THE HALF-LIFE OF PROGESTERONE IN THE PERIPHERAL BLOOD OF A EWE AT TWO STAGES OF GESTATION

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SUMMARY

[4-14C]Progesterone (10 μc, 154 μg.) was injected intravenously into a Suffolk ewe on the 115th day of gestation, and again a few hours before parturition. Within 5 min. of the injection, only about 1% of the radioactive progesterone was present in the circulating blood. During the ensuing 25 min., the fall in concentration was approximately exponential, the estimated half-lives being 7.3 ± 1.2 min. at day 115 and 8.1 ± 0.6 min. just before lambing. The difference between these half-lives is not significant.

Subject to a number of assumptions, these half-lives may be used as a measure of the rate at which progesterone is metabolized in the body. It is concluded that there are no pronounced changes in the rate of progesterone production or metabolism during the course of pregnancy in the ewe.

INTRODUCTION

Indirect evidence suggests that the influence of progesterone on the sheep myometrium declines before parturition (Bengtsson & Schofield, 1960). However, chemical estimations of progesterone in the peripheral blood of sheep have failed to show any significant decline in the concentration of the hormone itself before lambing (Short & Moore, 1959).

The purpose of the present experiment therefore was to investigate whether the rate of metabolism of progesterone is changed just before parturition. If there is no such change, it may be assumed that the rate of progesterone production remains substantially constant at this time. In addition it was hoped to obtain some information on the rate of secretion and metabolism of 20α-hydroxyprog-4-en-3-one, a progesterone derivative normally present in sheep peripheral blood.

MATERIAL AND METHODS

[4-14C]Progesterone (10 μc, 154 μg.; The Radiochemical Centre, Amersham) was dissolved in 1 ml. 50% ethanol and injected into the cephalic vein of a Suffolk ewe on the 115th day of gestation. The experiment was repeated 30 days later, a few hours before the ewe gave birth to a single live lamb.
On each occasion serial blood samples of 10 ml. were withdrawn from the jugular vein at repeated intervals during the hour following the injection. The first sample was not taken until 5 min. after the injection, so as to allow adequate mixing of the steroid in the circulation.

The plasma was separated by centrifugation, and extracted for progesterone and 20α-hydroxyprogren-4-en-3-one as described previously (Short, 1958). The plasma extracts were chromatographed on paper in the usual manner, together with authentic progesterone and 20α-hydroxyprogren-4-en-3-one as reference compounds. Appropriate areas of the chromatograms were cut out, eluted, and evaporated to dryness; the eluates were transferred to planchettes with several washings of light petroleum. Counts were made using a Nuclear Chicago thin end-window counter, with an estimated counting efficiency of 17% for carbon-14.

**RESULTS**

The results for progesterone at the two stages of gestation are shown in Fig. 1. The fall in concentration was approximately exponential, at least from 5 to 30 min. after injection. The half-lives during this period were 7.3 ± 1.2 min. at the 115th day of gestation just before parturition.
of gestation and $8.1 \pm 0.6$ min. just before parturition; the difference between these half-lives is not significant ($P > 0.05$).

There was slightly more radioactive progesterone circulating at any given time after the injection during mid-pregnancy than at term. Although this effect was not significant at the conventional 5% level, the probability associated with the significance test was quite small (approximately 10%); the effect, if real, might be explained by the slightly higher distribution volume for the injected steroid at term, due to the increased size of the uterine contents.

