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Progesterone Transportation in Blood

ARTHUR L. HASKINS, M.D., F.A.C.O.G., and HANS D. TAUBERT, M.D.

PROGESTERONE, whether secreted by ovary, placenta, or adrenal, probably enters the blood as an unconjugated steroid. Although variously estimated,[1, 2, 4, 5, 9] it is most likely that the amount of progesterone in the circulating peripheral blood of some animals and humans during pregnancy approximates 5–25 μg.%.

Several mechanisms for the transport of progesterone have been suggested. As a steroid the hormone could be carried in the fat compartment of the circulating blood plasma.

The aqueous solubility of progesterone is about 1500 μg.%.[3] This order of solubility exceeds the physiologic concentration of progesterone in the blood; therefore, aqueous solubility as a transport mechanism should be considered.

It has been proposed that progesterone, like some other steroids, exists in the circulating blood or is transported by a protein-binding mechanism. Hooker and Forbes[6] reported that 90% of plasma progesterone was protein-bound. The bound progesterone was considered to be biologically inactive and could be released by hydrolysis with hydrochloric acid. Westphal *et al.* published data indicating that the albumin binding of progesterone is an important transport mechanism. The protein binding of progesterone and other steroids was nicely elucidated by Sandberg *et al.*

It seems well established then, that progesterone does exist in the peripheral circulation, in a protein-bound form. The quantitative aspects of this binding mechanism were considered worthy of further exploration. The purpose of this experimentation was to examine the protein-binding mechanism involved in progesterone transport through the use of radioactive-tracer technics and electrophoresis. Specific attention was directed toward the quantitative aspects of the binding mechanism.

METHODS AND MATERIALS

Progesterone-4-C^{14} with a specific activity of 22 mc./mM was utilized as the steroid component of the binding experimentation.

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Several proteins from a variety of sources were used as the protein component of the binding. These included human serum from pregnant and nonpregnant women; human serum albumin (Armour); human serum albumin (Nutritional Biochemical); bovine serum albumin (Armour); and egg albumin. Rabbit serum was utilized in in-vitro and in-vivo experimentation.

The Nuclear-Chicago low-background gas-flow counter and scaler were used for the detection of radioactivity. The Nuclear-Chicago gas-flow counter analytical-count-rate meter were used for the scanning of radioactivity on the electrophoresis strip. Electrophoresis was carried out with a standard apparatus utilizing 500 v with *p*H maintained at 8.6 with a barbital buffer and Whatman No. 1 filter paper. Electrophoresis was maintained for 4 hr. Some electrophoresis experimentation was carried out utilizing cellulose acetate paper.

Progesterone-4-C^{14} in measured amounts, in 95% ethanol was evaporated to dryness. To this were added varying amounts of the protein substrate. The mixture was maintained at a constant temperature of 37°C. for 1 hr. For the in-vivo experimentation, progesterone-4-C^{14} in measured amounts was dissolved in 95% alcohol. The alcoholic solution was injected into the marginal ear vein of the rabbit. Blood was then taken at intervals by cardiac puncture.

Samples (0.01 ml.) of serum or specific protein fractions with the radioactive progesterone were placed on the electrophoresis strips. Upon removal from the electrophoresis apparatus, the strips were dried and scanned with the count-rate meter. Comparisons with the count-rate-meter findings following the staining of the strips with bromphenol blue were made. Other strips were then cut at 1-cm. intervals. The substance on the strip was eluted with 95% ethanol. These samples were counted and compared to the stained-strip and the count-rate-meter scanning recordings.

RESULTS

Human Serum Albumin, 5% (Armour)

Samples of radioactive progesterone were placed on the starting line of the electrophoresis strip. These were examined after 4 hr. of electrophoresis. The progesterone did not move from the starting line. Samples of serum albumin, 5%, were placed on the strip and were subjected to 4 hr. of electrophoresis. These strips were stained with bromphenol blue. The strip was examined with a densitometer. This showed that approximately 25% of the total albumin placed on a strip trailed behind the concentrated motile albumin. As the concentration of albumin was increased, the percentage of trailing decreased, although the absolute amount of albumin in the trail increased.

