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Studies of

WHITE BLOOD CELL PICTURE DURING REPRODUCTIVE PHASES IN HEIFERS AND COWS

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P A T N A.

I certify that this Thesis entitled "STUDIES OF WHITE BLOOD CELL PICTURE DURING REPRODUCTIVE PHASES IN HEIFERS AND COWS" has been prepared under my supervision by Shri Shrinivas.H. Dhulkhed, a candidate for the degree of M.Sc.(Vet.) with Gynaecology, Obstetrics and Artificial Insemination as major subject and that it incorporates the results of his independent study.

22.XII.67

(B.P.S.R.SINGH)

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I am sincerely grateful to the Government of Gujarat and to the Director of Animal Husbandry, Gujarat State, Ahmedabad for deputing me to undergo the course.

Shrinivas.H.Dhulkhed.

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INTRODUCTION

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INTRODUCTION

Reproduction is a luxary function of the body, not physiologically necessary for the life of the individual and usually not performed until the animal reaches nearly adult size and nutritive supplies are ample for the nutrition of both the dam and foetus.

But then, reproduction is a biological necessity and is highly essential for the propogation of one's own species. In domesticated animals, reproduction is of further importance - especially so in cattle - as that will bring them in calf and milk, the things for which the man has domesticating them since centuries past.

The economy of animal husbandry mainly depends upon the early maturation of the females, a high fertility rate, regular calvings - the reproductive efficiency - and maximum dairy production at minimum cost. It also seeks to eliminate the major diseases causing infertility or sterility or in any way causing reduced returns to the animal husbandry man.

For this, it is necessary to have a basic knowledge of the reproductive physiology and also the pathology of reproduction. During the last three decades only, the importance of the physiology of reproduction has been emphasised, so

as to utilize such an understanding in breeding better farm animals.

Further, it is essential to have a thorough knowledge regarding the various hormonal secretions, their functions, their control over reproduction and the various changes brought about by their action in the various tissues of the body of the cow. Zarrow (1962) is of the opinion that essentially all the hormones of the mammal are involved in some aspect of mammalian reproduction.

The various phases of reproduction and the hormones associated with it bring about many physiological changes in the body of the cow including biochemical and morphological changes in the blood which is one of the important tissues of the body.

Blood has been the object of study since ancient times. Leewenhock (1673) was the first to see the red blood corpuscles in the blood of man with his crude microscope. Blood lymphocytes were also discovered by him. Hewson (1737 - '79) detected the white blood corpuscles in the blood. Virchow (1845) discovered leucemia, a specific disease of the blood. Erlich (1891) was the first to stain the blood corpuscles with anyline dye. Romanowsky (1891) combined eosine and methylene blue to stain blood films which enabled to identify the various white blood cells.

In those days, haematology mainly dealt with the

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disease aspect only. Arneth (1921) and Schilling (1926) contributed much to the haematology by their famous work on the effect of diseases on the different kinds of blood cells and their regeneration. Arneth's index and Schilling's haemogram are still considered to be standard works in haematology.

In 1937-1946, world wide attention was focussed on the epoch work of Selye that stress factors bring about changes in the blood morphology. Selye (1937) noted that, normally exposure to stress causes both damage (shock) and defence (countershock) phenomena which he termed as "Alarm-Reaction". He further stated that adoptability is probably the most distinctive characteristic of life. Thus in 1946 he profounded his famous work of "General Adoptation Syndrome".

The "General Adoptation Syndrome" - "G.A.S."- is the sum of all non-specific systemic reactions of the body which ensue upon long continued exposure to systemic stress. This brings about the full play of pituitary - adrenocortical activity as a result of which many physiological changes in the body including biochemical and morphological changes in the blood are brought about. The haematologic response to stress will largely depend upon the proportion of adrenergic and glucocorticoid hormones discharged as well as upon the responsiveness of the whole blood cell system. This in turn depends upon conditioning factors such as hereditary, nutrition, specific action of stressors etc. It is mainly due to

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such differences as conditioning, that the same mechanism of exposure to stress can produce varying haematologic changes under different conditions.

This concept fundamentally changed the attitude towards the study of haematology; and it became essential to establish normal morphological values of blood under various reproductive physiological conditions causing such stresses.

During oestrus, the cow is under the influence of stress due to the increased activity of the gonadotropic and adrenotropic hormones of the anterior pituitary. The intensity of the behavioral oestrus is in direct proportion to the level of oestrogens secreted by the overy and then released in the blood. In cows which exhibit intense heat, the oestrogens are released in larger quantities, thus showing the effects of stress more, compared to the cows exhibiting feeble or silent heat due to a low level of oestrogens' secretion and release with less stress effects.

During pregnancy, the cow is subjected to various stress factors due to the development of the foetus, the changed levels of various hormonal secretions, the cessation of previous lactation etc. All these may have profound influence on the white blood cell picture.

During parturition, the cow is under the action of various hormones secreted from both anterior and posterior pituitaries, the adrenal cortex, the overy and the placenta;

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and hence under the influence of great stress.

Changes in the white blood in man resembling those brought about by "Stress" have been discovered in connection with normal sexual physiological conditions as ovulation, pregnancy and parturition(Bethell, Hartsaff and Farrell, 1943; Davis and Hulit, 1949; Andrews and Bonsnes, 1951; and Kullander, 1952).

Some workers have studied the effects of stress during certain various reproductive phases on the leucocytic picture in the cow(Canham, 1930; Morris,1944; Kerr et al., 1951; Moberg, 1955 in foreign countries; Agarwala, 1963 and Khajuria and Razdan, 1966 in India).

While studying the effects of lactation on the leucocytic picture, Canham (1930) showed that the total white cell count and eosinophil count increased just before milking.

Fraser (1930) recorded higher values of leucocytes for dry cows and lower figures for the dairy cows and the variation, he attributed to the milk production and to the pregnancy of the latter group.

Tokarj (1938) carried out studies to correlate the blood picture with the milk yield. He reported that the higher yielding cows had higher leucocytic count, higher haemoglobin values, a higher count of erythrocytes with larger diameters. He found that the percentage of lymphocytes rose from freshening to the period of maximum yield, while the neutrophilic

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percentage showed the reverse picture. He concluded that a high correlation existed between the blood picture and milk yield.

Tokarj's (1939) study on correlation between blood picture and constitution of Red German bulls is further interesting. He found that the "Eurysomic" (broader and more massive) type of bulls had greater number of erythrocytes per c.mm. , larger sized erythrocytes with more haemoglobin content percentage and higher leucocytic count compared to the "Leptosomic", (lighter and narrower) bulls and further that the milk yield of the progeny of the eurysomic bulls was higher. He, hence, advocated the breeders, to try to select and breed the eurysomic type of animals.

Groblewska (1955) reported a connection between the leucocyte index of a bull and the production level of his dam and further that the ratio of one granulocyte to two agranulocytes was characteristic of the "respiratory type" descending from dams of high yield compared to the "digestive type" of bulls from dams with lower milk yield and having a smaller ratio.

Groblewska (1960) further elucidated that breeding bulls having the neutrophil/lymphocytic index of 1:2 had daughters which gave higher milk yield than their dams, as compared to bulls having such an index of 1:3, produced daughters whose yields were less than their dams.

Tolle (1960) reviewing the literature on the correlation between blood group factors and heifer lactation,

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proved that such a relationship existed and that certain blood group factors had an arresting effect on the length of the lactation period.

Rendel (1960) while studying the relationship between blood group and production factors of cattle concluded that certain blood groups were associated with higher milk yield and fat percentages.

Agarwala et al.(1963) were of the opinion that blood characteristics such as increase in erythrocytic and monocytic counts and haemoglobin content were helpful in diagnosing pregnancy of three weeks in she-buffaloes.

Khajuria and Razdan (1966) stated that the leucocytic picture provided a diagnostic aid in reproductive physiological conditions.

The present work has been undertaken to study the blood picture in the sexually mature Tharparkar cows during various phases of reproduction, viz., oestrous cycle, pregnancy and parturition and to correlate those pictures with the various reproductive physiological conditions.

Such findings may serve as a base to study the pathological conditions of reproduction and the changes brought about by them in the blood picture.

Further with such knowledge, it should be helpful to determine which animals are the best able to adjust to a certain specified stress. The ability of agricultural and dairy

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animals to adjust to adverse situations with a minimum expenditure of energy should enhance their ability to expend a maximum energy towards desired agricultural and dairy production.

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REVIEW OF LITERATURE

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REVIEW OF LITERATURE

For the purpose of convenience, the review of literature has been arranged in the following sections:-

I. Normal white blood cell picture in cattle and the effect of age on it,

II. White blood cell picture during oestrous cycle,

III. White blood cell picture during pregnancy, and

IV. White blood cell picture in connection with parturition.

I. Normal white blood cell picture in cattle and the effect of age on it:

Many workers (Schultz, 1905; Canham, 1930; Fraser, 1930; Shankarnarayanan, 1937; Ferguson *et al*.,1945; Rusoff and Piercy, 1946; Storkebaum, 1951; and others) have investigated and established the normal white blood cell picture of cattle of various breeds under various environmental conditions.

All the authors agreed in general that there were individual variations of the white blood cell morphology, and that it varied in the same individual under various environmental and physiological conditions. Ferguson *et al*.(1945) found that variation in the blood picture was due to the individuality, and proposed that each animal of the species probably had a picture in health peculiar to itself, just as each

animal had its own size, form etc. It has also been proved that age, sex, breed, time of the day and seasons had also influences to a smaller or greater extent on the white blood cell picture.

Schultz (1905) observed that with increasing age, the white blood cell count decreased. Canham (1930) also made similar observations. Fraser (1930) concluded that young animals showed a higher total leucocytic count, a higher percentage of monocytes and neutrophils and a lower percentage of eosinophils; the latter appeared to increase in numbers from birth to adult age.

Nichols (1935) stated that the young pups of few weeks old had the total leucocytic count, double than the normal adult.

Reda (1951) working on Egyptian buffaloes found that the white blood cell count and neutrophils were higher in calves than in adults, while the lymphocytes were less. The eosinophils increased in parallel direction with the age, the lowest in the young and the highest in adults.

Hathoat (1953) found similar results in sheep.

Moberg (1955) working with large numbers of SRB-breed concluded that the total leucocytes decreased with increasing age. He found neutrophils higher in young animals while eosinophils increased with increasing age.

Holmann (1956) working with calves and heifers reported

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that the lymphocyte count increased from 33 percent at birth to 72 percent at 4 months of age, then decreased slowly to the adult level; while the eosinophil count rose from 1.5 percent from birth to about 10 percent by 22 months' of age.

Reda (1957) reported a higher total count in young sheep and a low level in adults, with lymphocytes, neutrophils and eosinophils corresponding to the same changes as in cattle from calfhood to adult age as reported by Fraser (1930).

Greatorex (1957) found a high leucocytic and erythrocytic count at birth of the calf and credited Sabin et al. (1936), who attributed this partly to the change in the circulation occurring at birth and partly to the incorporation of the placental blood into the vessels of the new born animal. He also found that the percentage of neutrophils was inversely proportional to that of lymphocytes in the calfhood upto the 8th month. Then, he observed a change towards a lymphocytic dominated picture and considered such change to be due to the onset of puberty and the rate of growth.

