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CHANGES IN PLASMA PROGESTERONE LEVELS DURING STORAGE OF HEPARINIZED WHOLE BLOOD FROM COW, HORSE, DOG AND PIG

By

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OLTNER, R. and L.-E. EDQVIST: *Changes in plasma progesterone levels during storage of heparinized whole blood from cow, horse, dog and pig.* Acta vet. scand. 1982, *23*, 1—8. — Progesterone concentrations in heparinized plasma harvested immediately after blood collection were compared with levels obtained after storage of the corresponding whole blood for 2 h, 4 h, 6 h, 1 day, 2 days and 5 days at room temperature and in a refrigerator. The blood was taken during the luteal phase from 4 dogs, 4 horses, 4 pigs and 8 cows. For 4 cows the storage time was extended to 9 and 20 days. No significant effect of whole blood storage time on plasma progesterone concentrations could be shown for dogs or pigs. For the horse a slight but significant decrease was demonstrated when the blood was kept at room temperature. For the cow, however, a dramatic decrease was observed even when the blood was stored in the refrigerator. Following incubation of cow's blood at room temperature, progesterone levels were close to zero after 1—2 days. By further extending the storage period, a reappearance of assayable progesterone could be elicited. For all species it was found that the storage of *plasma* at room temperature for 5—9 days did not change the progesterone concentrations.

progesterone; storage; cow; horse; pig; dog.

Vahdat et al. (1979) commented that greater emphasis has been placed on the validation of RIA methods for hormone determinations than on the possible influence of sample handling before analysis. In some cases the treatment and storage of samples prior to analysis can influence the final result more than does the precision and accuracy of the RIA method(s) employed. Thus it has been shown that in vitro incubation of bovine and ovine whole blood results in rapidly decreasing concentrations of the

blood plasma progesterone* content (e.g. *Short* 1958, *Van der Molen & Groen* 1968).

The present investigation was undertaken to further elucidate the pattern and magnitude of these changes in the cow. For purposes of comparison incubations were also made using blood from horses, dogs and pigs.

MATERIALS AND METHODS

Using sodium heparin as anticoagulant, (2 IE/ml blood), jugular vein blood was drawn from 4 bitches, 4 cows, 4 mares and 8 cows, all in the luteal phase of the oestrus cycle. After sampling, the blood was mixed thoroughly and distributed into tubes, 5—10 ml in each. Two tubes were immediately centrifugated (within 30 min of sampling) and the plasma was separated. One portion of the plasma was stored frozen (—18°C) until analysed and constitutes the 0-sample. For comparison the remaining 0-plasma was kept at room temperature for 5—9 days. Of the remaining tubes, containing whole blood, half were stored at room temperature and half in a refrigerator at 4°C. After 2 h, 4 h, 6 h, 1 day, 2 days and 5 days, respectively, plasma was separated by centrifugation and stored in the freezer.

The effect of a further extended storage time on the progesterone content was tested in 4 of the cows. Additional blood tubes were therefore stored for 9 and 20 days, respectively, before separation of the plasma.

All plasma samples from each individual animal were analysed (in duplicate) in the same assay to avoid interassay variation. The RIA method employed for the determination of progesterone has been described previously (*Bosu et al.* 1976, *Castellanos* 1979). The initial progesterone concentration of each animal, determined in the frozen 0-plasma, was defined as 100 % and the effects of storage on progesterone concentrations are therefore expressed as changes in mean % (and standard deviation, s) in relation to the individual initial value. The effect of storage time was analysed separately for each species and temperature of storage using the Statistical Analysis System (*Helwig*

* The following trivial names have been used throughout this paper:
20α-dihydroxyprogesterone: 20α-hydroxy-4-pregnen-3-one
20β-dihydroxyprogesterone: 20β-hydroxy-4-pregnen-3-one
progesterone: 4-pregnen-3,20-dione.