The comparison between the amount of progesterone moving with the albumin and the total recovery of progesterone from the individual strips is shown in Fig. 1. At concentrations from 5 to 80 μg.% of progesterone, the total albumin concentration of progesterone varied between 75 and 80%. All the progesterone added to 5% albumin, regardless of the concentration, exhibited some electrophoretic mobility. Progesterone not present in the albumin fraction was found to be present on the strip in the albumin trail. These concentrations of progesterone were considered to parallel, with generous tolerances, the physiologic blood levels found in the human during pregnancy.

The result of the addition of larger amounts of progesterone to 5% human serum albumin is shown in Fig. 2. The previously noted straight-line relationship between the amount of progesterone added to the albumin and the amount recovered from albumin undergoes a marked variation when physiologic concentrations are exceeded. The percentage of recovered radioactive progesterone from the albumin, which varied between 75 and 80% at physiologic

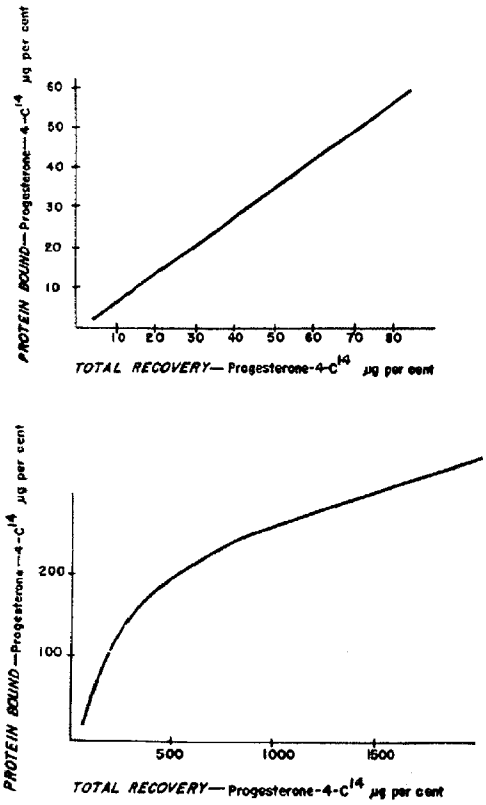

Fig. 1 (top) and **2** (bottom). Protein-bound progesterone compared to total progesterone content of 5% human serum albumin.

levels, changes to a maximum concentration of apparent binding of 20% when concentrations of 2000 µg.% are reached. It should also be noted in this experiment that all of the progesterone exhibited some electrophoretic mobility. Similar trailing of the albumin was demonstrated.

Human Albumin (Nutritional Biochemical)

Solutions of this human serum albumin of concentrations varying from 5 to 25% on no occasion imparted electrophoretic mobility to the radioactive progesterone. The mobility of the albumin in the electrophoresis apparatus was identical to that of the Armour's human albumin, but the progesterone did not move from the starting line.

Bovine Serum Albumin (Armour)

Five per cent solutions of this commercially obtained albumin failed to provide electrophoretic mobility to radioactive progesterone. The mobility of the albumin was similar to that of the human serum albumin as tested previously. The radioprogesterone remained at the starting line.

Egg Albumin

Ten per cent solutions of egg albumin in normal saline were added to radioactive progesterone as in the previous experimentation. Application of this mixture to the electrophoresis apparatus showed no motility of the progesterone. The albumin progressed satisfactorily along the electrophoresis strip.

Human Serum

A variety of human-serum specimens obtained from postmenopausal women, postpartum women, gravid women, and female cancer patients was added to radioprogesterone according to the standard technic. Physiologic concentrations of the radioprogesterone behaved in a manner similar to that of the human serum albumin experimentation. Upon exceeding the limits of physiologic concentrations, there was dispersion of the radioactivity throughout the strip into the globulin portions of the separated serum. The numerical results are shown in Table 1.