The interesting study of Kraft (1965) who studied the leucocytic picture of foetuses,reported that towards the end of second month of foetal life, lymphocytes predominated at first, and eosinophils were more numerous than neutrophils; but that from sixth month onwards, granulocytes began to increase until towards the end of foetal life, when the neutrophils out numbered the lymphocytes.

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Gayot and Cheverier (1966) working with 911 Charolias cattle came to the same conclusions that the white blood cell count decreased with increasing age.

Khajuria et al.(1966) reported that age of the animals had an influence on the values of the white blood cells.

Khajuria and Razdan (1966) studying the blood morphology of Indian cattle, concluded that the age of the animals was found to have a marked effect on the leucocytes.

II. White blood cell picture during oestrous cycle:

From his investigations on the leucocytic picture in cattle, Canham (1930) found that the total white cell count and neutrophils rose in connection with oestrus, with a slight concurrent increase in the eosinophils as well.

Lauda (1931) also recorded a frequent increase in the total white count during oestrus.

The white blood cell picture in a bitch during induced oestrus was studied by Bravo (1935). Initial lymphocytosis and eosinophilia was reported by him with neutrophils oscillating in both ways.

Satalov (1940) reported various degrees of leucocyte formation during oestrus, particularly during the period of ovulation.

The persistency of follicles and their effect on the blood picture was studied by Moberg (1965). He divided the

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animals into 4 catagories depending upon the extent of hormone secreted by the follicles and found : (i) a highly significant increase in the number of neutrophils and a low L/N ratio in cows with a female type of behaviour,and (ii) a highly significant increase in the number of lymphocytes and a high L/N ratio in cows with a male type of behaviour.

During involution of the corpus luteum, Tillmann and Gehring (1953) found lowest number of leucocytes. It increased at the beginning of oestrus cycle and decreased with follicular growth in the preovulation phase. Follicular maturation and ovulation produced rapid leucocytosis. Eosinophils were highest at the beginning of oestrus. They also found that the parenteral administration of oestrogens in horses slightly increased the number of neutrophils.

Moberg (1952, 1953) published results derived from a study on 27 oestrous cycles in 10 cows of the SKB-breed. He showed a rise in the total white count and neutrophils with a fall in the number of eosinophils.

Moberg (1955) further carried out investigations on the leucocytic picture in 9 SKB cows and 6 SRB heifers during 53 oestrous cycles. He reported an increase in the total white count owing to a significant neutrophilia. Eosinophils decreased in number on the day of heat and the day after heat. He further reported that the white blood cell picture in heifers had a tendancy towards lymphocytopenia, eosinophilia and

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neutrophilia during oestrus.

Moberg (1955) also found a very clear stress effect on the white blood cell picture due to overiectomy which caused a reduction in all types of leucocytes. He treated the overiectomised heifers with oestrogen which caused a neutrophilia, lymphocytopenia and eosinopenia and an increase in the total leucocyte count.

An interesting study was conducted by Pounden and Firebough (1956) on the effects of nervousness on conception during artificial insemination. They concluded that considerable nervousness or unusually placid reactions at the time of service might be less desirable as they caused reduced conception than the more usually observed responses.

Cole and Cupps (1959) expressed that changes in the physiological state of the animal during oestrous cycle might be expected to produce variations in peripheral blood values. They cited Anderson (1958) who found 10-12 percent increase in haematocrit values during pre-oestrus in dogs.

A decrease in milk yield in the majority of the cows conceiving to service - most probably due to stress factors - was reported by Bratonov et al. (1959). They further found the quantitative changes in milk production to be greater for buffaloes than for cows and were more pronounced when the signs of oestrus were marked.

Blood pressure measurements on Kostom and Friesion

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cows by Rubenkov (1959) showed a rise during oestrus.

Hammond (1927) studied mucus smears during oestrus in 12 Jersey heifers and found that polymorphonuclear leucocytes were a characteristic feature upto the 6th day of the cycle.

Newsam (1961) studying the mucus smears of normal heifers distinguished clearly neutrophils, eosinophils, and occasionally lymphocytes. At oestrus, the mucus was acellular, leucocytes reaching the maximum by 10th day of oestrous cycle.

Bassett (1962) studied the histology of the vulval skin of ferrets during various stages of oestrus and found large number of eosinophils which he suggested, were attracted to the site of extracellular protein synthesis to act as regulators.

Agarwala (1963) observed in Hariana cows, a high leucocytic value during proestrus and oestrus with a drop in the luteal phase. He recorded neutrophilia, eosinopenia with no change in monocytes and lymphocytes for the same conditions.

Kallela and Moberg (1965) fed plant oestrogens to intact and overiectomised ewes. Their results showed a marked tendancy towards eosinopenia and a slight tendancy towards neutrophilia as indicated by L/N ratio.

Nellore and Brown (1966) examined the vaginal smears and genital tract tissues during normal oestrous cycles in sheep and swine and reported neutrophilic leucocytosis during dioestrus and proestrus stages and a relative poucity of

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leucocytes in the vaginal smears close to the time of oestrus or ovulation. They suggested that this might be due to the inability of the leucocytes to penetrate freely into the increased epithelial barrier developed close to the time of ovulation.

Ross and Klebanoff (1966) during oestrous cycle in albino rats found eosinophils from the blood stream migrating into the uterine connective tissue. Just prior to, during and one day post oestrus, the eosinophils were lysed and phagocytosed by macrophages in the extra cellular space.

Solimen and Selim (1966) investigated blood picture in 120 female buffaloes and revealed that oestrus was characterised by leucocytosis, neutrophilia and eosinopenia.

III. White blood cell picture during pregnancy:

Schultz (1905) was the first to study the effect of pregnancy on the white blood cell picture in the cow. He did not find any changes in it. So also Obentrout (1934) and Romano (1935).

Bungener (1927) and Szepeshelyi (1934) observed an increase in the total leucocyte count in connection with normal pregnancy and especially so just prior to parturition.

Dieckmann and Wegner (1934) found an increase in both blood and plasma volume in the first trimester in humans with other changes in the blood.

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Morris (1944) studied the blood morphology of a single Shorthorn cow throughout the period of pregnancy. He noticed slight leucocytosis at the time the cow was drying off finally from the previous lactation, and again during the two weeks immediately prior to parturition. In both cases, it was mainly due to an increase in neutrophils. Lymphocytes and monocytes remained fairly constant, but eosinophils increased in the final stages of lactation.

Storkebaum (1951) studying on 107 healthy non-pregnant and 58 pregnant cows, reported a decrease in the lymphocytes and an increase in neutrophils as the pregnancy progressed.

McDonald et al. (1953) investigated the essentiality of the corpus luteum in pregnancy in cows and came to the conclusion that the corpus luteum is necessary to maintain pregnancy until approximately 200th day of pregnancy.

Moberg's (1953) findings were similar to Storkebaum's, in that he reported a slowly decreasing total white cell count during gestation, owing to a fall in the lymphocytes and an initial increase in the neutrophils.

Moberg (1955) studied 10 cows of SKB-breed during their pregnancy period. He reported an increase in the total white cell count upto the 3rd month; the neutrophils increased during the first half of pregnancy, fell and increased again until parturition; the eosinophils rose in the first month of gestation only, with a fall during the 3rd month; with the

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maximum in the 5th month; while the lymphocytes increased upto the 3rd month of gestation followed by a continuous decrease till parturition.

Siegrist (1956) gave the level of 17-hydroxy-corticosteroid level in animals under various kinds of stresses as given in the following table II.1.

TABLE II.1

Animal group	No. of animals studied	Plasma 17-hydroxycorticosteroids. Microgram %	
		Mean	Range
1. Pregnant, dry, within 6 weeks of parturition.	23	9.77	6.90 - 17.62
2. Milking, non-pregnant, within 6 weeks post-parturition.	20	4.58	2.14 - 8. 40
3. Ketotic cows.	10	7.48	2.46 - 10.51
4. Stressed cows.	21	11.95	6.58 - 21.78

Siegrist (1956) further stated that during increased stress, the body required - and the adrenal cortices under the corticotrophic stimulation of the anterior pituitary produce - increased amounts of adrenocorticoid steroids.

With similar type of investigations, Robertson and Mixner (1956) could detect 17-hydroxycorticosteroids on an

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average of 9.77 γ % with a range of 6.90 - 17.62 in the blood of dry, pregnant cows; while a dry, non-pregnant cows, the values were average 4.58 γ % with a range of 2.14 - 8.40.

Greatorex (1957) observed a drop in the total white cell count during the 4th and 5th month and a significant decrease in 50 % of the cows just before parturition. The differential count during gestation and parturition did not follow a regular pattern, but in general were similar to those observed by Moberg (1955).

Malek et al.(1958) studying the daily rythm of leucocytes in 103 pregnant women, found maximum leucocytes at 3.00 p.m. and a minimum of eosinophils and lymphocytes at 9 a.m. and again maximum at 3 p.m.

Anderson (1958) reported a 30-40 % increase in leucocytes during late gestation in dogs.

Patel et al.(1960) reported a higher eosinophil percentage in pregnant Kankrej cows compared to the non-pregnant cows.

Kuvin and Brecher (1962) studied the white blood cell picture in the last trimester of 88 normal pregnant women and found that 22 had myelocytes or metamyelocytes in their peripheral blood.

Pope et al.(1965) investigated the presence of oestrogens in the blood drawn from the jugular vein of cows during pregnancy. They could not detect it in cows pregnant for 2-3 months; but could detect oestrogen equivalent to 1 microgram

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of oestrone per litre of blood from cows 5-6 months pregnant and about 7 microgram from cows 7-9 months pregnant.

Roy et al. (1965) reported a significant decrease in the white blood cell count of ewes and goats by 120th day of pregnancy.

The study of Ullery et al.(1965) in 316 sheep showed highest relative eosinophil concentration during gestation.

After investigating 120 she buffaloes, Solimen and Selim (1966) reported that the last few months of pregnancy were accompanied by leucocytosis with neutrophilia, lymphocytopenia and eosinophilia.

Solimen et al.(1966) discovered an increase in the total gonadotropins during the 7th month of pregnancy in buffaloes while the Thyroid stimulating hormone (TSH) increased during the first month of pregnancy and decreased in the last two months of pregnancy.

IV. The white blood cell picture in connection with parturition:

Krisch (1923) was the first to study the changes in the white blood cell picture connected with parturition in cattle. His observations revealed, an increase in the total white cell count due to neutrophilia during a week before parturition; there was a sudden drop 1-2 hours after parturition, continuing for 1.5 - 4 days followed by another peak.

Bungener (1927), and Szepeshelyi (1934) also observed

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a rise in the total leucocytic count prior to parturition.

Canham (1930) recorded a higher leucocytic count just hours before parturition.

Bell and Morris (1934) investigating the oxytocic property of the blood in cows, found with few exceptions, a low level of oxytocin with an increase in the last week of pregnancy and a low level immediately after parturition.

