THE BIOLOGICAL HALF LIFE OF PROGESTERONE IN THE PERIPHERAL BLOOD OF COWS

Tatsuo IMORI

*Department of Surgery and Obstetrics, School of Veterinary Science, College of Agriculture
University of Osaka Prefecture, Sakai-shi, Osaka*

(Received for publication March 27, 1967)

Estimation of progesterone secretion in the peripheral blood of the cow would be of interest in the study of reproductive problems in cattle. This hormone has been proved to play an important role in the maintenance of a normal estrous cycle and pregnancy in this species also. So far as the secretion rate of steroid hormones is concerned, it could be assumed fairly well by determining the hormone level of the blood, the volume of distribution of the hormones, including the volume in the extravascular space, and the rate of metabolism of the hormones in animals. The estimated biological half life of progesterone in the circulating blood could be used as a good measure for the rate of metabolism of progesterone in animals, as suggested by PEARLMAN⁸⁾.

Some reports have appeared on the rate of disappearance of trace amounts of progesterone from the blood plasma following intravenous injection of this hormone labelled with the radioactive elements in human beings. Pearlman estimated that the biological half life of progesterone in the circulating blood of pregnant women must be 5 minutes or less. SANDBERG and SLAUNWHITE⁷⁾ have reported that the unconjugated steroids following intravenous injection of 4-¹⁴C-progesterone were cleared from the plasma at two rates with half lives of approximately 20 and 90 minutes, respectively. SOVIA *et al.*¹¹⁾ determined also two phases in the disappearance of radioactivity after intravenous injection of 4-¹⁴C-progesterone. In these two experiments, especially in that reported by SOVIA *et al.*, the primary phase represented a rapid decline or disappearance; it occupied approximately the first 4~5 minutes after intravenous injection. Following this phase, the clearance of the radioactivity progressed at much slower rate.

SHORT and EATON⁹⁾ indicated that the half life of progesterone in the maternal blood in the late pregnancy of woman should be extremely short, probably about 5 minutes or less.

Rather recently, SHORT and ROWELL¹⁰⁾ have reported that the estimated half life of progesterone in the peripheral blood was 7.3 and 8.1 in an ewe on the 115th day of gestation and another ewe at the time just prior to parturition, respectively.

MILLER *et al.*⁵⁾ indicated that the disappearance of radioactivity consisted of two phases in 2 pregnant and 9 non-pregnant cows after injection of ¹⁴C-progesterone or tritiated progesterone. They reported that the half life of progesterone was 25.4 and 21.1 minutes on the 60th and 130th day of gestation, respectively. The mean value of the half lives of progesterone in 9 non-pregnant cows was 36.6 minutes. However, they did not refer sufficiently to the stages of the estrous cycle in these cows. They regarded the decline in the second phase as the real clearance rate of progesterone in their experiment.

The objectives of the present study were to determine the biological half life (1/2

turn over (T.O.) time) of free steroids in the peripheral blood of cattle after intravenous injection of ^{14}C -progesterone and to estimate the rate of metabolism of this hormone in the estrous cycle and gestation in this species.

MATERIAL AND METHODS

Five dairy cows (Holstein-Friesian), weighing approximately 450~540 kg, were employed for this experiment. Three of them were non-pregnant lactating ones and tested at four different stages of the luteal phase of the cycle. The other two were pregnant and tested on the 65th and 154th day of gestation. The labelled hormone used was 4- ^{14}C -progesterone of 20 or 25 μCi (125 to 171 μg , according to lot; supplied by New England Nuclear Corp., Boston, Mass., U.S.A.) dissolved in 5 ml of ethanol and diluted to a 30 ml amount with physiological saline. It was injected into the external jugular vein of each cow. On each occasion, serial blood samples (numbering 8 to 9) of about 115 ml each were withdrawn from the jugular vein into a flask containing oxalate as anticoagulant, at various intervals over a period of 47 to 80 minutes following injection.

The first sample was collected 5 minutes after injection, except in one case where 10 minutes after injection. Immediately, it was centrifuged at 4,000 rpm at 5~8°C for full 20 minutes. As a result, a total of 50~55 ml of plasma was separated. The plasma was first saponified by 1.2 ml of 20 % sodium hydroxide solution, and then extracted with a double volume of ether which contained 3 % of ethanol. After washing with one-half volume of distilled water, it was dehydrated over sodium sulfate. The pooled extract of ether was then evaporated to dryness under reduced pressure and with a stream of nitrogen gas.

