between Days 19 through 24 (3). Using sensitive bioassay techniques, MCG can be detected in urine as early as Day 12 or 13 and its presence in measurable amounts has been noted through Day 38 or 39 (7). Bioassays have the disadvantage of requiring 3 days before a result is available. An additional drawback is the necessity for keeping animals for assay purposes. The immunologic test offers speed and convenience.

Despite the accuracy of 85% attained in this study between Days 20 and 33, the suitability of this immunologic test for the routine diagnosis of pregnancy in the monkey is open to question for two reasons. The ring that forms the end point of a positive test is not so distinct as it is when human urine or serum is used. It takes some experience to read the test. Of more importance is the failure to obtain positive tests earlier than Day 20. By this time, an experienced investigator can tell by rectal palpation whether the monkey is pregnant.

Summary. A commercial human pregnancy test was used to diagnose pregnancy in the monkey. Between Days 20 and 33 of pregnancy 17 of 20 tests (85%) were positive. In nonpregnant monkeys 42 of 48 tests were negative giving a false positive rate of 12.5%. From limited data it appears that this test is unable to detect MCG prior to Day 20 of pregnancy and this limits its usefulness.

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Received Jan. 14, 1970. P.S.E.B.M., 1970, Vol. 134.