Tokarj (1938) found that the percentage of lymphocytes rose from freshening to the period of maximum yield, while the neutrophilic percentage showed the reverse trend. He reported that the higher yielding cows have higher leucocytic count, higher haemoglobin values, a higher count of erythrocytes with larger diameters. He concluded that a high correlation existed between the blood picture and milk yield.

Ferguson et al.(1941) studied 24 Holstein - Friesion cows for their white blood cell picture before, during and after parturition. They found the total number of leucocytes decreased significantly immediately after parturition and increased later to precalving period. The percentage of both neutrophils and lymphocytes remained unchanged while the eosinophils decreased at parturition, with monocytes behaving in the opposite direction to eosinophils.

Morris (1944) found an increase in eosinophils immediately prior to parturition with leucocytosis.

Selye (1946) found that severe haemorrhage caused neutrophilia and leucocytosis.

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Kerr et al. (1949) reported the cross over of neutrophils and lymphocytes at the time of parturition and soon after it, and a marked absolute reduction in the number of lymphocytes immediately after calving. These returned to the normal state by the 5th day.

Garm (1950;1951) observed eosinopenia, lymphocytopenia and neutrophilia before parturition. These changes ended at parturition with considerable rise in the total count.

Shaw (1956) said that cow at, and immediately after parturition was subjected to considerable stress as shown by the marked decrease in the blood eosinophils during the parturient period.

Grab and Peterson (1953) noticed increases in respiration, heart rates and temperature on the day of calving. The animals quickly returned to the normal by the day following calving.

Hays and VanDemark (1953) stimulated the cervix and vulva of cows and found that it caused the release of oxytocin.

Merryl and Smith (1954) observed a relative decrease in the eosinophils and lymphocytes and likewise an increase in the neutrophils in connection with parturition.

Merryl and Smith (1954) investigated the reaction of leucocytes in parturition and after the administeration of ACTH. The study revealed an increased leucocytic count due to neutrophilia...

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accompanied by lymphocytopenia and eosinopenia. Similar results with higher and greater variations were also obtained with ACTH administration. The authors concluded that this was due to an increased secretion from the adrenal cortex in response to stress of parturition in the cow.

Moberg (1953, 1955) found that the total count increased gradually during the last three weeks prior to parturition and was caused by a rise in the neutrophils. The highest total white and neutrophil counts were observed during actual labour and immediately after delivery after which there were sudden falls to nearly normal levels. The number of eosinophils fell with approaching parturition, reaching to its lowest point during labour. The day after parturition, the eosinophils reached to an isolated single peak, fell again and during the next three weeks rose to about the normal level. The number of lymphocytes did not undergo considerable changes during the period.

Holmann's (1955) findings were similar to that of Moberg's. He also found leucocytosis in which the neutrophils doubled their previous numbers 12 hours before calving. This neutrophilia persisted for about 24 hours and then subsided. Lymphocytes also fell by 12 hours after calving. Holmann could not detect any difference in the eosinophilic count.

Bajwa et al. (1956) failed to detect any regular pattern in the white blood picture in the ketotic cows.

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Moberg (1956) studying the leucocytic picture in cows with retained foetal membranes detected leucopenia due to neutropenia.

In his study on the bone marrow changes at parturition in cows and in cows with retained foetal membranes, Winquist (1956) detected a shift to the left in the neutrophilic granulocytes indicating increased production.

Paterson's (1957) investigations with parturient cows revealed a higher mean concentrations of 17-hydroxycorticosteroids before parturition, the highest being found 1-2 weeks prepartum; he also found a significant increase in leucocytes and neutrophils 1-2 weeks prepartum with sharp decrease at about parturition; there was significant eosinopenia 1-2 weeks prepartum while lymphocytic changes were not significant.

Blockburn et al.(1959) carried out feeding trials with hay and silage to parturient cows; but they found no difference in the eosinophil picture upto calving as both the groups showed a marked decrease of eosinophils from 29-35 days precalving to the time of calving and for further 7 days after calving.

Rubenkov (1959) measured higher than normal blood pressure in the last stage of pregnancy and in the beginning of calving and which fell during 12 hours after calving with a further rise on the 2nd day.

Straub et al.(1959) also reported a significant

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elevation in the total leucocyte cound at about parturition mainly due to a marked increase in the neutrophils with an increase in the lymphocytes as well; eosinophils were depressed and monocytes increased. By the day following calving, the total count had fallen which was mainly due to a fall in both the neutrophils and lymphocytes.

Otsuka and Nakajami (1961) found a pronounced decrease in the number of circulating eosinophils at parturition in cows with recovery to normal in about 40 days.

Gier et al. (1962) detected large number of neutrophils accumulated in sluffing in the uterus of cows during the first fortnight after calving, in the placental mass etc. They detected lymphocytes also in most of the normal uteri.

Agarwala (1963) working with Hariana cows recorded almost constant total count in connection with parturition, with increase in lymphocytes after calving and with acute neutrophilia and eosinopenia.

Roy et al. (1965) studied the white blood cell picture in various reproductive phases in sheep and goats. They detected an increased leucocyte count during parturition and ascribed it to acute neutrophilia accompanied by moderate lymphopenia and eosinophilia. After 6 hours of parturition in sheep, they detected further rise in the total leucocyte count due to increased number of eosinophils, neutrophils and lymphocytes; while in goats they found a decrease in the total count

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due to reduction in neutrophils accompanied by a slight fall in lymphocytes as well.

Ullery et al.(1965) also studied sheep haematology and found a marked drop in eosinophils at parturition in a manner typical of the reaction to stress.

Solimen and Selim (1966) studied the blood picture of 120 female buffaloes during different reproductive phases and found leucocytosis with lymphopenia, eosinopenia and neutrophilia indicating typical moderate stress.

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MATERIALS IN GENERAL AND METHODS

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MATERIALS AND METHODS

MATERIALS (In general)

For the purpose of this study, heifers and cows of the Tharparkar breed belonging to the Government Cattle Farm, Patna were utilised. The material has been divided into the following four catagories:-

(A) To establish the normal white blood cell picture in sexually mature female cattle, 40 cows and 20 heifers representing a random sample of the herd of the farm were selected. Cows in parturient stages were not included in the study.

(B) To study the white blood cell picture during oestrous cycles, cows were selected on the basis of their regular reproductive cycles, excluding the cases of pathological conditions like cystic overies etc. Twenty complete oestrous cycles in 12 cows were studied. Blood samples of another 10 cows during the period of oestrus only were also taken and included in the study. The above animals include some heifers as well.

(C) To establish the leucocytic picture during pregnancy, a minimum of 6 cows in each month of pregnancy (a total of 73 cows) were selected on the basis of service records and confirmed by rectal examinations. The animals so selected and studied include some heifers as well.

(D) To study the leucogram of cows in parturition, blood samples from 6 cows during the period of three weeks prior to and three weeks after parturition were collected and studied. The cows were selected from the lot which were due for calving.

The above cows were free from tuberculosis, brucellosis and Johne's disease as proved by regular tests carried out by the Livestock Research Station, Patna. The cows were free from parasites and were clinically healthy and in good condition.

All the animals were kept under the existing management and animal husbandry practices. They were given concentrate mixture containing equal parts of ground nut cake, wheat bran and gowar meal with mineral mixture and common salt and computed as per prescribed feeding standards. Chaffed greens (Para grass) and hay were given ad libitum. Watering was also allowed ad libitum. Exercise in the form of grazing was allowed in the morning hours. The milking hours were between 4-00 to 5-00 in the morning and 3-00 to 4-00 in the evening.

The investigations were carried out from May,1967 to September,1967. The average maximum and minimum temperatures, the average relative humidity and the rainfall during the above months are given in table III-1.

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TABLE - III.1

Average temperature, relative humidity and rainfall during the study period.

Months	Average temperature in F°		Av. relative humidity in %	Total rainfall in m.m.
	Maximum	Minimum		
May, 1967	100.7	76.5	87	29.0
June "	101.6	80.9	76	21.3
July "	93.3	79.6	87	158.4
Aug. "	89.6	78.0	91	213.4
Sept. "	89.1	77.1	96	476.1

The original data was obtained from the Central Potato Research Station, Sahaynagar, Patna.

METHODS

Collection of blood:

The blood was collected regularly between 7-00 and 8-00 in the morning before the animals were allowed water and feed or were allowed to go out for grazing.

The blood was collected from the jugular vein in the standing position. The animal was controlled in a service crate to avoid any excitement to the animal.

A small area over the jugular vein in the middle of the neck was closely clipped, wiped with cottonwool and painted over with rectified spirit. A sterilized hypodermic

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stainless steel needle, size no.18 (B.G.) with uniform bore was used for insertion into the vein. In most of the times, only one bold thrust was sufficient to bring out the blood in a continuous flow. The first few drops were discarded. Then one millilitre of blood was collected directly from the needle into a small vial containing the dried crystalls of the double oxalates. During and soon after the collection of the blood, the vial was gently rotated and shaken so as to have a proper mixing of the anticoagulants with the blood to avoid clotting.

Anticoagulants used:

The double oxalate crystalls of anticoagulants were prepared according to Heller et al.(1934) as follows:-

Potassium oxalate	...	0.8 gms.
Ammonium oxalate	...	1.2 gms.
Glass distilled water..		100 ml.

0.1 ml. of the above solution was dispensed into each small sterilized vial and evaporated to dryness in a hot air oven at 60°C.

Preparation of smears:

After collection of 1 ml. of blood into the vial containing the anticoagulants, a drop of blood was taken on a clean slide directly from the flow. Two smears per sample were

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prepared from it on clean, dry and nongreasy slides and rapidly dried in the air. For the drawing of good smears, thin slides with smooth edges and rounded off corners were used (Schalm, 1961).

From the above materials, total leucocytic counts and differential counts were made as per standard techniques.

Total white blood cell counts:

This was performed immediately after collection of the blood samples. The technique described by Stitt et al. (1938), Napier and Dasgupta (1945) and Schalm (1961) were followed.

The total white blood cell count was made in "Improved Neubauer Chambers" using Thoma white blood cell pipettes and Turk's fluid as dilutor.

The Turk's dilutor was prepared as described by Stitt et al. (1938) as follows:-

Glacial acetic acid	...	1.0 ml.
1 % Acq. Gention violet solu.		1.0 ml.
Distilled water	...	100 ml.

Both the chambers of the haemocytometer were charged with the diluted blood of each sample. The whole ruled area of the haemocytometer was counted using a mechanical hand tally counter. The calculations were done as per standard methods.

The results were expressed in thousands per cubic millimeter of blood.

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Differential leucocytic counts:

The smears were stained by the "Wright's stain" (B.D.H.) as per prescribed standard technique. The white blood cells were counted under oil immersion lense. One hundred cells from each of the two smears were counted. The "battlement or palisade" method advocated by MacGregor et al. (1940) was adopted. The results were expressed in percentages in relation to the total white blood cell count.

The absolute values of various leucocytes were then calculated from the results of total and differential counts.