The dried extract was then dissolved into 25 ml of n-hexane and applied onto a column prepared by aluminum oxide which was similar to the column suggested by Loy *et al.*⁴⁾

The solvent system consisted of n-hexane, 10 % chloroform-hexane, and 100 % chloroform in order. The last 100 % fraction was collected and evaporated to dryness under reduced pressure and with a stream of nitrogen gas on the warm water bath (45~50°C).

Estimation of radioactivity was performed in a Packard automatic liquid scintillation spectrometer. The detection efficiency was carefully determined experimentally by use of an internal standard of benzoic- ^{14}C -acid.

Each sample was dissolved in 20 ml of toluene containing 0.4 % of POP and 0.004 % of POPOP for the scintillation counting.

The observed counts per minute (cpm) were corrected to a disintegration per minute (dpm) scale by use of the detection efficiency. Correction was also made for a possible slight quenching effect by recounting after an addition of the known amount of ^{14}C -progesterone (about 1,000 cpm in 0.2 ml of toluene) and proper calculations followed. The averaged loss in the whole procedure was determined by use of 4 blood plasma samples of 50 ml each from a steer, which had been collected at a slaughter house and to which had been added a tracer amount of ^{14}C -progesterone (about 17,000 cpm) before extraction. The averaged loss was 14.4 % at the time just prior to the step of radioactivity counting. Correction was made finally by this amount.

The rate of clearance of the radioactivity from the blood plasma was determined by plotting counts per 50 ml sample of plasma *versus* time in minutes at which the sample was removed following injection.

The biological half life at each of the two phases of clearance was obtained from a line drawn through the plots at each clearance phase.

RESULTS

Results are shown in Table 1 and Figs. 1~6. As shown in these figures, the radioactivity observed indicates an initial increase until around 10 minutes after injection, followed by a rapid decrease until about 15 to 25 minutes after injection. The first decreased phase was then followed by another decline of the second phase of much slower rate. In general, the first phase clearance of radioactivity was rapid, especially in the 2 pregnant and 3 cyclic cows at each stage of day 8 of a 20-day cycle (Cow No. 216), of

Table 1. Biological Half Life of Injected 4-¹⁴C-progesterone in the Peripheral Blood of Cows (in Minutes)

Cow No.	Stage	Dose of ¹⁴ C-progesterone injected (μCi)	1/2 T.O. time of injected ¹⁴ C-progesterone in blood (in minutes)		
			First phase	Second phase	
216	cyclic, day 8 (20-day cycle)	25	2.8	11.6	(Fig. 1)
210	cyclic, day 12 (21-day cycle)	25	2.2	10.0	(Fig. 2)
18	cyclic, day 17 (23-day cycle)	20	3.4	27.6	(Fig. 5)
18	cyclic, day 19 (23-day cycle)	25	11.1	22.4	(Fig. 6)
3	65 th day of pregnancy	25	2.4	10.7	(Fig. 3)
4	154 th day of pregnancy	25	2.3	15.0	(Fig. 4)

Fig. 1. 1/2 T.O. Time of Progesterone (Cow No. 216) on the 8th Day (20-day Cycle); 25 μCi of 4-¹⁴C-progesterone Was Injected (i.v.)

1st phase: 2.8 min.
2nd phase: 11.6 min.

Fig. 2. 1/2 T.O. Time of Progesterone (Cow No. 210) on the 12th Day (21-day Cycle); 25 μCi of 4-¹⁴C-progesterone Was Injected (i.v.)

1st phase: 2.2 min.
2nd phase: 10.0 min.

Fig. 3. 1/2 T.O. Time of Progesterone (Cow No. 3) at 65th Day of Pregnancy; 25 μ Ci of 4- 14 C-progesterone Was Injected (i.v.)

1st phase: 2.4 min.

2nd phase: 10.7 min.

Fig. 4. 1/2 T.O. Time of Progesterone (Cow No. 4) at 154th Day of Pregnancy; 25 μ Ci of 4- 14 C-progesterone Was injected (i.v.)

1st phase: 2.3 min.

2nd phase: 15.0 min.

Fig. 5. 1/2 T.O. Time of Progesterone (Cow No. 18) on the 17th Day(23-day Cycle); 20 μ Ci of 4- 14 C-progesterone Was Injected (i.v.)

1st phase: 3.4 min. 2nd phase: 27.6 min.

Fig. 6. $1/2$ T.O. Time of Progesterone (Cow No. 18) on the 19th Day (23-day Cycle); $25 \mu\text{Ci}$ of $4\text{-}^{14}\text{C}$ -progesterone Was Injected (i.v.)