& Council 1979). To describe the data the following model was used:

$Y_{ijk} = \mu + a_i + t_j + e_{ijk}$

where

Y_{ijk} = the ijkth observation
μ = general mean
a_i = effect of the ith animal ($i = 1, 2, \ldots 4$ or $1, 2, \ldots 8$)
t_j = effect of the jth storage time ($j = 1, 2, \ldots 7$ or $1, 2, \ldots 9$)
e_{ijk} = residual term with variance σ_e^2

A reduction in plasma progesterone concentration in cow's blood following storage could be explained as reflecting a change in the distribution of progesterone between plasma and erythrocytes (*Vahdat et al.* 1979). To test this hypothesis whole blood from 2 cows was incubated at 20°C with added tritiated progesterone (1,2,6,7-^{3}H(N)-progesterone, 20,000 dpm/ml blood; New England Nuclear). Plasma was separated immediately (0-sample) and after 2 h, 4 h, 6 h, 1 day, 2 days and 5 days of storage. Until analysed, the plasma was kept frozen. Changes in the amount of antibody reactive ^{3}H-progesterone in the samples were estimated by using the standard assay procedure including extraction with petroleum ether but without adding tracer. In addition, the total radioactivity of each plasma sample was measured in the scintillation counter. In both cases the individual original counts were considered as 100 %.

RESULTS

Dog and pig

Mean plasma progesterone concentrations in 0-samples were 22.3 nmol/l (range 11.1—36.2) and 63.8 nmol/l (range 40.4—86.9) for dogs and pigs, respectively.

Considering the intra-assay variation, no obvious changes in the plasma progesterone occurred even when the blood was stored at room temperature (Fig. 1 a, b).

Horse

The mean plasma progesterone concentration in the 0-samples was 24.2 nmol/l (range 23.5—24.8). Although the variation between animals was marked, a definite decrease in the progesterone levels after storage at room temperature could be observed (Fig. 1 c). When compared with original values this de-