As lymphocyte/neutrophil ratio (L/N ratio) seems to be of some interest and importance, the ratio between the lymphocytes and neutrophils was calculated in each case.

Statistical analysis:

The different data of this study were subjected to different statistical tests, depending upon the situations.

Analysis of variance:

To test the effect of oestrus, pregnancy and parturition on the leucocytic picture of the blood, the data were subjected to 'F' test. The following models of analysis of variance have been used (Snedecor, 1961).

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Model - I

Source of variation.	D.F.	S.S.	M.S.	F.
Between cows	C - 1	B	$\frac{B}{C-1} = X$	$\frac{X}{Y}$
Within cows	N - c	A-B	$\frac{A-B}{N-c} = Y$	
Total	N - 1	A		

Model - II

Between cows	C - 1=b	B	$\frac{B}{b} = X$	$\frac{X}{Z}$
Intervals	I - 1=c	C	$\frac{C}{c} = Y$	$\frac{Y}{Z}$
Error	a - b-c= n	A-B-C	$\frac{A-B-C}{n} = Z$	
Total	N - 1 =a	A		

Critical difference:

Wherever 'F' test was significant, critical difference was obtained to find out the significance of individual mean differences. The following formula has been used.

$$\text{C.D. at } 5/1\% = t_{05}/t_{01} \text{ error df } X \sqrt{s^2\left(\frac{1}{n1} + \frac{1}{n2}\right)}$$

Where S^2 was error ms, n1 and n2 were observations of different groups.

<u>'t' test</u>:

To test the significance of two means, 't' has been used. Before proceeding to the actual test, equality of S_1^2 and S_2^2 was tested to satisfy the assumption of the same variation in two samples. To test this $\frac{S_1^2}{S_2^2}$ or $\frac{S_2^2}{S_1^2}$ (n1-1, n2-1) has been calculated. Wherever the 'F' was insignificant, the variance of two samples were pooled to get the better estimate of variance.

$$S^2 = \frac{(n1-1)\ S1^2 + (n2-1)\ S2^2}{n1 + n2 - 2}$$

$$\text{Now 't'} = \frac{\bar{X}1 - \bar{X}2}{S\sqrt{\frac{1}{n1 + n2}}} \quad (n1 + n2 - 2)$$

Where $\bar{X}1$, $\bar{X}2$ were two means to be tested and S was the S.D.

Standard error, standard deviation and coefficient of variance were calculated as per formulae advocated by Snedecor (1961).

The following notations have been used to mark the degree of significance in this work:-

N.S. = not significant
* = significant at 5% level
** = significant at 1% level
*** = significant at 0.1% level.

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NORMAL WHITE BLOOD CELL PICTURE

I N

THARPARKAR HEIFERS AND COWS

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THE NORMAL WHITE BLOOD CELL PICTURE IN THARPARKAR HEIFERS AND COWS

MATERIALS

Blood samples from 20 heifers and 40 cows were obtained for study of the normal white blood cell picture. The animals represented a cross-section of the herd. Care was taken in that no parturient animal was included in this study.

All the 20 heifers were between 2-4 years of age, while the details of the 40 cows were as follows:-

	Age
(a) Cows below 5 years ...	5
(b) Cows between 5-10 years	26
(c) Cows above 10 years ..	9

	Calvings
(a) Cows upto 2 calvings	11
(b) Cows from 2-5 calvings	21
(c) Cows with more than 5 calvings	8

The methods used for the white blood cell count have been described in chapter III "Materials and Methods".

RESULTS

The mean total count and differential counts along

with standard error and coefficient of variation of heifers and cows were worked out. These results are mentioned in tables IV-1 and IV-2.

TABLE IV-1

The normal white blood cell picture in Tharparkar heifers.

	Total count	Lymphocytes	Monocytes	Neutrophils.	Eosinophils	Basophils
		Absolute values				
Minimum	6600	4161	151	1741	327	0
Maximum	18122	12500	897	6449	1927	272
Mean	12303	7530	345	3420	947	61
S.D. ±	5516	7784	190	1112	530	88
S.E.	1224	1730	42	247	118	19
		Percentage values				
Minimum		48.5	1.5	16.5	5.0	0
Maximum		74.5	6.0	45.0	14.0	2.0
Mean		61.2	2.8	27.8	7.7	0.5
L/N ratio		2.2				
C.S.S.		383.50	67.14	413.04	176.76	99.36
S.D. ±		0.59	0.10	0.64	0.27	0.15
S.E.		0.13	0.02	0.14	0.08	0.03
C.V.		1.15	1.03	2.02	1.67	3.64

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TABLE IV-2

The normal white blood cell picture in Tharparkar cows

	Total count	Lymphocytes	Monocytes	Neutrophils	Eosinophils	Basophils
			Absolute values			
Minimum	4376	2626	149	1206	219	0
Maximum	13625	7686	863	4905	1943	126
Mean	9120	5212	346	2682	839	36
S.D. ±	1356	1172	183	844	136	53
S.E.	215	186	29	134	21	8
			Percentage values			
Minimum		34.0	1.0	21.0	2.5	0
Maximum		71.5	8.5	42.5	17.0	2.0
Mean		57.2	3.8	29.4	9.2	0.4
L/N ratio		1.9				
C.S.S.		652.43	389.63	414.80	446.95	157.21
S.D. ±		0.51	0.30	0.32	0.35	0.12
S.E.		0.08	0.04	0.05	0.05	0.01
C.V.		1.04	2.67	0.97	1.98	3.14

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The average total count and differential counts in the two groups were subjected to 't' test to see whether the two groups differ significantly with respective different counts. These results are given in table IV-3.

TABLE IV-3

The results of 't' test of total and differential counts in heifers and cows

Group	d.f.	Mean difference	Calculated 't'
Absolute values			
Total counts	58	3183	3.91***
Lymphocytes	58	2323	13.96***
Monocytes	58	1	-
Neutrophils	58	738	2.86**
Eosinophils	58	108	1.22 N.S.
Percentage values			
Lymphocytes	58	4.0	23.00***
Monocytes	58	1.0	23.28***
Neutrophils	58	1.6	8.15***
Eosinophils	58	1.5	16.07***

A. Absolute numbers of leucocytes:

The mean total leucocytic count in heifers was

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significantly higher (12303) compared to that of the cows (9120).

The average number of lymphocytes in the circulating blood in heifers was 7530 compared to 5217 in cows, showing the same decreasing tendancy as total leucocytes, with increasing age and was in parallel with it. The decrease in the adult cows was highly significant.

There was no difference in the level of monocytes between heifers (345) and cows (346).

The neutrophils also showed a highly significant fall with increasing age, the average numbers in heifers being 3420 while cows had 2682. The level of L/N ratio which was 2.2 in heifers dropped down to 1.9 in cows.

The eosinophils showed a slight but not significant drop in the count with increasing age.

No change in the values of basophils was observed.

B. Relative numbers of leucocytes:

The lymphocytes showed a lower percentage with increasing age, the decrease being highly significant.

The monocytes showed a higher percentage with increasing age, heifers showing 2.8 % while the cows had 3.8 %. The increase was highly significant.

With neutrophils, the percentage difference was highly significant, with heifers showing a lower value of 27.8 % and

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cows 29.4 %, even though the absolute value showed a decrease.

The eosinophils showed an increase in the parallel direction with increasing age. The percentage of eosinophils in heifers was 7.7 % and in cows 9.2 %, the increase in cows being highly significant.

DISCUSSION

For the convenience of comparison, the white blood cell picture worked out by other authors is arranged in table IV-4.

From the above observations, it may be concluded in general that the leucocytic count along with the absolute numbers per c.mm.^{3} of lymphocytes and neutrophils decreased with increasing age; while the percentages of monocytes, neutrophils and eosinophils increased with increasing age. There was no substantial change, in the absolute values of monocytes.

Schultz (1905), Canham (1930), Fraser (1930), Moberg (1955), Holmann (1956), Greatorex (1957), Gayat and Cheverier (1966) and Khajuria and Razdan (1966) showed similar results in cattle.

The works of Nichols(1935) in dogs, Reda (1951,1967) in buffaloes and goats, Hathoat (1953) in sheep also showed a similar variation, Sturgis and Bethel(1943) while reviewing the blood picture in man reported similar findings.

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TABLE IV-4

Review of investigations of the white blood cell picture on normal cattle

Authors		No. of animals	Total count	Percentage				
				Lymphocyte	Monocyte	Neutrophil	Eosinophil	Basophil
1		2	3	4	5	6	7	8
Works by Foreign Authors								
Schultz	(1905)	39	9300	-	-	-	-	-
Dimmock & Thompson	(1906)	-	-	54.2	1.4	30.4	13.1	0.6
Kacko	(1927)	200	-	54.0	4.0	33.0	8.0	1.0
Kohanawa	(1929)	12	~~9600~~ - ~~22300~~	51.7	4.3	33.0	10.9	0.1
Canham	(1930)	14months adult ♀	9600 - 22300	54.0	4.0	33.0	8.0	1.0
"	"	Adult ♀	5000 - 19900	53.0	4.0	33.0	9.0	1.0
Fraser	(1930)	41	7800	51.0	6.9	30.3	11.3	0.5
Zemljic	(1936)	50	7008	52.5	5.8	32.1	9.0	0.1
Jones	(1943)	41	10961	-	-	-	-	-
Ferguson, Irwin & Beach	(1945)	25	8912	41.2	7.9	34.7	14.9	0.6
Rusoff & Piercy	(1946)	46	8411 - 10268	54.5 -57.3	5.5-6.4	29.4-32.6	6.4-7.3	0.4-0.6
Braun	(1946)	49	9347	56.1	7.8	27.8	8.4	0.7

TABLE IV-4(contd.)

1		2	3	4	5	6	7	8
Drago	(1949)	60	7800	60.3	9.3	25.8	6.7	-
Moser	(1951)	50	-	50.3	8.8	32.4	8.1	0.4
Storkebaum	(1951)	107	6636	64.1	1.8	31.9	2.1	0.1
Hathoat, Gobba & Moustafa	(1956)	25	10816	64.7	4.4	26.6	3.7	0.1
Greatorex	(1957)	49	9100	57.0	2.0	30.0	11.0	0.0
Gourley	(1959)	60	10100	48.5	12.3	28.1	9.7	1.4
Smith	(1959)	40	11220	49.7	6.2	30.8	12.9	-
Schalm (Review)	(1961)	Cows	4000-12000	45.0 - 75.0	2.0-7.0	15.0-45.0	2.0-20.0	0 - 2.0
du Toit(S.Africa)	-	Adult cattle	-	49.0	3.7	38.8	8.0	0.5
Seargent & others	-	39	-	59.5	7.5	22.0	10.0	0.9
Hoffner	-	German adult cattle	-	51.0	7.5	36.0	5.0	0.5

TABLE IV-4(contd.)