TABLE 1. DISTRIBUTION OF PROGESTERONE-4-C^{14} IN HUMAN SERUM AFTER INCUBATION AT 37°C. AND ELECTROPHORESIS

Total progesterone (µg.%)	*Albumin progesterone (µg.%)*	*% progesterone in albumin*
1645	220	13.4
850	180	21.3
236	102	42.9
230	110	46.8
193	86	44.4
98	48	50.6
72	56	76.5
16	13	80.4
6.8	5.2	75.6

This might have been interpreted as globulin binding of progesterone except for the observation that the distribution of the radioactivity in whole serum was identical with those in which only serum albumin was used.

Repetition of this experiment using cellulose acetate electrophoresis paper resulted in excellent protein fractionation. The radioprogesterone did not migrate from the starting line.

Intravenous Administration of Radioactive Progesterone to the Rabbit

It has been shown that the intravenous administration of progesterone in rabbits results in a rapid disappearance of the progesterone in the circulating blood. It was therefore necessary to use a large amount of radioactive progesterone and to remove the blood from the circulation within seconds after its administration to demonstrate protein binding.

Following the administration of approximately 20 mg. of radioactive progesterone to the marginal ear vein of the rabbit, blood was obtained by cardiac puncture within 15 sec. The total radioactivity of the blood thus removed indicated the progesterone content to vary between 16 and 216 μg.%. The electrophoretic treatment of rabbit serum under these conditions resulted in a similar distribution of radioactivity to that obtained with the human serum albumin (Armour) and whole blood serum. The results of this limited experimentation are shown in Table 2.

TABLE 2. DISTRIBUTION OF PROGESTERONE-4-C^{14} IN RABBIT SERUM AFTER INTRAVENOUS ADMINISTRATION AND ELECTROPHORESIS

Total progesterone (μg.%)	*Albumin progesterone (μg.%)*	*% progesterone in albumin*
216	68	31.5
45	16	35.5
16	13	81.3

DISCUSSION

It has been shown in previous experimentation that the total progesterone content of human or other animal blood may be removed by ether extraction. This suggests that when protein binding occurs, the bond is easily disrupted. Consideration was given to the thought that the lipid content of human serum albumin might be responsible for the transport of progesterone. Accordingly, a number of human serum albumin fractions were extracted with ethyl ether prior to the addition of radioactive progesterone. The ether-treated albumin fractions continue to bind the progesterone in a manner similar to the untreated samples. In addition to this, electrophoresis strips with radioprogesterone and human serum albumin were subjected to fatty-acid-staining technics. The result of this was a failure to demonstrate lipid substance. It appears then that the binding mechanism is independent of lipid substances. The mechanism by which the protein binding occurs is unproved at the moment but may be the result of electrostatic or exchange valance forces.

It is apparent from both the in-vitro and the in-vivo experimentation that the steroid progesterone is carried in the blood in physiologic levels in human serum albumin and in rabbit serum albumin. The small amount of progesterone not present in the main body of albumin during electrophoresis is found in the trailing portion and approximates or parallels the amount of albumin in the trail. This suggests that the total progesterone content of blood is to be found in the albumin. As physiologic levels are exceeded, the albumin compartment is unable to provide electrophoretic motility to the excess progesterone to the extent of allowing for total migration of the molecule. It appears that a mechanism is present which imparts partial electrophoretic motility to even excessive amounts of the steroid.

SUMMARY

Radioactive progesterone was added to human serum, human serum albumin, bovine serum albumin, and egg albumin. Radioprogesterone was injected intravenously in rabbits. Electrophoresis of the serum mixtures was accomplished.

These studies indicated that physiologic concentrations of progesterone are carried in the albumin component of human and rabbit serum. When the physiologic limits are exceeded, progesterone escapes from this compartment in increasing amounts.

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