

A STUDY OF THE REACTION OF THE WHITE BLOOD  
CORPUSCLES IN BOVINES AT PARTURITION WITH A  
CONSIDERATION OF THE EVIDENCE OF THE ACTION  
OF THE ADRENAL CORTICAL HORMONE (CORTISONE)

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(With 13 Figures in the Text)

When carrying out researches on the infection of cattle with *Trichomonas foetus* we observed that animals sensitized either by infection or by vaccination with *T. foetus* antigen lost the skin sensitivity at the time of parturition.

In the sensitive animal, circulating antibody fixes in the skin and when antigen or hapten is injected intradermally a measurable bleb is formed. An animal whose skin reacts in this way is considered to have been sensitized to the antigen in question (Kerr, 1944).

The inhibition of the skin response could be brought about by a number of different means; e.g. specifically by the injection of further doses of antigen of appropriate magnitude and non-specifically by the injection of cortisone (C11 hydroxy steroids) or sphingomyelin and at parturition.

All these agents produced a very similar effect on the lymphocyte/neutrophil ratio. The lymphocytes were depressed in number and the neutrophils usually rose. In other words, we found associated with the loss of skin sensitivity the type of white corpuscle response characteristic of the reaction to the injection of cortisone (White & Dougherty, 1946; Hills, Forsham & Finch, 1948; Kerr, McGirr & Robertson, 1949).

It seemed of interest to study the white blood corpuscles in a larger number of cases at parturition.

#### MATERIAL AND METHODS

Blood was taken by syringe from the jugular vein. Films were made and dried in air, fixed in methyl alcohol, and stained with Giemsa's stain.

From each film 200 white blood corpuscles were counted. Total white cell counts were made in citrated blood in the Neubauer counting chamber. Consecutive differential total counts were plotted graphically. The important thing in this work is the timing of samples which involves close observation of the animal. Counts must be made over the actual calving period and for 48 hr. or thereabouts afterwards.

\* The work was carried out while one of us (M.R.) was in receipt of a grant from the Agricultural Research Council.

OBSERVATIONS

In the normal bovine lymphocytes are consistently and regularly more numerous than neutrophils. Moderate excitement, such as alarm at being handled, or being driven too rapidly or a struggle at the time of bleeding, may produce a slightly raised neutrophil count. Acute excitement or fear may send the neutrophil count up very high. The most astonishing neutrophil counts can be found in animals after transportation in a motor van. The animals used for these observations were expertly handled and became very tame and indifferent to the taking of samples.

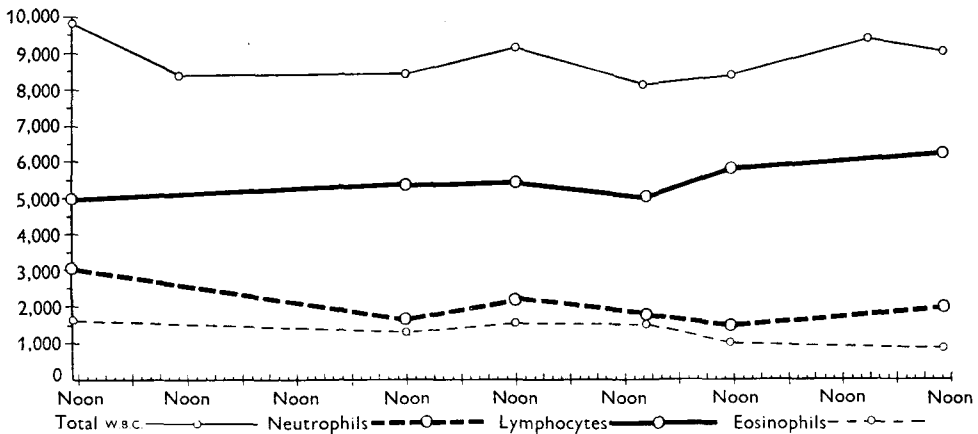


Fig. 1. Differential (white blood corpuscles) counts. Normal heifer. Middle period of oestrous cycle. (E9.)

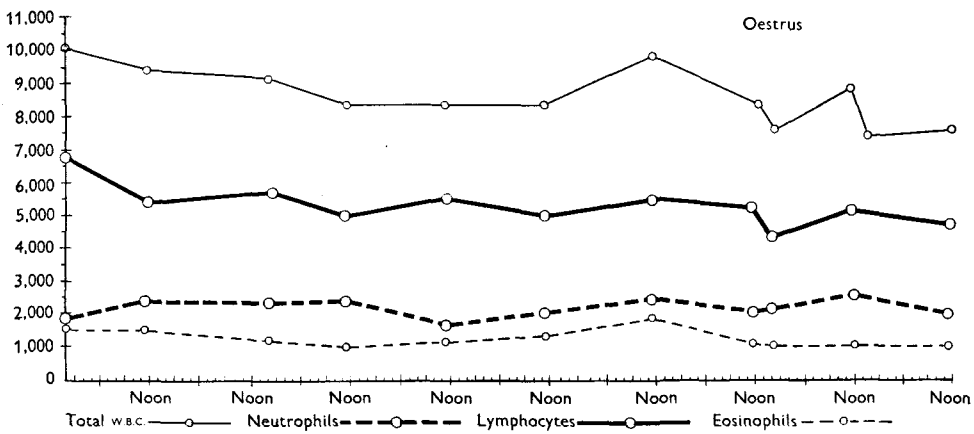


Fig. 2. w.b.c. count. Oestrus in normal heifer. Same animal as in Fig. 1 (E9).