1		2	3	4	5	6	7	8
				Works of the Indian Authors				
Shankarnarayanan	(1937)	Sindhi cows	12840	57.0	including monocytes	31.0	11.0	-
"	"	Gir cows	12000	70.0	"	23.0	8.0	-
Mullick & Pal	(1943)	Hariana cows	9100	-	-	-	-	-
Govindan Nayyer	(1949)	S.Indian cattle	8100	68.0	6.0	23.0	3.0	-
Patel, Mithuji & Shah	(1960)	Kankrej cows	11610	-	-	-	-	-
Mishra & Biswal	(1961)	30 Orissa cattle	9440	66.0	4.0	25.0	5.0	-
Agarwala	(1963)	Hariana cows	11530	48.9	4.7	38.8	7.6	-
Mithuji,Shukla & Patel	(1966)	Kankrej cows	12630	67.0	4.0	23.0	6.0	-

Dacie (1956) wrote that higher levels of leucocytes were observed in childhood with predominance of lymphocytes. Trautmann and Fiebiger (1957) also stated the same thing in regard to dogs, cattle, rabbits and chickens.

Greatorex (1957) who studied in detail the haematology from calfhood to adult age in dairy cattle, cited Winquist (1954) who reported that calves at birth were under great stress due to the reaction of the pituitary-adrenocortical activity of the calf and that of the mother as well, before parturition. This stress was indicated by the reduced percentages of lymphocytes, monocytes and eosinophils at birth in calf. Greatorex found that the stress to be a transient one as the leucocytes showed increased values within a few days afterwards. His observations showed that characteristic changes occurred between five and nine months of age. During this period, the percentage of neutrophils was reduced and that of lymphocytes increased, while eosinophils and monocytes showed definite increases. He considered such changes to be associated with the approach or onset of puberty and the changes in the rate of growth. This stage corresponds to the heifers of about 2-3 years of age in the Indian cattle.

Lymphocytes have been considered to be closely connected with formation of antibodies and their release, and have a protective function in the body. As the new born calf has a low level of lymphocytes due to the undeveloped state of its lymphatic organs, the necessity of feeding the colostrum which

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contains antibodies to the new born for the first few days seemed to be justifiable. There seemed to be a rapid development of the lymphatic organs and hence lymphocytes within few days as shown by Greatorex (1957). The lymphocyte dominated leucocyte picture in heifers i.e. in young age was in line with the greater protection needed at that age. The lowered absolute lymphocyte value in cows might be attributed to the atrophy of the lymphatic organs in the adult age especially thymus at puberty. While reviewing the literature concerning the hormonal influence on the lymphatic apparatus, Dougharty (1952) stated that cortisone, pregnancy and lactation also influenced the involution of the lymphatic tissues, especially of the thymus.

Khajuria and Razdan (1966) found that the percentage of neutrophils rose from 24.00 % in immature heifers to 25.00 to 26.25 % in pregnant heifers and further rose to 27.50 to 29.12 % in cows in first lactation. In the present study also, the percentage of neutrophils which was 27.8 % in heifers rose significantly to 29.4 % in cows. From this, it might be concluded that pregnancy and lactation had a positive influence on the neutrophil ratio in the circulating blood.

The percentage of eosinophils also showed an increase with increasing age. This increase might be attributed to three factors. Spiers (1958) suggested that eosinophils were involved in antibody formation. With the atrophy of the thymus and other lymphatic organs decreasing the number of circulating lymphocytes, the demand for antibody formation was met with an

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increase in the eosinophil numbers. Secondly that though the haemopoitic activity was reduced in adult animals where in all granulocytes were formed, the level of eosinophils in the circulating blood might be replinished from those formed in the intestinal wall(Godlowski,1951). Thirdly the decreased or sluggish activity of pituitary-adrenal complex in adult cattle (Moberg, 1955).

It is well known that the bones and the haemopoitic system connected with it are in developing stage from birth to maturity when it levels off to a maintenance activity only. The high total and differential counts in the young might be attributed to this growing activity of the haemopoitic system, compared to the lower levels of white blood cells in the adult age due to the lessened activity.

The average total leucocytic count of 12303 per c.mm. in heifers was slightly higher than the figures of 11,116 and 10900 per c.mm. of blood reported by Moberg (1955) and Greatorex (1957) respectively for foreign breed heifers; it tallied with the figures of 11388-12671 reported for immature and pregnant heifers by Khajuria and Razdan (1966).

The average total count, 9120 per c.mm. of blood of cows closely tallied with the figures reported by Schultz(1905), Canham (1930), Furguson et al.(1945), Brown (1946), Greatorex (1957) and many others for foreign breed cattle; and Mullick and Pal (1943) for Hariana cows, Mishra and Biswal (1961) for Orissa cattle. The figure was lower than 12.840 and 12000 for

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Sahiwal and Gir cows, 11610 for Kankrej cows, 11530 for Hariana cows and 12630 for Kankrej cows reported by Shankarnarayanan (1937), Patel et al.(1960), Agarwala (1963) and Mithuji et al. (1966) respectively.

The lymphocyte percentage of 61.2 % in heifers concurred with that of Khajuria and Razdan (1966) who reported 61-62 % for heifers in humid season. It was slightly lower than that reported by Moberg (1955).

The adult cows showed 57.2 % of lymphocytes. This finding was in close confirmation with the findings of many workers including Schultz (1905), Shankarnarayanan (1937), Brown (1946), Drago (1949), Greatorex (1957), Schalm (1961) and many others. Some authors have reported still higher and lower figures.

The percentage of monocytes in cows which was 3.8 %, was similar to those of almost all Indian authors except Govindan Nayyer (1949) who reported a slightly higher figure. It also tallied with that reported by Kohanawa (1929), du Toit, Canham (1930), Hathoat,(1956) and Schalm (1961) etc.

The heifers showed a neutrophilic percentage of 27.8. It concurred with the figure of 27 % reported by Greatorex (1957) for 2 year heifers and was not far away from 24.00 to 26.25 % reported by Khajuria and Razdan (1966) for heifers; but it was higher than the figure reported by Moberg (1955) for heifers of SRB -breed.

The adult cows showed 29.4 % of neutrophils. This corresponded well with the figures of Fraser (1930), Rusoff and

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Piercy (1946), Braun (1946), Bathoat,(1956), Greatorex (1957), Smith (1959), Schalm (1961) for foreign breeds and Shankar-narayanan (1937) for Singhi and Gir cows. The other Indian authors except Agarwala (1963) obtained slightly lower figures, while Agarwala obtained a figure of 38.8 %, because his study probably included parturient cows as well.

The eosinophil percentage of 7.7 in heifers coincided with Moberg's (1955) figures but was slightly higher to those reported by Khajuria and Razdan.

There was great variation in the figures of eosinophil percentages for adult animals reported by many authors. The percentage of 9.2 for adult cows seemed to be midway in the figures reported. It agreed with those reported by Kohanawa (1929), Canham (1930), Fraser (1930), Zemljic (1936),Shankar-narayanan (1937), Braun (1946), Greatorex (1957), Gourley(1959), Schalm (1961) and others.

Indian authors have not reported on the percentage of basophils. The percentage of 0.5 and 0.4 for heifers and cows respectively agreed with those reported by other authors.

The relative and absolute values obtained for the cows representing the random sample of the farm and arranged in table IV-2 have been taken as normal values for the herd and have been used as such for comparison with the leucocytic picture during oestrous cycle, pregnancy and parturition.

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WHITE BLOOD CELL PICTURE

DURING OESTROUS CYCLE

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WHITE BLOOD CELL PICTURE DURING OESTROUS CYCLE IN HEIFERS AND COWS

MATERIALS

The leucocytic picture in 20 complete oestrous cycles in 12 cows was studies. The study also included the leucocytic picture of another 10 cows during oestrous period only. Blood samples were taken every 3rd or 4th day during the whole cycle except during the oestrus period when the blood samples were taken daily. The details of the animals under study are given below:

	Age
(a) Heifers between 2-3 years	3
(b) Cows below 5 years ...	3
(c) Cows between 5-10 years	10
(d) Cows above 10 years	6

	Calvings
(a) Heifers	3
(b) Cows upto 2 calvings ...	8
(c) Cows from 2-5 calvings	8
(d) Cows with more than 5 calvings ...	3

The methods adopted have already been described in chapter III.

RESULTS

The mean leucocytic values along with S.D.,S.E. and C.V. of cows in different stages of oestrous cycle were obtained. These results are shown in table V-1.

The data of leucocytes were subjected to 'F' test to see whether different stages of oestrous cycle differ significantly with respective different values. From 'F' test, it was concluded that the different stages differ significantly, Table V-2. Since 'F' was significant, critical difference was obtained and presented in table V-3. The different leucocytic values in various stages of oestrous cycle were compared with normal values and subjected to 't' test; the results are shown in table V-4.

The leucocytic mean values obtained are represented in graphs V-1 and V-2.

Total leucocytic count:

From the results in table V-1, it was observed that the total leucocytic count was highest (10778) on the day of heat. On the second day of heat - the day of ovulation,there was a slight but not significant reduction in the total count which came down to normal level on the day after ovulation and remained so in the whole luteal phase except the period of 10-12 days after ovulation when there was a significant increase.The

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follicular phase of the cycle showed another significant rise (table V-3) and remained so in the proestral period (table V-1).

Lymphocytes:

The lymphocyte percentage showed a significant fall from the normal value on the day of ovulation (table V-4). The value rose highly significantly (table V-4) from the normal in the first part of the luteal phase and fell in later part of the same phase and remained so in the follicular phase. The fall was highly significant from the normal value (table V-4).

Further the fall in the lymphocyte percentage on the day of ovulation compared to the day of heat was also significant (table V-3). The rise in the lymphocytic value after ovulation was highly significant (table V-3).

The absolute values of lymphocytes remained almost the same throughout the oestrous cycle except a slight drop in the last part of the luteal phase (table V-1).

The L/N ratio was lowest on the day of ovulation and remained about the normal level in the luteal phase.

Monocytes:

The percentage of monocytes remained rather significantly high during oestrus phase and parts of the luteal phase when compared to the normal values (table V-4). The percentage value fell significantly in the middle of the luteal phase (table V-3). The absolute values also fluctuated

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likewise (table V-1).

Neutrophils:

The neutrophil percentages showed higher values during the follicular phase and lasted till ovulation(table V-1). The luteal phase showed significantly lower percentages of neutrophils, compared to the normal values (table V-4). The critical difference analysis revealed a significant drop in the neutrophil percentages in the middle of the luteal phase (table V-3). The absolute values showed similar tendancies,with the lowest value at the end of the luteal phase(table V-1).

Eosinophils:

The eosinophil percentage showed a highly significant lower value from the normal on the day of heat(table V-4). The percentage showed a significant rise on the day of ovulation from that of the day of heat (table V-3).

The increase in the percentage of eosinophils was highly significant during the last days of the luteal phase by both 'C.D.' and 't' tests (table V-3 and V-4).

The absolute values were more than normal during the follicular phase but were normal in the first half of the luteal phase (table V-1). The absolute number of eosinophils, then increased and reached the highest in the last days of the luteal phase; but were reduced in the beginning of the follicular phase (table V-1).

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TABLE V-1

Variations in the total and differential counts of the white blood cell picture during oestrous cycle.