1st phase: 11.1 min. 2nd phase: 22.4 min.

day 12 of a 21-day cycle (No. 210) and of day 17 of a 23-day cycle (No. 18). In these 5 cows, except No. 18, corpora lutea should be considered to be functionally active. In cow No. 18, rectal palpation of the ovaries revealed that the corpus luteum was still large as it was in the functional stage, but that it was a little harder than usual or that it was going to shrink. In other words, it appeared to have begun regressing in the palpation of the corpus luteum *via* rectum on the day of the experiment (day 17 of a 23-day cycle).

In these 5 cows, the half life of the injected radioactive progesterone was strikingly short, or 2.2 to 3.4 minutes at the first clearance, but was settled down to 10.0 to 27.6 minutes at the second phase of clearance. In only one case in this experiment, at day 19 of a 23-day cycle in cow No. 18, both clearance rates were rather low, being with half life of 11.1 minutes at the first and 22.4 minutes at the second phase. A crossing result was obtained however, between two different days of cycle in the same cow No. 18; at the second phase clearance, the half life of the sample at day 19 was shorter than that at day 17 when the corpus luteum appeared to be functional though partially.

Table 1 shows the half life of progesterone intravenously injected, in the peripheral blood in all the experimental cows.

DISCUSSION

MILLER *et al.*⁵⁾ have regarded the first rapid clearance of radioactivity as mixing or equilibration phase. The author however, would like to have a different conception. The initial increase until around 10 minutes following injection in this experiment may have to be considered as the equilibration phase. This conception could be introduced

from the results given by SOVIA *et al.*¹¹⁾ in the human being and by SHORT and ROWELL¹⁰⁾ in the ewe. Especially the latter reported a rapid clearance of progesterone and 20α -hydroxypregn-4-en-3-one (20α -ol), both radioactive, after intravenous injection of ^{14}C -progesterone.

Furthermore, in his experiment, with intravenous injection of 50 and 100 mg of cold progesterone, BRUSH¹⁾ indicated also a very rapid conversion of progesterone into 20α -ol in the ewe.

SHORT and ROWELL¹⁰⁾ mentioned further that the independent clearance rate of both radioactive progesterone and 20α -ol in the pregnant ewe, following intravenous injection of ^{14}C -progesterone presented an appearance of almost a single phase, except a fall in concentration of 20α -ol in the blood of a sample collected on the 115th day of gestation. Clearance of progesterone and 20α -ol started at a time around 5 minutes after injection. In the ewe, the half life of progesterone was 7.3 and 8.1 minutes, and that of 20α -ol, 15.4 minutes.

The half life of 20α -ol seems to be approximately twice as long as that of progesterone in their experiment. It could be assumed that if these two clearance rates had been combined together, the whole clearance rate would have shown a two-phase appearance. From these facts and the results obtained from this experiment, the author would like to regard that the clearance of injected progesterone started at the first phase, accompanying conversion of some of the hormone in 20β -ol, in this experiment. The radioactivity observed in the plasma in this experiment should include that of progesterone itself, of 20β -ol⁹⁾, and possibly of trace amounts of a few other metabolites which are extractable with ether.

Thus, if the first clearance phase could be regarded as the starting clearance phase of injected progesterone, the results would show the very short half life of progesterone in the peripheral blood in cows, even though the endogenous progesterone level in the peripheral blood seemed to be rather low in this species^{2,3,8)}.

There seemed to be no significant difference in metabolic rate of progesterone between the functional luteal stage of the cycle and the early and middle gestation period in cows. Only at the regressing stage of the luteal phase, the metabolic rate of progesterone appeared to slow down.

Again, SHORT and ROWELL¹⁰⁾ have introduced an improved type of study concerning estimation of the half life of steroid hormones. By this type of study, the conversion and also the separated clearance rate or biological half life could be explained with much more simplicity. Efforts will be made to lead further studies to this direction.

SUMMARY

Cows at different luteal stages of the ovarian cycle and in gestation were injected intravenously with $4\text{-}^{14}\text{C}$ -progesterone, so that the biological half life of injected progesterone may be estimated radiochemically. A two phase clearance of radioactivity was observed in the blood of each cow. A very rapid clearance of radioactivity was noted especially in cows at the functional luteal stage of the cycle and in the early and middle gestation period. There seemed to be no significant difference in clearance rate of progesterone between these two different stages in each cow. In these stages when progesterone secretion must be active, the biological half life of injected ^{14}C -progesterone was determined as short in general. It was very short at the first clearance phase or 2.2 to 3.4 minutes but was settled down to 10.0 to 27.6 minutes at the following phase. In a cow at the regressing luteal phase, the biological half life was rather long, being

11.1 minutes at the first phase and 22.4 minutes at the second phase.