WHITE BLOOD CELL COUNTS FROM A NORMAL ANIMAL DURING THE OESTRUS CYCLE

It was considered necessary to a proper understanding of the white blood corpuscle relations to observe a normal animal through the oestrous cycle.

E9 (Figs. 1, 2). The animal, E9, was a heifer which was ovulating at normal intervals. Counts were made from the 16th day before the appearance of oestrus

to the 3rd day after. Figs. 1 and 2 also show the relatively stable level of counts in a normal animal. We have never observed any marked reaction during oestrus in the animals we have studied.

#### WHITE BLOOD CELL COUNTS BEFORE, DURING AND AFTER CALVING

Of fifteen animals observed, six were regarded as typical in that they calved normally, their white blood corpuscle counts showing a drop in lymphocytes and a rise in neutrophils. From these six animals seven complete records were obtained, for one calved twice in consecutive years. These animals fall into two groups: Group 1, consisting of four cows *K 2*, *E 5*, *K 6* and *D 4*, which showed a 'cross-over' of the lymphocyte/neutrophil ratio after calving; and Group 2, consisting of cow *E 11*, and on two occasions cow *D 13* which showed a reversed ratio before parturition. These seven sets of observations are considered below.

Of the remaining nine animals, four conformed to the pattern of Group 1, but are not considered in detail because their counts were not complete; and two, cows *E 1* and *K 3*, are considered separately because their behaviour was atypical. The remaining three animals are disregarded because, owing to the inconvenient time of calving and the intervention of other duties, counts were not obtained from them over the crucial period.

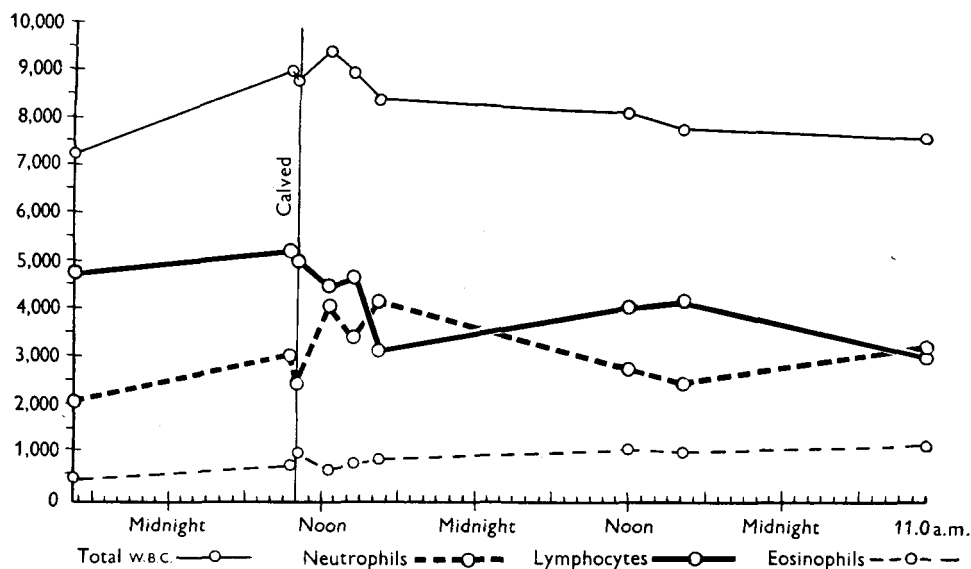


Fig. 3. w.b.c. count during calving period. Note lymphopenia at 6 hr. (*K 2*.)

#### Group 1. Animals in which the cross-over takes place after calving

*Cow K 2* (Fig. 3). An example of relatively late cross-over. This animal had a high lymphocyte count at all times and the relation of the cells 18 hr. before calving and at two counts made 30 min. before removal of the calf and a few minutes after, were not substantially different from that observed during the previous 2 months. At 6 hr. after calving the cross-over had taken place. It was not very extensive

but it was quite definite. This animal had been vaccinated during the last months of pregnancy and had become skin sensitive. The skin response was much reduced 6 hr. after calving and was negative at 48 hr. It was still negative on the 4th day but the sensitivity of the reaction had returned when a test was made 5 days later on the 9th day.

It should be noted that the testing substance is a hapten with apparently no antigenic properties. It was prepared by Dr Morgan and Dr Feinberg from crude freeze dried trichomonas organisms. This substance causes no appreciable alteration in the immune relations in the animal and has been of the greatest service in this research.