Leucocytes	Particulars	Day of heat	Day of ovulation.	After ovulation 1-3 days	4 - 6 days	7 - 9 days	10 - 12 days	13-15 days	16-19 days
1	2	3	4	5	6	7	8	9	10
				Absolute values					
Total count	Minimum	6122	6832	6410	6832	7332	7310	4688	7210
	Maximum	17254	17675	14444	18566	11300	13344	11088	15866
	Mean	10778	10444	9871	9878	9107	10277	8719	10403
	S. D. ±	2584	2851	2273	3559	1319	2165	2753	2510
	S. E.	445	594	710	1112	412	676	1251	643
	C. V. %	23.97	27.29	23.02	36.02	14.48	21.06	31.57	24.12
Lymphocytes	Minimum	2261	2184	2888	2835	3876	3775	1782	2141
	Maximum	9835	12618	9894	12830	7080	8379	6101	11740
	Mean	5896	5097	6150	6154	5018	5745	4839	5441
	S. D. ±	2039	2151	1807	2979	1088	1732	1685	2308
	S. E.	370	422	392	930	340	541	702	563
	C.V. %	34.58	42.20	29.38	48.40	21.68	30.14	34.82	42.41

TABLE V-1(contd.)

1	2	3	4	5	6	7	8	9	10
				Absolute values(contd.)					
	Minimum	153	123	192	135	257	230	234	172
	Maximum	1116	884	669	821	904	771	902	820
Monocytes	Mean	517	439	424	425	519	411	514	468
	S. D. ±	243	182	159	215	210	176	306	200
	S. E.	44	37	36	65	67	59	127	50
	C. V. %	47.00	41.45	37.50	50.58	40.46	42.82	59.53	42.73
	Minimum	1768	950	1442	1257	1716	2076	1004	1875
	Maximum	6470	6363	5880	3796	4690	4087	3050	4523
	Mean	3244	3384	2655	2539	2668	2795	2058	3048
Neutrophils	S. D. ±	1250	2435	1128	795	942	594	770	1776
	S. E.	227	507	250	256	303	170	296	403
	C. V. %	38.53	71.95	42.48	31.31	35.30	21.25	37.41	58.26

TABLE V-1(contd.)

1	2	3	4	5	6	7	8	9	10
				Absolute values(contd.)					
Eosinophils	Minimum	313	296	227	362	440	538	831	467
	Maximum	1943	3192	1406	1771	1498	2673	3101	2273
	Mean	938	982	721	889	792	1079	1316	1040
	S. D. ±	428	661	336	421	359	614	795	499
	S. E.	77	137	76	131	112	192	305	127
	C. V. %	45.62	67.31	46.60	47.35	45.32	56.90	60.41	47.98
Basophils	Minimum	0	0	0	0	0	0	0	0
	Maximum	272	179	112	82	129	122	83	129
	Mean	47	53	40	36	25	50	25	57
	S. D. ±	73	57	32	28	41	42	34	46
	S. E.	13	12	7	8	13	13	14	12
	C. V. %	155.31	107.54	80.00	77.77	164.00	84.00	136.00	80.70

TABLE V-1(contd.)

1	2	3	4	5	6	7	8	9	10
					Percentage values				
Lymphocytes	Minimum	33.0	26.0	48.5	41.5	41.0	45.0	46.5	40.0
	Maximum	73.0	63.5	77.5	72.0	69.5	68.5	64.5	62.5
	Mean	54.7	48.8	62.3	62.3	55.1	55.9	55.5	52.3
	S. D. ±	0.02	0.54	0.70	0.82	0.64	0.44	0.24	0.51
	S. E.	0.003	0.11	0.17	0.27	0.20	0.14	0.09	0.14
	C.V. %	0.36	1.10	1.12	1.31	1.16	0.78	0.43	0.97
	L/N ratio	1.8	1.5	2.3	2.4	1.8	2.0	2.3	1.7
Monocytes	Minimum	1.5	1.0	2.0	1.0	3.5	1.5	3.5	2.0
	Maximum	12.0	7.0	8.0	9.5	8.0	8.0	10.0	8.0
	Mean	4.8	4.2	4.3	4.3	5.7	4.0	5.9	4.5
	S. D. ±	0.22	0.15	0.24	0.29	0.16	0.15	0.18	0.16
	S. E.	0.04	0.03	0.05	0.09	0.05	0.04	0.08	0.04
	C. V. %	4.58	3.57	5.58	6.74	2.80	3.75	3.05	3.55
Neutrophils	Minimum	14.0	17.0	10.5	9.5	18.0	21.0	17.0	18.0
	Maximum	46.0	54.0	45.5	48.5	41.5	38.0	30.0	45.0

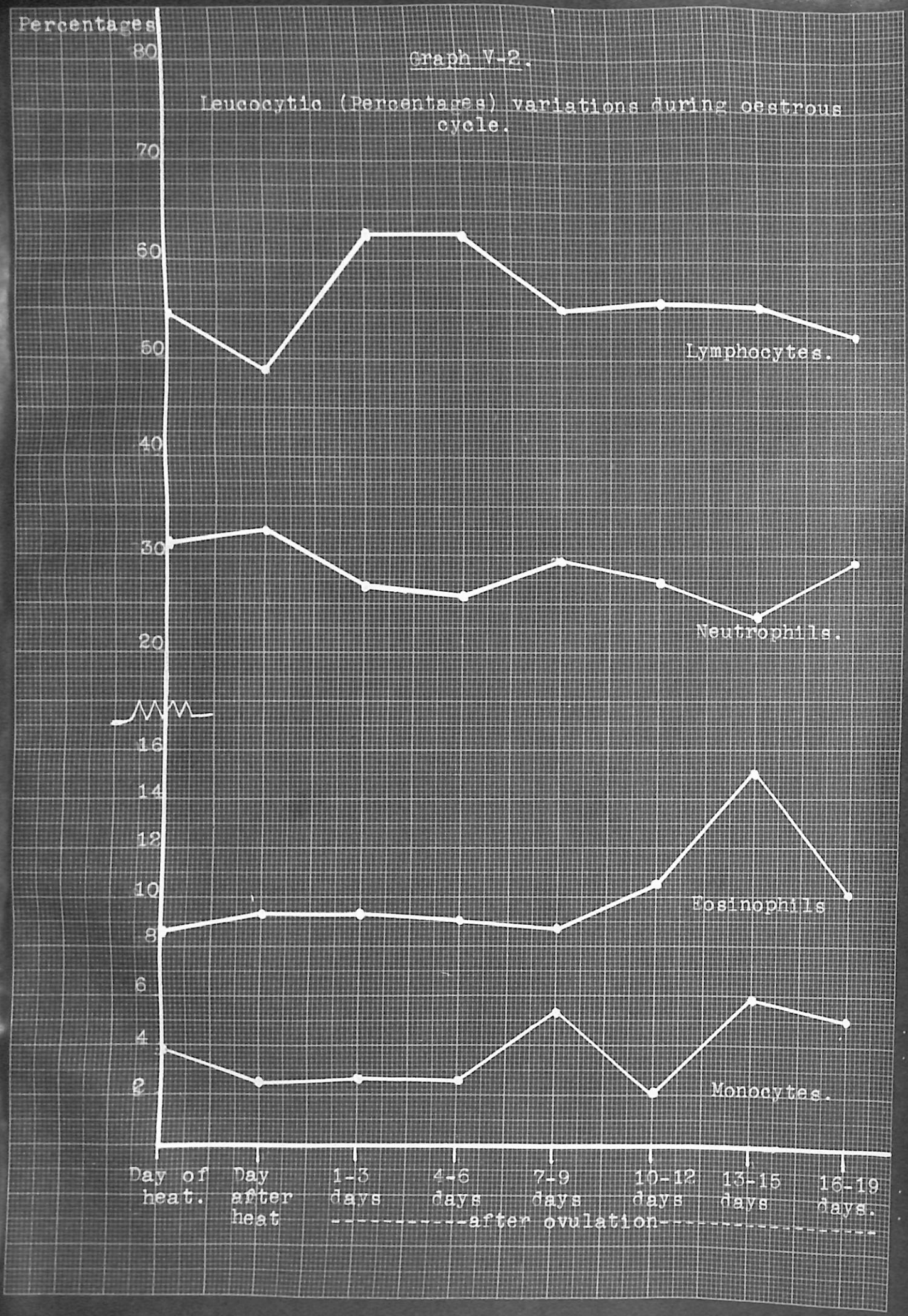
TABLE V-1 (contd.)

1	2	3	4	5	6	7	8	9	10
				Percentage values (contd.)					
Neutrophils (contd.)	Mean	30.1	32.4	26.9	25.7	29.3	27.2	23.6	29.3
	S. D. ±	0.99	1.10	0.96	1.30	0.70	0.35	0.31	0.67
	S. E.	0.18	0.23	0.22	0.41	0.21	0.11	0.12	0.15
	C. V.%	3.28	3.39	3.56	5.05	2.38	1.28	1.31	2.28
	Minimum	3.0	3.0	2.5	4.0	5.5	6.0	9.5	3.5
	Maximum	17.0	24.0	18.5	22.5	18.5	21.5	23.0	24.0
Eosinophils	Mean	8.7	9.4	7.3	9.0	8.7	10.5	15.1	10.0
	S. D. ±	0.54	0.67	0.42	0.59	0.24	0.42	0.46	0.37
	S. E.	0.11	0.14	0.09	0.19	0.07	0.13	0.19	0.09
	C. V. %	6.20	7.12	5.75	6.55	2.75	4.00	3.04	3.70
	Minimum	0	0	0	0	0	0	0	0
	Maximum	2.0	1.0	1.5	1.0	1.5	1.0	1.0	2.5
Basophils	Mean	0.44	0.51	0.41	0.36	0.28	0.49	0.29	0.55
	S. D. ±	0.13	0.10	0.10	0.06	0.09	0.03	0.46	0.03
	S. E.	0.02	0.01	0.02	0.01	0.02	0.01	0.20	0.008
	C. V. %	29.5	19.6	24.3	16.66	32.14	6.12	15.86	5.45

