IN THE LABORATORY

The Solubility of Progesterone in Saline[1]

THOMAS R. FORBES and CHARLES W. HOOKER

Department of Anatomy, Yale University

The mode of transport of the steroid hormones of the ovary, the testis, and the adrenal in the blood has been held to be a not inconsiderable problem, inasmuch as these compounds are generally regarded as insoluble in water, and blood is essentially an aqueous medium. The problem is perhaps most striking with respect to the luteal hormone, progesterone, which is probably the least active of the glandular steroid hormones, the international unit being 1.0 mg. Few precise measurements of the solubility of steroid hormones in water appear to have been made, however, and the data on the concentrations of these hormones in circulating blood are not entirely satisfactory.

Using a bioassay procedure that regularly detects 0.0002 μg of progesterone (*1*) it has been found that random samples of blood plasma from pseudopregnant rabbits, pregnant mice, a pregnant woman, and a monkey in the luteal phase of the menstrual cycle contained 5.5–8.0 μg of progesterone/ml. Approximately 90% of the hormone was biologically active without fractionation and was readily extracted with ether or acetone. The remaining approximately 10% appeared to be bound to protein and showed no activity until freed by partial hydrolysis with acid. The most frequently observed level, 6.0 μg, of "free" progesterone is equivalent to 0.0006 gm/100 ml, a ratio that would ordinarily be considered to represent insolubility. Accordingly, attempts have been made to ascertain the solubility of crystalline progesterone in 0.9% NaCl solution. Saline rather than distilled water was chosen to simulate the ionic concentration of the blood and to provide an isotonic material for injection.

As a first test, an excess of progesterone was placed in each of three flasks, and approximately 100 ml of 0.9% saline was added. One flask was stored at room temperature, the second at 37° C, and the third at 59° C. The flasks were allowed to stand with occasional shaking for 24 hrs. At the end of this time a sample was drawn from the supernatant fluid in each flask and immediately assayed by intrauterine injection into ovariectomized mice according to our regular procedure (*1*).

[1] This investigation was aided by grants from the Committee for Research in Problems of Sex, National Research Council, and from the Fluid Research Fund of the Yale University School of Medicine.

The progesterone was supplied by Ciba Pharmaceutical Products, Inc., through the courtesy of Ernst Oppenheimer and F. L. Mohr.

The sample stored at room temperature assayed 0.5 μg/ml; the sample at 37° C, 6.0 μg/ml; and the sample at 59° C, slightly more than 6.0, but less than 6.6 μg/ml.

To test the validity of assay of progesterone in aqueous solutions, two solutions of progesterone were prepared at 37° C and assayed. The first was made to contain 3.0 mg/liter (3.0 μg/ml), and the second, 6.0 mg/liter (6.0 μg/ml). The assay values were 3.0 and 6.0 μg/ml, respectively.

Two attempts were made to determine solubility at 37° C gravimetrically as well as by bioassay. In the first, 15.0 mg of crystalline progesterone was placed in a 1-liter volumetric flask that was then filled to mark with 0.9% aqueous NaCl. The flask was placed in the incubator at 37° C and stirred mechanically for 16 hrs. After an additional 8 hrs in the incubator, the fluid was filtered through a Gooch crucible to determine the quantity of undissolved progesterone. This was found to be 8.4 mg, indicating that 6.6 mg had dissolved. A small portion of the filtrate was assayed, with a finding of 6.0 μg/ml. Efforts to obtain a satisfactory value for the weight of the evaporated filtrate were unsuccessful. In the second determination the same procedure was followed except that 10.0 mg of progesterone was the starting quantity, the storage period was 30 days, and the agitation was occasional and by hand. Here the material retained by the Gooch funnel was 1.0 mg, indicating that 9.0 mg had dissolved. The filtrate assayed slightly more than 8.0 μg/ml. Again, a satisfactory value for the weight of the evaporated filtrate was not obtained. The agreement in both determinations between bioassay and the partial gravimetric determination seems to be reasonably good.

Apparently the solubility of crystalline progesterone in saline at 37° C is approximately 6.0–9.0 μg/ml, or 6.0–9.0 mg/liter. The solubility at room temperature is clearly much less, and increasing the temperature above 37° C did not significantly increase the quantity dissolved. It is not apparent why storage for 30 days appreciably increased the amount dissolved over that in solution at the end of 24 hrs.

Although the data on plasma levels include but a few physiologic states, no level of "free" progesterone thus far encountered exceeds the solubility of this compound in water (saline) at body temperature. If this circumstance obtains after further study, it may prove unnecessary to postulate any device other than physical solution in water for the transport of the biologically active progesterone in the blood.

Reference

1. HOOKER, C. W., and FORBES, T. R. *Endocrinology,* 1947, **41,** 158–169.