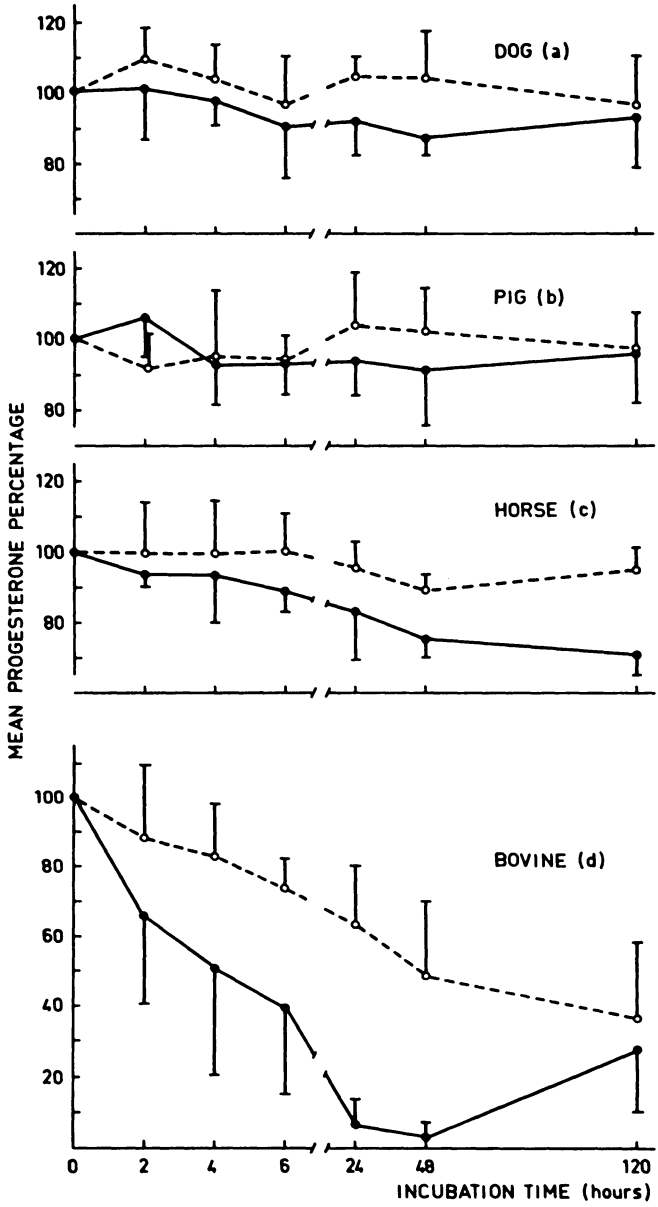

Figure 1. Mean plasma progesterone percentage (and s) after storage of whole blood from 4 dogs, 4 pigs, 4 horses and 8 cows at 20°C (●——●) and at 4°C (○----○).

crease was highly significant ($P < 0.001$) following 2 and 5 days of storage.

Cow

For the 8 cows, the mean plasma progesterone concentration in the 0-samples was 15.4 nmol/l (range 12.2—20.7). Following 2 h and 6 h of incubation at 20°C and 4°C, respectively, the decrease in progesterone concentration was highly significant ($P < 0.001$; Fig. 1 d). When the blood was stored at room temperature, progesterone levels were close to zero after 1—2 days. From the 2nd to the 5th day of storage, however, a significant increase ($P < 0.01$) was observed. In the 4 cows where the incubation period was extended, the progesterone increase was further accentuated at 9 days (44.8 ± 4.0 %, mean % ± s). Following storage of the blood at 20°C for 20 days, however, a decrease occurred (34.9 ± 19.0 %). As can be seen in Fig. 1 d the decrease in progesterone levels in blood kept at 4°C was slower than at 20°C but approximately linear. Extending the storage time at 4°C to 9 and 20 days, thus further reduced plasma progesterone levels to 32.8 ± 11.4 % and 20.2 ± 13.1 %, respectively.

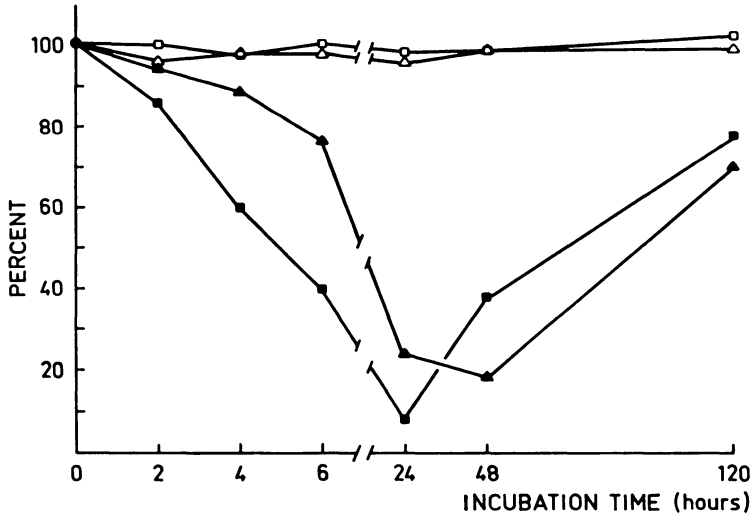

Figure 2. Total plasma radioactivity (□——□; △——△) and antibody-bound ^{3}H-progesterone (■——■; ▲——▲) from 2 cows after incubation (20°C) of whole blood with added ^{3}H-progesterone.

When ^{3}H-progesterone was incubated with whole blood from 2 cows (at 20°C) a marked decrease in the plasma levels of antibody-bound radioactivity took place during the first day (Fig. 2). After 5 days of incubation, however, the amounts of bound radioactivity were higher than at 1 and 2 days.

In Fig. 2 it can also be seen that the total radioactivity in plasma separated after increasing duration of incubation did not change.

General

For all species studied it was found that the progesterone levels in 0-plasma kept frozen or at room temperature for 5—9 days before analysis were very similar and well within the variation of the assay.

DISCUSSION

Storage of heparinized blood from dogs and pigs for 5 days did not markedly affect the plasma progesterone levels, which agrees with the findings of *Van der Molen & Groen* (1968) and *Holdsworth* (1980).