In general, the secretion of progesterone seemed to be very rapid in the cow, though the blood level of this hormone was considered rather low in this species.

ACKNOWLEDGMENTS

This study was supported partially by fellowship grants from the Rockefeller Foundation and the Population Council, both in New York, N. Y. The author gratefully acknowledges these supports. It is also his great pleasure to appreciate the consideration and help given by Dr. C. W. TURNER and his staff of the Department of Dairy Husbandry, University of Missouri, Columbia, Missouri, U.S.A.

REFERENCES

- 1) BRUSH, M. G. (1963): *J. Endocr.* **26**, 65~73.
- 2) GOMES, W. R., ESTERGREEN, V. L., FROST, O. L. and ERD, R. E. (1963): *J. Dairy Sci.*, **46**, 533~558.
- 3) IMORI, T. (1967): Unpublished data.
- 4) LOY, R. G., McSHAN, W. H. and CASIDA, L. E. (1957): *J. biol. Chem.*, **229**, 583~597.
- 5) MILLER, W. R., WILLIAMS, R., PIPES, G. W. and TURNER, C. W. (1963): *J. Dairy Sci.*, **46**, 1402~1404.
- 6) PEARLMAN, W. H. (1957): *Ciba Foundation Colloq. Endocr.*, **11**, 233~248.
- 7) SANDBERG, A. A. and SLAUNWHITE, W. R. (1958): *J. clin. Endocr.*, **18**, 253~265.
- 8) SHORT, R. V. (1958): *J. Endocr.*, **16**, 426~428.
- 9) SHORT, R. V. and EATON, B. (1959): *J. Endocr.*, **18**, 418~425.
- 10) SHORT, R. V. and ROWELL, J. G. (1962): *J. Endocr.*, **25**, 369~374.
- 11) SOVIA, K. U., HUSKINS, A. L. and MCCAFFERY, R. E. (1959): *Acta endocr. (Kbh.)*, **31**, 400~404.

ウシの末梢血中の Progesterone の Biological Half Life について

藺 守 龍 雄

大阪府立大学農学部家畜外科学教室
(昭和 42 年 3 月 27 日 受付)

Steroid Hormone の分泌率を推定する一手段として、静脈内に注入した微量の外性ホルモンが血中から消失する率を測定する方法、すなわち Biological Half Life 測定法が、近時唱えられ出した。

筆者は、 $4\text{-}^{14}\text{C}$ -Progesterone の $20\sim 25\ \mu\text{Ci}$ ($125\sim 171\ \mu\text{g}$ に相当) をウシの静脈内に注入し、注射後 $47\sim 80$ 分の間に $8\sim 9$ 回の静脈血 (各 115ml) を採取、約半量の血漿を分離してから、これをエーテルで抽出、アルミナ・カラムで部分的精製を行なったのち、Tri-Carb liquid scintillation spectrometer にかけて、血中からの放射能量の消失率を測定し、放射性 Progesterone の biological half life ($1/2\ \text{T.O. time}$) を算出した。

その結果、ウシでは $1/2\ \text{T.O. time}$ はきわめて短いことがわかった。従って Progesterone 分泌は盛んであることが推定される。

とくに黄体開花期と妊娠中のもの (初, 中期) で

は、biological half life の値に有意差はみられなかった。注射された Progesterone の血中からの消失は、二相性を示した。これは主として $20\beta\text{-OH}$ -Progesterone をきわめて早く代謝物として作り出す結果と考える。その第 1 相における $1/2\ \text{T.O. time}$ は、黄体開花期のもの 3 例 (No. 18 の第 17 日目は退行期にはいろいろとする時期と考えられた) と妊娠の 2 例 (第 65 と 154 日目) では、 $2.2\sim 3.4$ 分というきわめて短い値を示した。第 2 相に至って、これらは $10.0\sim 27.4$ 分となった。

黄体退行期の一例 (No. 18 の第 19 日目) では、第 1 相で 11.1 分、第 2 相では 22.4 分を示した。

ウシの静脈血中 Progesterone の量自体は低いと見なされてはいるが、分泌率および代謝速度については、実験値は分泌が盛んで代謝率も速いことを示したものだといえる。