TABLE V-2

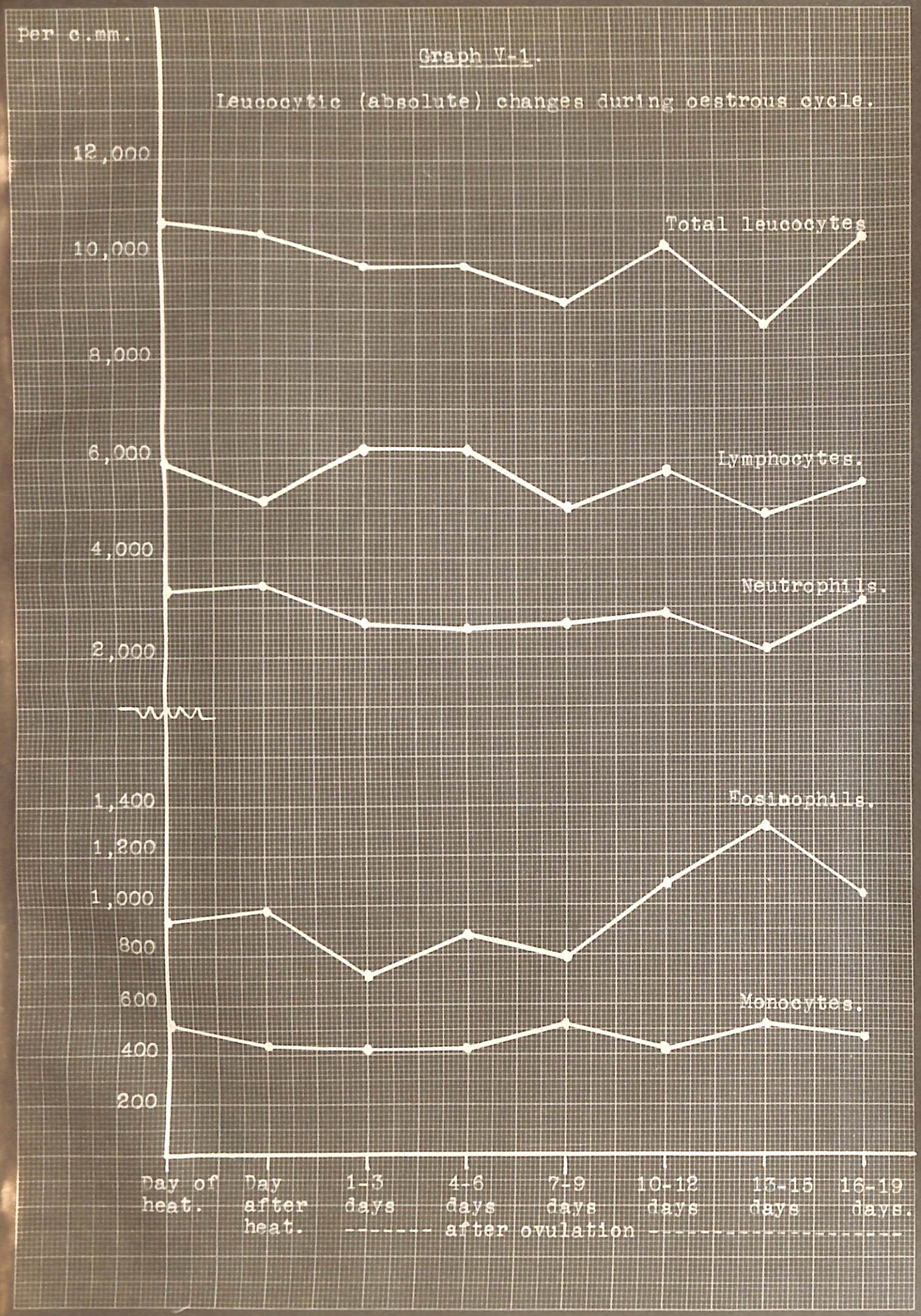
Analysis of variance of white blood cell picture during oestrous cycle in Tharparkar cows.

Source of variation	D.F.	Total count		Percentage values									
		M.S.	F.	Lymphocytes		Monocytes		Neutrophils		Eosinophils		Basophils	
				M.S.	F.	M.S.	F.	M.S.	F.	M.S.	F.	M.S.	F.
Between cows	30	9689293	1.7*	66.59	4.2**	12.74	2.55*	71.02	4.5**	18.01	3.1**	2.71	0.17 N.S.
Between intervals	7	5438537	0.9NS	88.74	6.5**	8.36	1.67NS	41.07	2.6*	155.94	27.40**	1.89	0.12 N.S.
Error	86	5696095		13.46		5.0		15.69		5.69		15.53	
Total	123												



Graph V-2.

Leucocytic (Percentages) variations during oestrous cycle.


Per c.mm.
Graph V-1.
Leucocytic (absolute) changes during oestrous cycle.
12,000
10,000
8,000
6,000
4,000
2,000
1,400
1,200
1,000
800
600
400
200
Total leucocytes
Lymphocytes.
Neutrophils.
Eosinophils.
Monocytes.
Day of heat.
Day after heat.
1-3 days
4-6 days
7-9 days
10-12 days
13-15 days
16-19 days.
after ovulation

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Basophils:

The basophils - relative and absolute - in general showed higher values during the follicular phase and lower values in the luteal phase (table V-1).

From 'F' test, (table V-2), it was concluded that there was significant difference between the cows in respect of total leucocytes, percentages of lymphocytes, monocytes, neutrophils and eosinophils. The percentages of lymphocytes, neutrophils, and eosinophils differed significantly between the intervals where as total leucocytes and monocytes percentages did not show any significant difference.

DISCUSSION

The previous investigations by other workers on the white blood cell picture in oestrous cycle had shown varying results.

The present study had shown higher total count at the time of oestrus in particular and in follicular phase in general. Canham (1930), and Lauda (1931) found an increase in the total leucocytic count during oestrus. Feja (1941) in cows and Tillmann and Gehring (1953) in cows and horses found similar variation in the total count. The latter authors reported an increase at the beginning of oestrous cycle and decrease with follicular growth in preovulation phase. The variations in total count found in the present study were similar to

these observations. These authors further reported that the total white cell count fell down to the lowest level during involution of the corpus luteum. In the present work also, the total white cells showed the lowest value at the end of the luteal phase. Moberg (1952, 1953 and 1955),confirmed the findings of the earlier workers, while Satalov (1940) had reported earlier, higher leucocytic formation during the period of ovulation. The findings of Agarwala (1963) in Hariana cows and of Solimen and Selim (1966) in she buffaloes were quite similar to the findings of the present study.

The relative and absolute values of lymphocytes showed great variations in the oestrous cycle; the lymphocytopenia at the time of oestrus disappeared by the beginning of the luteal phase and again reappeared in the follicular phase. Feja (1941), Tillmann and Gehring (1953) recorded similar findings earlier in cows. Moberg (1955) did not find such variation in cows; while in heifers, he recorded a lower value on the day of heat. Bravo (1935) reported on the other hand, lymphocytosis in a bitch with induced heat. Agarwala (1963) did not find any difference in the lymphocytic value during oestrous and dioestrus phases.

It has been mentioned under results that L/N ratio was the lowest on the day after heat followed by a gradual rise to normal in the luteal phase. Moberg (1955) after studying the L/N ratio in two families of cows, pointed out that cows showing strong symptoms of heat have a lower L/N ratio than

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those exhibiting weak symptoms.

The higher monocytic values in the dioestrus period of the present study corresponded to similar findings of Agarwala (1963) while Moberg and others have not referred to these values.

Canham (1930) reported an increase in the neutrophilic count at oestrus which reflected in the increase of the total count. Similar findings have been obtained in the present investigations also. Moberg (1955) reported the peak value for neutrophils in cows and heifers in oestrus; while in the present study, the neutrophils attained the peak on the day of ovulation. The neutrophilic picture in the luteal phase obtained in the present study corresponded to similar findings of Moberg's (1955). Neutrophilia during oestrus was also reported by Agarwala (1963) in cows, and Solimen and Selim (1966) in female buffaloes.

The significant fall in the percentage of eosinophils on the day of heat obtained in this study had also been recorded by other workers including Tillmann and Gehring (1953), Moberg (1952) and Agarwala (1963) in cows. Solimen and Selim (1966) found similar decreases in connection with oestrus in female buffaloes and Bravo (1935) obtained the same results in a bitch with induced oestrus. Andrews and Bonsnes (1951) found no significant variation in the number of eosinophils during the sexual cycle in women. Canham (1930) on the other hand reported eosinophilia in connection with oestrus in cows.

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In the present study, the eosinophil percentage rose significantly on the day of ovulation from the level on the day of oestrus, and the absolute values also showed a rise. Moberg's (1952) work also showed a rise from 4.0 % on the day of heat to 4.3 % on the day of ovulation. In his further work, Moberg (1955) referred to a decrease in the absolute values during the above period, but at the same time failed to mention about the percentage values. Canham's (1930) report of a rise in eosinophils probably corresponded to the day of ovulation and to similar findings of the present investigations.

Denecke and Josam (1927) noted neutrophilia in dogs as a result of oestrogen administration. Tryslowitz and Dingemanse (1941) obtained similar results with initial leucocytosis in dogs. Gaudlitz (1952) administered oestrogen to cows and found neutrophilic leucocytosis with increase of eosinophils in some cases. Kahn (1953) obtained similar results when leucocytosis occurred after administration of oestrogen; but when leucopenia was observed it was due to lymphocytopenia. Moberg (1955) carried out similar experiments in overiectomised heifers. Overiectomy caused postoperative reduction in all types of leucocytes while oestrogen treatment to the same heifers caused neutrophilia with rapid recovery to normal values, lymphocytopenia and eosinopenia. Similar results have been reported by Feja (1941) in cows, Castrodale et al. (1941) in rat, rabbit and dogs and in humons by Feuchtinger (1942) and others.

Cole and Cupps (1959) cited Hansel (1958) who

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reported that the follicular growth was maximum by 18-21 days postoestrus. Roberts (1956) stated that the content of oestrogen was maximum in the matured follicles. The effects of stress caused by oestrogen secretion mediated through the neurohumoral control over the pituitary-adrenal complex and directly on the haemopoietic system (Moberg, 1955) might be expected to produce maximum changes in the white blood cell picture during the maximum secretion of oestrogen, i.e. during oestrus and upto ovulation. The results obtained in the present study followed the above pattern.

As is well known, progesterone inhibits the formation of follicles in the overy and thereby oestrogen secretion through its inhibitary action over FSH production from the anterior pituitary. Moberg (1955) studying the effects of progesterone to the oestrogen conditioned overiectomised heifers, stated that progesterone inhibited the influence of oestrogens; so that the lymphocytopenia and eosinopenia caused by the earlier administered oestrogen, returned to normal more rapidly after the progesterone treatment. He further stated that progesterone had no definite influence on neutrophils. Moberg reasoned that the favourable effect of progesterone leading to the recovery of the lymphocytic values could be due to its neutralizing effect on the action of oestrogen on lymphopoiesis. In the same way, the cause for the increase in the eosinophil count could be due to the reduced activity of the pituitary-adrenal complex - reduced stress effect - due to the absence of

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oestrogen during the luteal phase. The sudden recovery of the leucocytic values in the early luteal phase followed by the normal values in the luteal phase proper due to the influence of progesterone could be easily understood in view of the above reasoning.

Newsam (1961) found the vaginal mucus smears of heifers at oestrus to be acellular. Nellore and Broun (1966) found similar state in vaginal mucus smears and genital tract tissues in sheep at oestrus and suggested that this might be due to the inability of the leucocytes to penetrate the increased epithelial barrier developed close to the time of ovulation. If so, the same barrier might be acting as protective wall against the genital tract infection during oestrus.

Hammond (1927), Newsam (1961) and Nellore and Broun (1966) found neutrophils, eosinophils and occasionally lymphocytes in the vaginal mucus smears and genital tract tissues in the luteal phase. This showed that the white blood cells migrate from the circulating blood into the genital tract tissues and then in the mucus in the luteal phase of the oestrous cycle. This partly explains the reduction in total and differential counts in the circulating blood during the luteal phase.