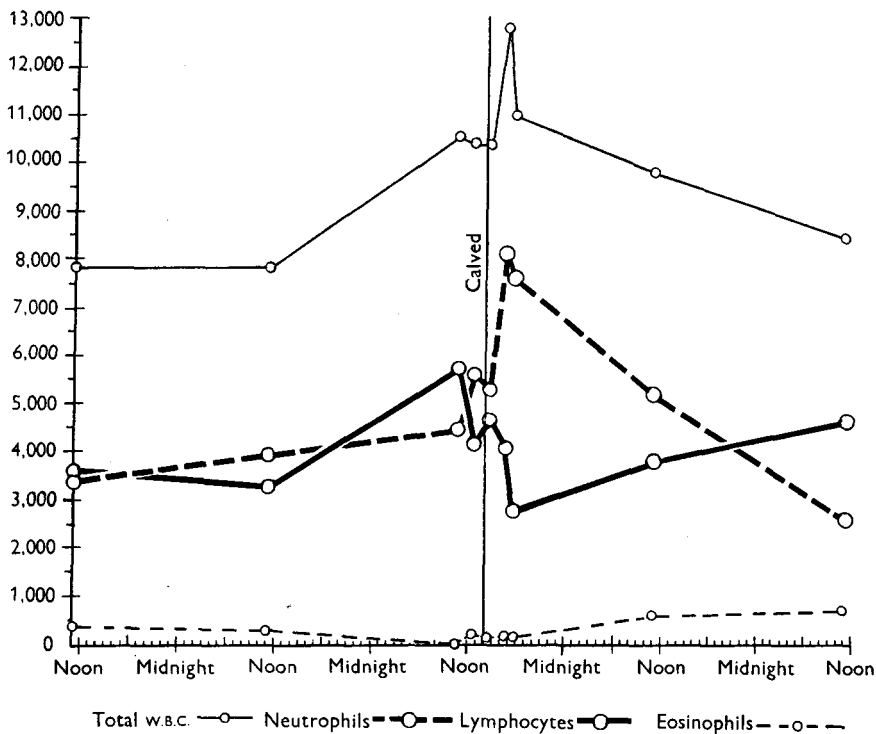


Fig. 4. w.B.C. count during calving period. Marked cross-over of neutrophils and lymphocytes. Lymphopenia at  $3\frac{1}{2}$  hr. (E5.)

*Cow E 5* (Fig. 4). The animal was a normal heifer with her first calf. In the 2 days before calving, lymphocytes and neutrophils approached one another, but 4 hr. before calving when parturition was beginning and the totals had risen somewhat the lymphocytes were above the neutrophils. The decrease in the number of lymphocytes had started 2 hr. later, but the lymphopenia was not developing fully until  $3\frac{1}{2}$  hr. after the delivery of the calf. It is not certain that the peak of the cellular reaction was noted in this example.

*Cow K 6* (Fig. 5). This animal was a normal cow which had already had several calves. Counts had been taken from the 13th day before calving up to the 3rd day and the graph, which is not reproduced, ran a steady course with totals between 6500 and 8000. The lymphocytes were 4000 to 5000 and neutrophils

rather low, between 1000 and 2000. Twenty-four hours before calving (Fig. 5) this state of affairs was maintained. Half an hour before the actual delivery of the calf the lymphocytes and neutrophils had approached each other, and 4½ hr. later the lymphocytes had begun to drop and the neutrophils to rise. At 6 hr. there was a marked rise in the neutrophils and a slight rise in the lymphocytes which, however, was probably not significant as the lymphocytes were at a definitely low figure 24 hr. after calving. At 48 hr. the white corpuscles had resumed the normal pre-calving relation.

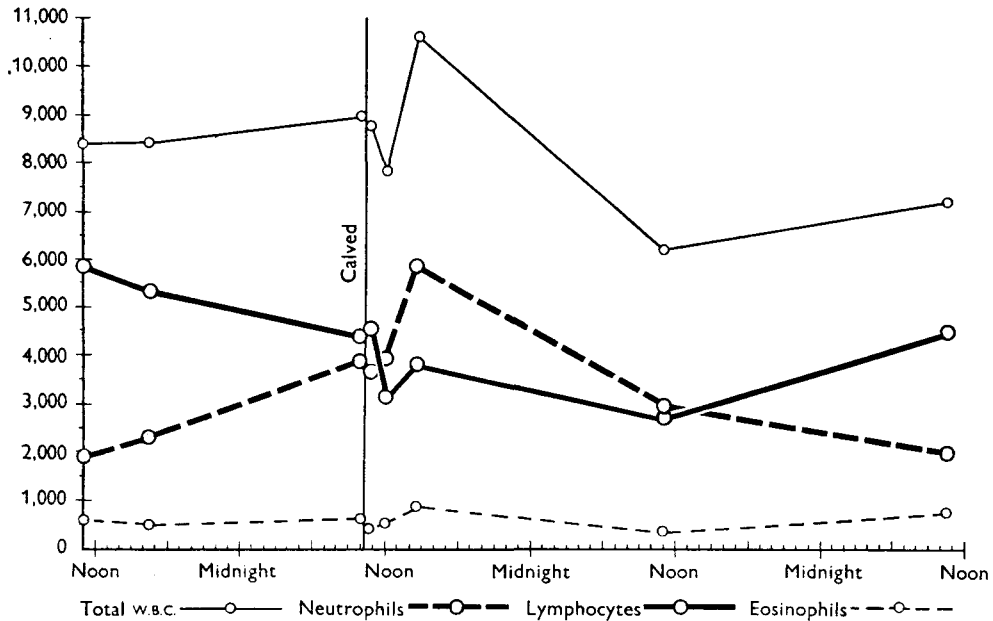


Fig. 5. w.b.c. count during calving period. Cross-over of neutrophils and lymphocytes. (K 6.)