The results for 20α-hydroxyprogren-4-en-3-one at the two stages of gestation are shown in Fig. 2. The values on the 115th day do not allow estimation of a half-life since they are too variable, and the samples were not taken over a sufficiently long period of time. At term, the fall in concentration was approximately exponential for the period from 5 to 30 min. after injection, the half-life being $15.4 \pm 2.9$ min.
DISCUSSION

The distribution of progesterone between the peripheral blood and the extravascular tissues of the normal ewe is represented diagrammatically in Fig. 3. In the steady state, the rate of secretion of progesterone into the blood (A) is equal to the net rate of flux from the blood into the tissues (C). This net rate of flux is in turn equal to the rate at which the hormone is metabolized and excreted from the tissues (B). Thus the net rate at which progesterone diffuses out of the blood may be used as an indirect measure of the rate at which it is metabolized. Such a calculation involves making a number of assumptions:

1. The only way in which progesterone can enter the system is by secretion directly into the blood stream.

2. The metabolism of progesterone by the blood itself is negligible. Most of the metabolism takes place after the steroid has diffused out of the circulation into extravascular tissues, e.g. liver cells.

3. Progesterone is removed from the extra-vascular tissues chiefly by metabolism, and to a lesser extent by re-entry into the peripheral blood. No unmetabolized progesterone is excreted as such in the urine or faeces.

It seems likely that there are no great errors entailed in making these assumptions. In addition, it must be assumed that within a few minutes of the administration of radioactive progesterone, it becomes completely mixed with the endogenous progesterone in the peripheral blood and the extra-vascular tissues, and is handled by the body as if it were the natural hormone.

In the present experiments, the normal state of equilibrium was undoubtedly disturbed by the intravenous injection of a large dose of radioactive progesterone (154 μg.) relative to the amount normally present in the blood (15 μg.). In calcu-
lating this latter figure it has been assumed that progesterone in blood is confined to the plasma, that the normal concentration in the peripheral blood is 0·5 µg./100 ml. plasma (Short & Moore, 1959), and that the total circulating plasma volume is about 3 l.

An initial rapid diffusion must occur after the injection. If the 10 µc injected were mixed instantaneously in the blood stream, the initial concentration would be 12 800 counts/min./10 ml. plasma. However, within 5 min. of the injection the concentration was only 150 counts/min./10 ml. plasma (see Fig. 1), indicating that almost 99% of the injected dose had already been removed from the circulation. Thus the total amount of progesterone present in the extra-vascular tissues is probably about 100 times the amount that is present in the blood.

After this initial rapid disappearance, the subsequent rate of removal of the radio-active progesterone from the circulation was much slower. Since it appeared to be approximately exponential, it seems likely that the system had reverted to the equilibrium state depicted in Fig. 3. Once equilibrium has been restored, the total amount of progesterone circulating in the peripheral blood is probably only slightly higher than normal, because the amount of steroid injected is likely to represent only a small fraction of that already present in the extra-vascular tissues. In this connexion it is interesting to recall that the concentration of progesterone in at least one extra-vascular tissue, fat, greatly exceeds the concentration in the blood (Zander, 1959).

Although the calculation of half-lives from the data obtained in this study necessitates making a number of assumptions, the results at the two stages of gestation in the same animal should be directly comparable. It can therefore be stated with some confidence that there are no marked differences in the rate of progesterone metabolism at the two stages of pregnancy studied. Furthermore, the progesterone half-lives are in fact longer than those found in two non-pregnant ewes, where the values were about 4 minutes (Short, 1961). Since the blood progesterone levels are similar in pregnant and non-pregnant ewes (Short & Moore, 1959), this would suggest that no increase in progesterone production is required to maintain a pregnancy. Also, any decrease in the influence of progesterone on the myometrium at term is not likely to be due to any alteration in the rate of secretion or peripheral metabolism of progesterone.

The present study also suggested that some of the injected progesterone was metabolized to 20α-hydroxypregn-4-en-3-one; the concentration of this steroid was almost as high as that of progesterone within 5 min. of the injection. Much of the 20α-hydroxypregn-4-en-3-one normally found in the peripheral blood of sheep is therefore probably formed from the metabolism of progesterone, rather than being secreted as such by the endocrine glands. This is borne out by the observation that there is very little 20α-hydroxypregn-4-en-3-one relative to progesterone in the ovarian vein blood of sheep, whereas the concentrations of the two hormones are almost equal in the peripheral blood (Short & Moore, 1959).
REFERENCES