The initial decrease in progesterone levels observed in cow's blood also tallies with the findings of others (*Short* 1958, *Delahaut et al.* 1979, *Vahdat et al.* 1979, *Holdsworth* 1980, *Owens et al.* 1980). Following incubation of blood at 40°C, *Vahdat et al.* (1979) found an increase ($P < 0.05$) in progesterone between 6 h and 24 h. At the lower temperature used here (20°C) the increase did not occur until after 1—2 days, but was very pronounced (Figs. 1 d and 2).

Short (1958) related the initial decrease to a conversion of progesterone to a product tentatively identified as 20β-dihydroxyprogesterone. *Van der Molen & Groen* (1968) working with different animal species, identified the metabolite as 20α-dihydroxyprogesterone. They also showed that the transformation was glucose dependent and that in the absence of glucose, 20α-dihydroxyprogesterone was oxidized back to progesterone. Measurements of glucose in the cow samples in the present study agree well with this concept. In the blood kept at room temperature, glucose concentrations were thus approximately 0 after 2 days, whereas when the blood was kept in the refrigerator only minor decreases in plasma glucose levels were observed even after 5 days of storage.

No attempt was made here to identify the conversion product, but it would seem most likely, that the decrease in progesterone in cow's blood during storage is due to 20α-hydroxysteroid dehydrogenase activity associated with the blood cell fraction.

When ^{3}H-progesterone was added to cow's blood the constant radioactivity in plasma harvested after increasing periods of time shows that binding of progesterone to erythrocytes does not supply an explanation for the decreasing progesterone concentrations. The decrease in antibody-bound radioactivity with time, however, indicates the conversion of progesterone into a product with a low affinity for the antibody (probably 20α-dihydroxyprogesterone).

CONCLUSION

It has been established that in the four species studied, no obvious metabolism of progesterone occurred in plasma separated from the blood cell fraction. In the case of whole blood from dog and pig, time and temperature of storage had a negligible effect on the plasma progesterone levels. Progesterone levels in plasma obtained from horse blood kept at room temperature declined with time, but from a clinical point of view the changes are of minor importance.

When bovine blood is considered, however, it is obvious that storage time and temperature excert considerable influence on the progesterone level. If this is not taken into account, incorrect conclusions can be drawn. It is therefore imperative to separate the plasma rapidly after blood collection, or to inhibit the enzymatic conversion activity in the whole-blood samples (*Delahaut et al.* 1979, *Holdsworth* 1980). Even if these precautions have not been taken, however, an answer to the question whether or not a cow has an active corpus luteum can still be obtained by storing the blood sample at room temperature until it is 5—9 days old before centrifugation and plasma removal.

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SAMMANFATTNING

Förändringar i plasmaprogesteronnivån vid lagring av hepariniserat helblod från ko, häst, hund och gris.

Progesteronkoncentrationen i heparinplasma avskiljd omedelbart efter provtagningen jämfördes med nivåer erhållna efter lagring av motsvarande helblod i 2 h, 4 h, 6 h, 1 dag, 2 dagar och 5 dagar i rumstemperatur och i kylskåp. Blod togs från 4 hundar, 4 hästar, 4 grisar och 8 kor som alla befann sig i luteal fas. För 4 av korna utsträcktes lagringstiden till 9 och 20 dagar. Hos hund och gris påverkades inte plasmaprogesteronkoncentrationen märkbart vid lagring av helblodet. Hos häst påvisades en relativt lindrig men statistiskt signifikant nedgång efter lagring i rustemperatur. För ko kunde däremot konstateras en dramatisk nedgång i plasmaprogesteronkoncentrationen även efter kylskåpslagring av blodet. Vid rumslagring var progesteronnivåerna nära noll efter 1—2 dagar för att därefter åter öka.

För alla undersökta djurslag gällde att lagring av *plasma* i rumstempertur i 5—9 dagar inte påverkade progesteronkoncentrationen.

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