*Cow D4* (Fig. 6). This animal was a sensitized cow. She had calved on 28 July 1945 and 21 October 1946, but counts were not made. She was very carefully observed during the last month of pregnancy in 1948 with her third calf, and white blood corpuscle counts were begun 13 days before calving. It was noted that this animal showed signs of being about to calve at any time during the last month before actual parturition took place. Generally speaking, the cows had given little evidence of pre-calving signs. She was in perfect health and calving was without difficulty.

The graph of the pre-calving period showed slightly low lymphocytes (3000–4000) from the 13th day to the 7th day. During the last 5 days lymphocytes rose to the 5000 level with some tendency to temporary approaches of the neutrophils to very nearly the same number. Sixteen hours before calving (Fig. 6) lymphocytes and neutrophils were both high and very close together. The cow calved at 3.30 a.m. and 30 min. later the count showed a very high total produced by an increase in neutrophils. The lymphocytes remained steady at between 5000 and 6000. The peak of the cellular reaction occurred 7 hr. later when the lymphocytes dropped

from 5300 to 2976. At 10 hr. the cells began to approach one another in number, the lymphocytes increasing and the neutrophils declining. This process continued through the next 24 hr. and the normal relations of lymphocytes to neutrophils were re-established on the 3rd day.

The skin response declined gradually during the pre-calving period, showing a rise of 5 mm. at 13 days, 4 mm. at 7 days, and of 3 mm. at 12 hr. before the delivery of the calf, and no response at all 10 hr. after calving. The return of sensitivity

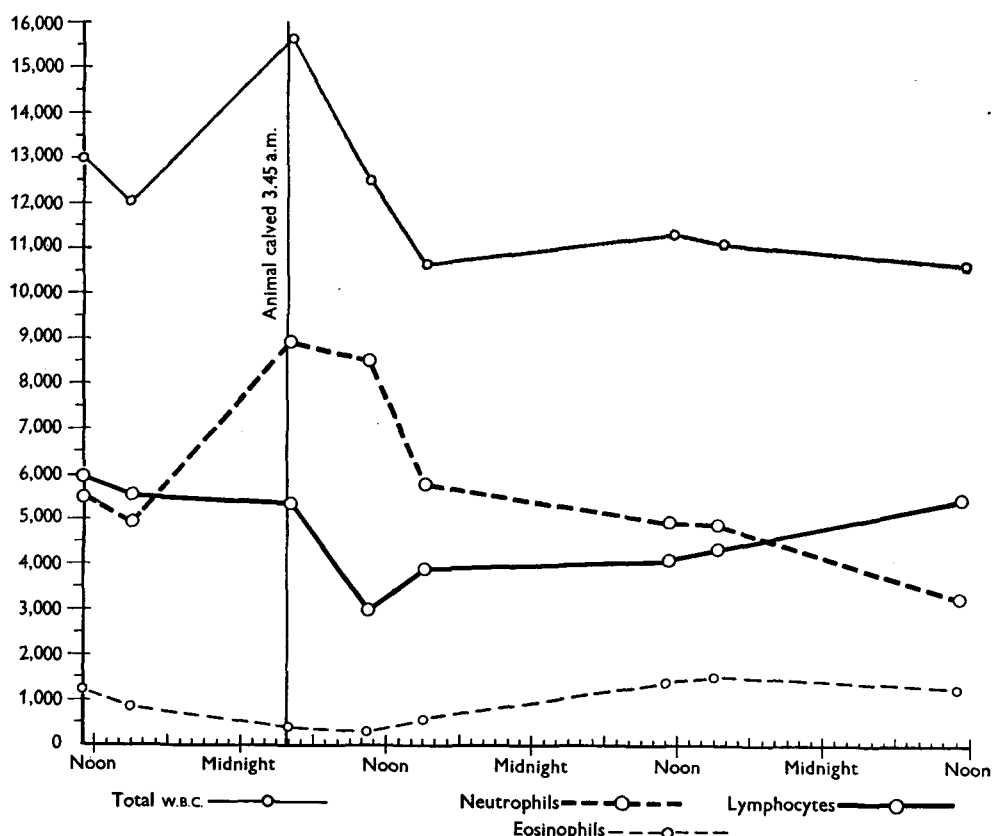


Fig. 6. w.b.c. count during calving period. Neutrophils rise at time of delivery. Lymphocytes remain steady. Note lymphopenia at 7 hr. (D4.)

was slow in this case. The response was still poor 9 days after calving. When tested 1 month after parturition sensitivity had returned and the skin measurement showed a rise of 5 mm.

In these four characteristic graphs it should be noted that the peak of the reaction takes place in every case about 6 hr. after calving. This is what would happen if the adrenal cortical stimulation or the release of cortisone took place at the time of delivery.

In four more animals counts were incomplete, but evidence of the cross-over of lymphocytes and neutrophils was clear. One of these animals, *K4*, had been sensitized and showed inhibition of the skin reaction at the calving period.

Group 2. *Animals coming into parturition with the lymphocytes and neutrophils already in the reverse position*

*Cow E11* (Fig. 7). This was a normal in-calf cow. She calved quickly and extremely easily on the 3rd day after her arrival. The lymphocytes were low (3740) 2 days before calving. The neutrophils were already slightly above the lymphocytes. After calving, both sets of cells rose somewhat. The neutrophils began to drop in number 4 hr. after delivery and at 24 hr. the lymphocytes numbered 5368 and the neutrophils 1936, thus re-establishing the usual normal arrangement of the white blood corpuscles. There was an early lymphopenia which was not further accentuated after calving. This pattern suggests a gradual change in the relation of the cells before calving and no critical phase at the actual time of delivery.

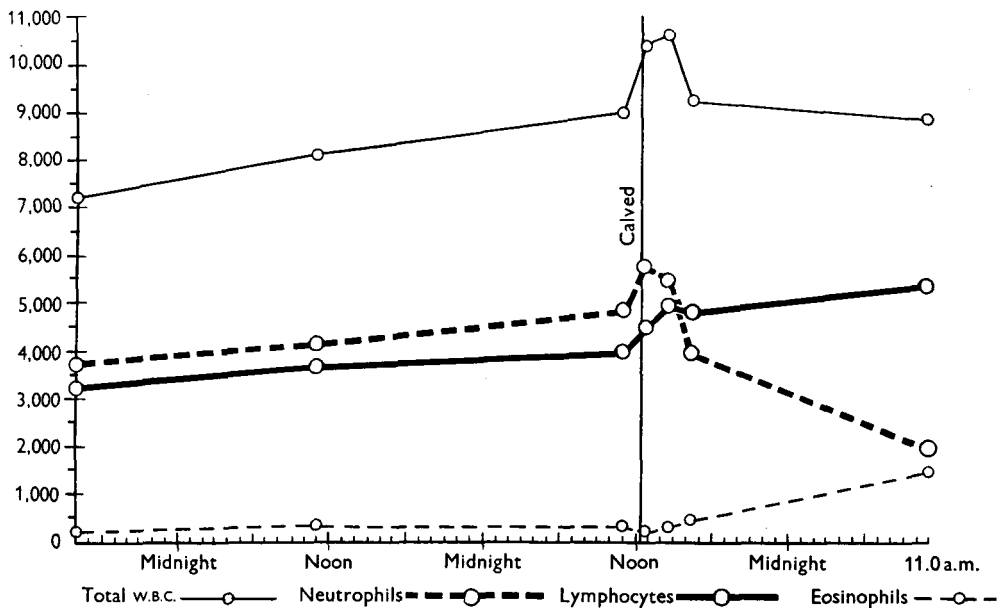


Fig. 7. w.b.c. count. Early cross-over of lymphocytes and neutrophils. Parturition was extremely easy. No further drop in lymphocytes after delivery of the calf. (*E11*.)

*Cow D13*. Two more graphs starting before calving in the reverse position were produced, this time from one animal, *D13*, in two consecutive years. In 1948, *D13* (Figs. 8, 9) had a normal distribution of white blood corpuscles 11 days before calving. The total white blood corpuscle count was 8200, the lymphocytes were 4500, the neutrophils were 2500 and the eosinophils were 1200. These relations were maintained for the next 6 days. The condition began to change 5 days before calving, and the lymphocytes dropped in number. They were now on the 3000 line instead of at 4000–5000. Nine hours before calving the lymphocytes dropped to 2900 and the neutrophils began to rise. The lymphocytes remained at this low level while the neutrophils rose to 6000 immediately after calving. Eight hours after parturition the lymphocytes were still very low and the neutrophils were high.

The lymphocytes rose after this, but the relation between them and the neutrophils remained reversed for several days.

*D13* was a sensitized animal. The inhibition of the skin reaction was complete when a test injection was made 15 hr. after delivery. Counts made for various purposes during the ensuing year showed a general tendency to relatively high neutrophil counts although the lymphocyte/neutrophil ratio remained above unity in the normal position.

*Cow D13*. In April 1949 *D13* calved again (Fig. 10). A graph was made of the total differential white blood corpuscle counts starting from what proved to be the 10th day before calving. The total white blood corpuscle counts were running at a level of 7000–8000. The lymphocytes were on the 3000 mark just below the neutrophils so that they were already in the reversed position and low in number.

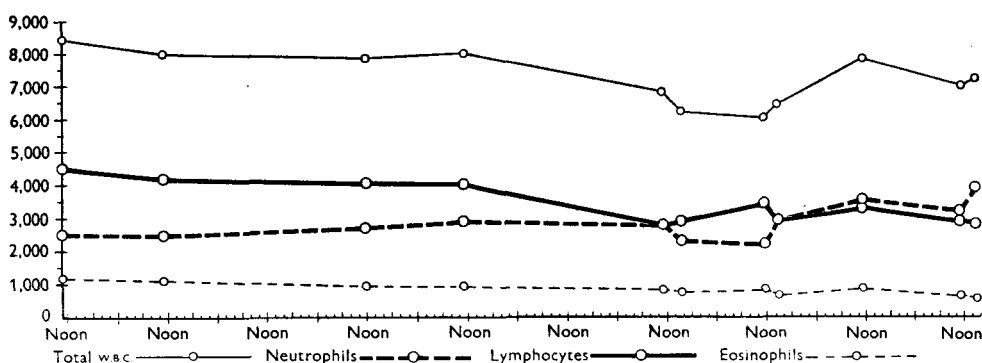


Fig. 8. W.B.C. count during pre-calving period 11 days before calving. Note drop in lymphocytes and rise in neutrophils at end of this period. (*D13*, 1948.)

This condition still persisted at 24 hr. before calving. At the moment of delivery (Fig. 10) the totals had risen and both lymphocytes and neutrophils were involved in the rise, the neutrophils being above the lymphocytes. One hour after delivery the lymphocytes were falling so that at 3½ hr. the lymphocytes were 2300 and the neutrophils 6210. This is, in fact, a typical post-calving cross-over condition with definite lymphopenia. Twenty hours later the picture had not changed very much, but at 44 hr. the lymphocytes were 4400 and the neutrophils were still high. This animal, when examined later, showed the normal lymphocyte/neutrophil ratio of more than unity, but the neutrophils were more numerous than is usual in bovines.

In this animal the skin reaction was sensitive giving a rise of 10 mm. on intradermal injection with the hapten at 5 days before calving. The reaction was inhibited and after calving showed only a rise of 2.5 and 2 mm. (which is below the positive level) when tested at 20 and 44 hr.

Two aberrant graphs remain to be considered.

*Cow E1*. The first *E1* (Fig. 11) was derived from the counts of a heifer with her first calf. The parturition was apparently without unusual clinical incidents. The animal calved at 2 a.m. and 15 min. later the lymphocytes and neutrophils were practically equal in number at 3700 with the total at 8600. Eight hours later the





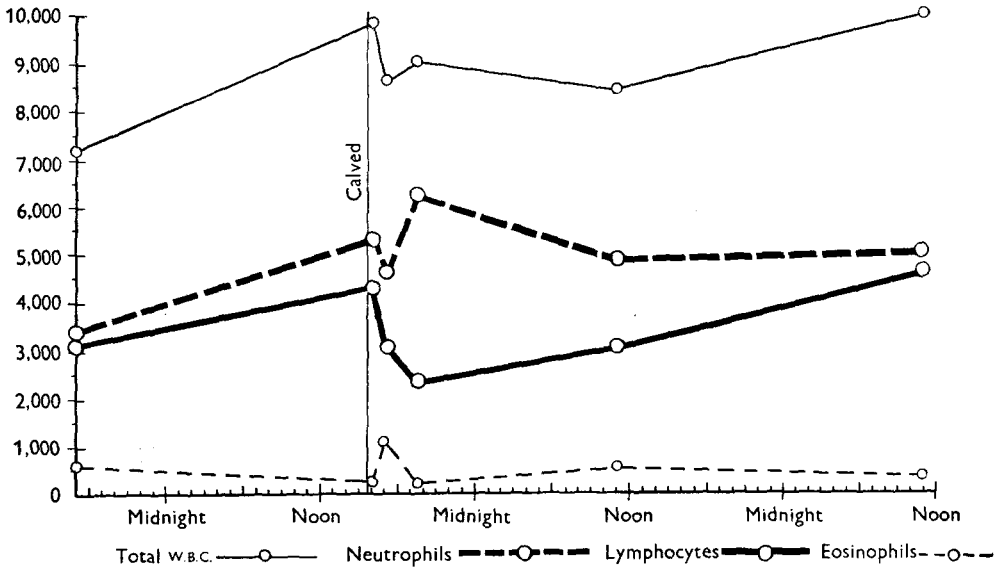


Fig. 10. w.b.c. count during calving period. Note early rise in neutrophils. Note the marked drop of lymphocytes at 3½ hr. Lymphopenia still noticeable at 20 hr. after calving. (D13, 1949.)

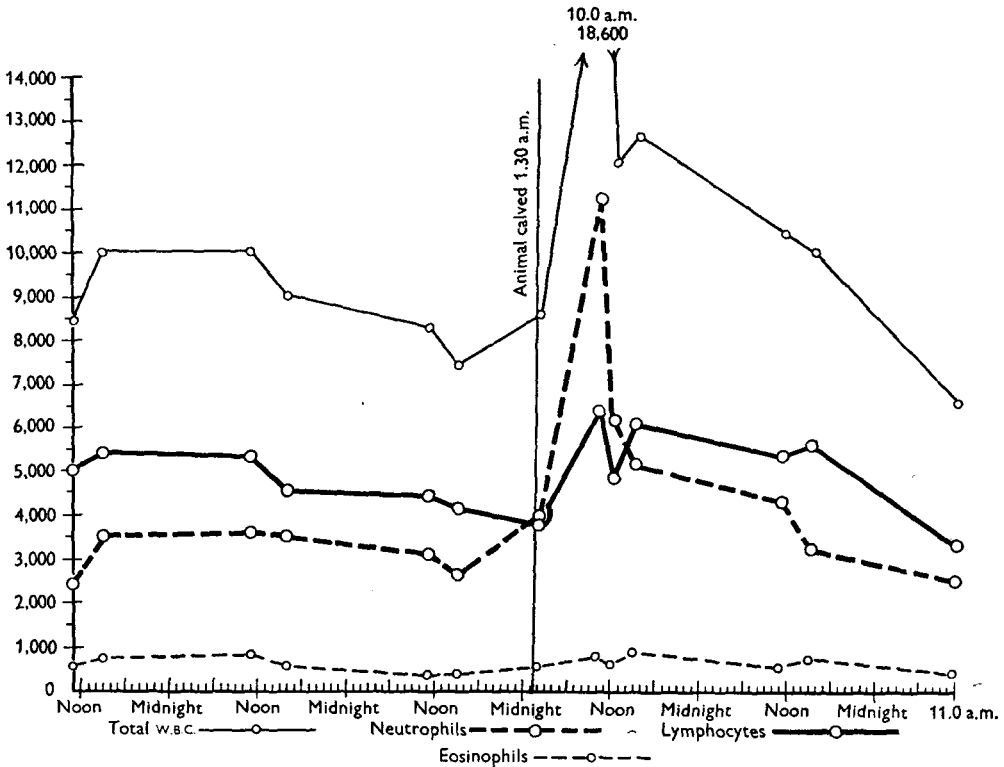


Fig. 11. w.b.c. count during calving period. Aberrant reaction. Very great increase in total count 8 hr. after calving. Neutrophils very much increased. No lymphopenia. (E1.)

count showed a very marked rise, the total now being 18,600. Both lymphocytes and neutrophils had increased in numbers; the high totals were, however, due to the neutrophils which numbered 11,600. The neutrophils dropped rapidly after that and 13 hr. after calving they were below the lymphocytes. In this graph there was certainly a cross-over of lymphocytes and neutrophils, but the absence of any reduction in the number of lymphocytes alters the character of the reaction.

We cannot put forward any interpretation of this graph except to suggest that whatever supervened in the first hours after calving masked any tendency for the usual drop in number of the lymphocytes to be developed.

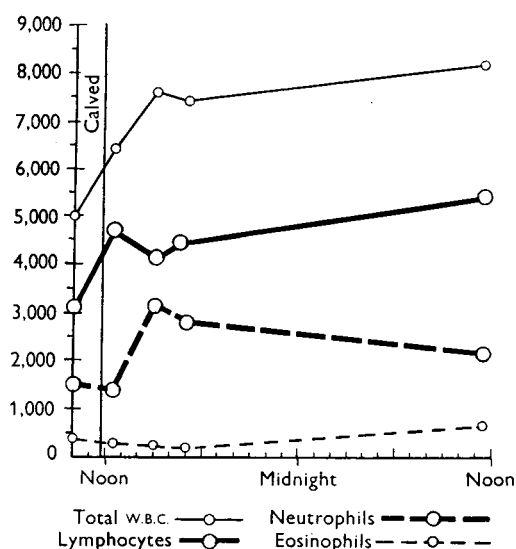


Fig. 12. w.b.c. count at premature calving due to infection with *Brucella abortus*. No cross-over of neutrophils and lymphocytes. No post-partum lymphopenia. (K 3.)

*Cow K 3.* The second aberrant graph (Fig. 12) is of particular interest as parturition occurred 1 month before term and was found to be due to a *Brucella abortus* infection. The calf was viable, but was very small and definitely premature. The cow was observed about 2 hr. before calving and a count was made at once. The total count was low. The relation of the lymphocytes and neutrophils was normal. Three quarters of an hour after calving the neutrophils had not risen though there was an increase in the lymphocytes. Three hours after calving there was an approach of the neutrophils to the lymphocytes, but the graph shows no cross-over and is quite different in character from the normal calving graphs. There is no resemblance to the cortisone type of graph (Fig. 13) which suggests that parturition was not brought about by the same hormone sequence as that which terminated the pregnancy in the other animals studied. It should be noted that in practically all the typical graphs there is a drop in the number of eosinophils at about the peak of the white blood corpuscle reaction. We did not, however, make any special study of the eosinophils as we considered that in the bovine the lymphocytes and neutrophils gave a much more striking and reliable indication of the white blood corpuscle reaction in the animal.

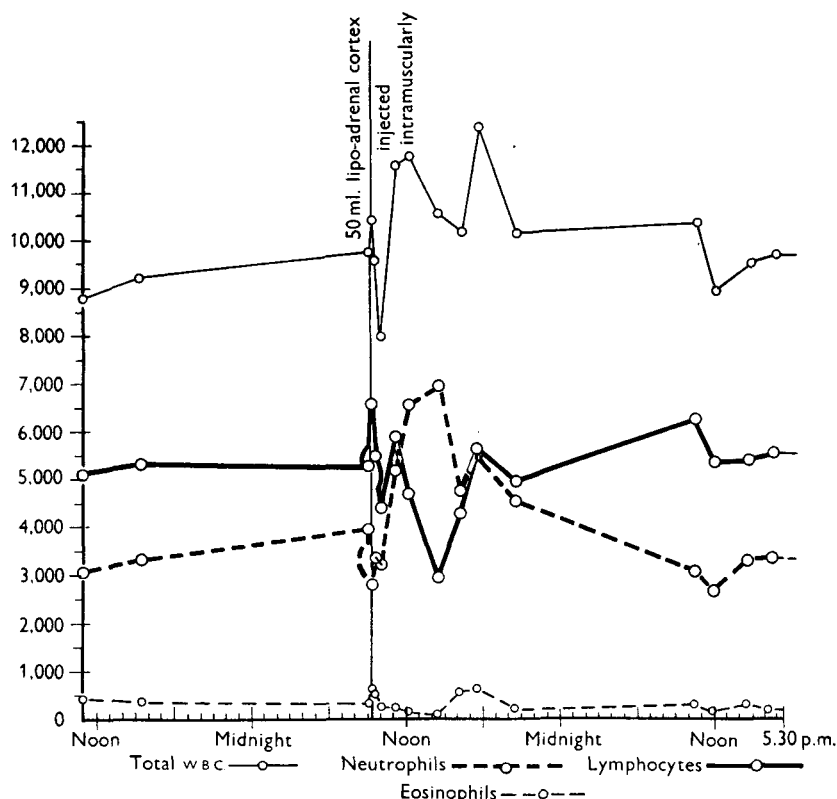


Fig. 13. w.b.c. count response to the intramuscular injection of 50 ml. of adrenal cortical steroids. Cross-over of lymphocytes and neutrophils. The peak of the lymphopenia is at 6 hr.

#### DISCUSSION

At the present time the sequence of hormone activities which brings about the termination of pregnancy and the initiation of parturition in the bovine remains obscure, at least in detail. A decrease in the production of progesterone (luteal hormone) and an increase in that of oestrogen seem to occur towards the end of pregnancy. It is also considered that the pituitary-adrenal-cortical complex is probably involved, but the actual interplay of the hormones is not at all clear (Robson, 1947).

It has become evident in recent years that the adrenal cortical hormone (C 11 hydroxy steroids) produces as one of its reactions a temporary but absolute lymphopenia (White & Dougherty, 1946; Hills *et al.* 1948; Valentine, Craddock & Lawrence, 1948).

Recent experimental work on the pituitary gland in rabbits has shown that restraint or subcutaneous farradic shocks (Colfer, de Groot & Harris, 1950) produce the same type of white blood corpuscle response as that dealt with here, namely an absolute lymphopenia and a rise in granulocytes. This work was carried further (de Groot & Harris, 1950), and it was shown that direct stimulation of the

anterior pituitary gland produced a marked lymphopenia and in short could elicit the cortisone type of white blood corpuscle response.

In the studies on the inhibition of the skin reaction in cattle sensitized to *Trichomonas foetus* antigen the present authors connected the blood corpuscle response, notably the lymphopenia and the inhibition of the skin reaction, with the direct action of cortisone itself (Kerr *et al.* 1949).

In the present study it appears that the blood corpuscle response, particularly the lymphopenia, and the reduction of the sensitivity of the skin reaction afford evidence of the outpouring of C 11 hydroxy steroids (cortisone type) probably at the actual crisis of the final stage of the expelling of the foetus. The evidence for this last is that the delay after the actual delivery of the calf of the appearance of the peak of the reaction in the white blood corpuscles corresponds with that found in the experimental injection of the hormone into the bovine (Fig. 13 and Kerr *et al.* 1949).

In the period before the initiation of parturition there seems to be in certain cases some degree of cortisone effect varying very much with the individual animal. The tendency for the lymphocytes to fall and the neutrophils to rise and the beginning of the reduction in sensitivity all point in that direction. The animals in which parturition itself is initiated with the cells already in the reverse position show this tendency in a more developed condition, but even in them, unless the lymphopenia is already well developed as in Fig. 9, the final stage of parturition further depresses the number of the lymphocytes and produces a more intense cortisone effect grafted on to the earlier tendency (see Fig. 10).

Fig. 7 (*E*11) is interesting, as here the early appearance of the reverse position in the lymphocytes and neutrophils was probably evidence of some adrenal cortical activity, but the absence of any crisis at the actual delivery as shown by the extreme ease of calving is reflected in the absence of any evidence of a late outpouring of C 11 hydroxy steroids.

The aberrant picture produced by *K*3 in which the pregnancy was terminated prematurely by the action of the *Brucella abortus* infection is also striking as showing the white blood corpuscle counts from a birth in which there was apparently no stimulation of the adrenal cortical hormone.

The graphs reproduced here give no information as to how the outpouring of cortisone is produced. The part played by the anterior pituitary gland can be inferred, but our observations go no further than the recording of the characteristic effect on the white blood corpuscles and the inhibition of the skin reaction. This work also leaves quite unprejudiced the question of the co-operation of other hormones including those of the adrenal cortical complex such as desoxycorticosterone which has no action on the white blood corpuscles. The observations recorded do, however, afford in our view positive evidence that cortisone is one of the hormones at work in the last stage of parturition in the normal bovine. We also connect the desensitization of the skin found at this time with the action of cortisone.

## SUMMARY

A study was made of the reaction of the white blood corpuscles in bovines at parturition.

Graphs made from the differential total counts of the white blood corpuscles from shortly before calving and at intervals through the period of parturition and for 24–48 hr. after calving show a definite absolute lymphopenia with a rise in the neutrophils.

The type of graph produced resembles that found in bovines when cortisone is injected.

The peak of the white blood cell reaction usually takes place about 5–9 hr. after the delivery of the calf. This is also the period of the peak reaction in the cellular relations after injection of cortisone.

The observations recorded seem to afford positive evidence that C 11 hydroxy steroids (cortisone) are poured out in the last stage of parturition in the normal bovine. The desensitization of the skin in animals which were sensitized to *Trichomonas foetus* antigen which was found at this time is considered to be connected with the action of the hormone.

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