On the other hand, Bessette (1962) in ferrets and Ross and Klebanoff (1966) in rats reported that eosinophils migrate into the genital tract tissues during oestrus. Spiers (1955) suggested that eosinophils were involved in antibody

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formation. Then it was likely that the protective epithelial barrier in the genital tract spoken of by Nellore and Broun (1966) might be reinforced by eosinophils during oestrus against infection.

Dougharty and White (1947) were of the opinion that the lymphocytopenia produced after the administration of ACTH - induced stress might also be used an index of alarm reaction. The typical reaction of eosinophils to the pituitary adrenal complex has also been advised as an index of stress reaction by Selye (1946), Archer (1957) and many others, and is known as 'Thorn test'.

Viewed from these tests, the reaction of the white blood cell picture was typical of the effects of stress due to the influence of various hormones secreted during oestrus.

Pounden and Firebough (1956) reported that considerable nervousness or unusually placid reactions at the time of service or artificial insemination resulted in reduced conception. Bratanov et al.(1959) found that cows and buffaloes exhibiting marked symptoms of oestrus yielded less milk than those animals exhibiting normal symptoms. From these observations, it seemed that animals exhibiting medium stress effects were better suited for breeding and milk production than the animals exhibiting either very high or quite placid reactions of excitement and nervousness due to stress effects.

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WHITE BLOOD CELL PICTURE

DURING PREGNANCY

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THE WHITE BLOOD CELL PICTURE DURING PREGNANCY

MATERIALS

A minimum of 6 cows and/or heifers per each month of pregnancy were selected for purpose of studying the white blood cell picture during pregnancy. In all 73 animals - 9 heifers and 64 cows - were studied. The selection was made on the basis of their service records confirmed by actual rectal examinations. The details of the animals were as follows:-

	Age
(a) Heifers, 2 - 3 years	9
(b) Cows below 5 years	8
(c) Cows between 5 - 10 years	40
(d) Cows above 10 years	16

	Calvings
(a) Heifers	9
(b) Cows upto 2 calvings	20
(c) Cows with 2 - 5 calvings	31
(d) Cows with more than 5 calvings	13

The methods used have been the same as described under chapter "Materials and Methods".

RESULTS

The data analysed as per methods mentioned in chapters III and V have been arranged in tables VI-1, VI-2, VI-3 and VI-4. The mean values of total and differential counts have been represented in graphs VI.1 and VI.2.

Total count:

In general, the total white cell counts rose gradually reaching the maximum of 12489 per c.mm. of blood by 6th months; then decreased and levelled during the 7th (10283) and 8th (10292) months. The count came down to slightly below normal (9005) during the 9th month,(table VI-1). From table VI-2, it was observed that the total white cell count varied highly significantly between months. From critical difference analysis (table VI-3), it was observed that the total count rose significantly during the 6th month and suddently dropped down during the 7th month (table VI-1). The drop was highly significant(table VI-3). Again there was a significant drop during the 9th month,(table VI-3). When compared to the normal values (table VI-4) the total counts during 5th, 6th and 7th months showed highly significant upward trend, while the values for 1st, 4th and 8th months did not show any significant variations from the normal.

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TABLE VI-1

Changes in the total and differential counts of the white blood cells during pregnancy

Leucocytes	Particulars	Months								
		1st	2nd	3rd	4th	5th	6th	7th	8th	9th
1	2	3	4	5	6	7	8	9	10	11
		Absolute values								
Total count	Minimum	7566	6176	8510	7532	9188	8375	6666	6550	6376
	Maximum	11076	11376	13022	14722	14332	16254	15066	13010	12400
	Mean	9070	8833	9874	10368	10973	12489	10283	10292	9005
	S.D. ±	1431	1608	1854	2822	1691	3390	2221	2441	1927
	S.E.	596	578	713	1175	650	1303	602	1017	550
	C.V. %	15.77	18.20	18.77	27.21	15.41	27.15	21.59	23.71	21.39
Lymphocytes	Minimum	4161	4632	4681	4506	4410	5826	3333	3990	2455
	Maximum	7643	6089	7227	10085	6851	10932	8286	7351	7296
	Mean	5415	5406	5993	6397	6002	7668	5573	6175	5205
	S. D. ±	1419	561	884	2087	858	2170	1715	1526	1302
	S. E.	591	200	340	869	330	834	463	636	372
	C. V. %	26.20	10.37	14.75	32.62	14.29	28.29	30.77	24.71	25.01

TABLE VI-1(contd.)

1	2	3	4	5	6	7	8	9	10	11
Monocytes	Minimum	151	114	177	169	232	266	56	162	191
	Maximum	474	423	863	459	491	1138	1112	585	504
	Mean	263	274	405	290	351	400	401	288	315
	S. D. ±	141	102	248	113	157	314	311	158	118
	S. E.	58	36	95	47	60	121	84	66	34
	C. V. %	53.61	37.22	61.23	38.96	44.72	78.50	77.55	54.86	37.46
Neutrophils	Minmum	1462	957	1037	1431	2060	2543	1890	1048	1020
	Maximum	2907	4266	3842	3313	6449	5364	4972	3448	5022
	Mean	2231	2296	2478	2519	3413	3185	3156	2573	2692
	S. D. ±	508	960	966	619	1406	1164	828	855	1054
	S. E.	211	342	371	258	540	447	223	356	301
	C. V. %	22.77	41.81	38.98	24.57	41.19	36.54	26.23	33.22	39.15
Eosinophils	Minimum	573	432	222	763	602	251	236	655	327
	Maximum	1382	1059	1533	1913	1373	2763	1900	1675	1028
	Mean	1079	804	908	1120	1064	1099	884	1173	720
	S. D. ±	343	289	470	440	284	843	394	390	173
	S. E. ±	143	103	180	183	109	324	106	162	49
	C. V. %	31.78	35.94	51.76	39.28	26.69	76.70	44.57	33.24	24.02

TABLE VI-1 (contd.)

1	2	3	4	5	6	7	8	9	10	11
Basophils	Minimum	0	0	0	0	0	0	0	0	0
	Maximum	118	80	65	151	184	162	116	131	124
	Mean	19	28	24	49	47	65	48	38	34
	S. D. ±	48	31	30	57	65	65	37	58	44
	S. E.	20	11	11	23	25	25	10	24	12
	C. V. %	252	110	125	116	138	100	77	153	129
					Percentage values					
Lymphocytes %	Minimum	52.5	53.5	53.0	54.0	48.0	42.0	50.0	47.5	38.5
	Maximum	69.0	75.0	77.0	68.5	60.0	72.0	64.0	68.0	69.0
	Mean	59.7	61.2	60.7	61.7	54.7	61.4	54.3	60.0	57.8
	S. D. ±	0.31	0.37	3.00	0.16	0.22	0.99	0.16	0.49	0.73
	S. E.	0.13	0.13	1.10	0.06	0.08	0.38	0.04	0.20	0.20
	C. V. %	0.51	0.60	4.94	0.25	0.40	1.61	0.29	0.81	1.26
	L/N ratio	2.4	2.3	2.4	2.5	1.7	2.4	1.7	2.4	1.9
Monocytes %	Minimum	0.0	1.0	2.0	1.5	1.0	2.0	1.5	1.5	2.0
	Maximum	5.0	4.0	6.0	4.5	5.0	7.0	9.5	4.5	5.0
	Mean	2.9	3.1	4.1	2.8	3.2	3.2	3.9	2.8	3.5
	S. D. ±	0.24	0.10	0.24	0.10	0.13	0.16	0.39	0.10	0.10
	S. E.	0.10	0.03	0.09	0.04	0.05	0.06	0.10	0.04	0.02
	C. V. %	8.27	3.22	5.85	3.57	4.06	5.00	11.00	3.57	2.85

TABLE VI-1(contd.)

1	2	3	4	5	6	7	8	9	10	11
Neutrophils	Minimum	18.5	15.5	14.0	16.0	21.0	20.0	22.5	16.0	16.0
	Maximum	35.5	37.5	30.0	33.0	45.0	33.0	36.0	36.5	45.0
	Mean	24.6	26.0	25.1	24.3	31.1	25.5	30.7	25.0	29.9
	S. D. ±	0.59	0.44	0.40	0.46	0.59	0.26	0.19	0.49	0.85
	S. E.	0.24	0.15	0.15	0.19	0.22	0.10	0.05	0.20	0.24
	C. V. %	2.39	1.69	1.59	1.89	1.89	1.01	0.61	1.96	2.84
Eosinophils %	Minimum	7.0	7.0	2.5	8.5	3.5	3.0	3.0	9.5	3.5
	Maximum	17.5	11.5	13.0	13.0	14.0	17.0	17.0	13.5	13.5
	Mean	11.9	9.1	9.2	10.8	9.7	8.8	8.6	11.4	8.0
	S. D. ±	0.31	0.10	0.59	0.11	0.37	0.59	0.35	0.08	0.24
	S. E.	0.13	0.03	0.22	0.04	0.14	0.23	0.09	0.03	0.07
	C. V. %	2.60	1.09	6.41	1.01	3.81	6.70	4.06	0.70	3.00
Basophils %	Minimum	0	0	0	0	0	0	0	0	0
	Maximum	1.5	1.0	0.5	2.0	2.0	1.0	1.0	2.0	1.0
	Mean	0.21	0.32	0.24	0.47	0.43	0.52	0.47	0.37	0.38
	S. D. ±	0.15	0.09	0.06	0.22	0.18	0.15	0.08	0.24	0.12
	S. E.	0.06	0.03	0.02	0.09	0.07	0.06	0.02	0.10	0.03
	C. V. %	71.42	28.12	25.00	46.80	41.86	28.84	17.02	64.86	31.57

TABLE VI-2

Analysis of variance of white blood cell picture in pregnant Tharparkar cows.

Source of variation	D.F.	Total count		For percentage values									
				Lymphocyte		Monocyte		Neutrophil		Eosinophil		Basophil	
		M.S.	F.	M.S.	F.	M.S.	F.	M.S.	F.	M.S.	F.	M.S.	F.
Between months	8	10035428	6.07**	19.45	1.10 NS	4.36	0.41 NS	27.48	1.51 NS	14.42	1.34 NS	1.85	0.42 NS
Within months	64	1652566		17.66		10.49		18.10		10.70		4.31	
Total	72												

TABLE VI-3

Mean and critical differences of total leucocytic count during pregnancy

Between months	Mean difference		Calculated C.D. @ 5 %	@ 1 %
First and second	236.41	NS	1355.63	-
Second and third	1040.46	NS	1306.21	-
Third and fourth	493.91	NS	1403.20	-
Fourth and fifth	605.67	NS	1403.20	-
Fifth and sixth	1515.95	*	1355.63	1812.05
Sixth and seventh	2204.86	**	1174.07	1569.36
Seventh and eighth	8.24	NS	1228.68	-
Eighth and ninth	1286.33	*	1254.99	1677.52

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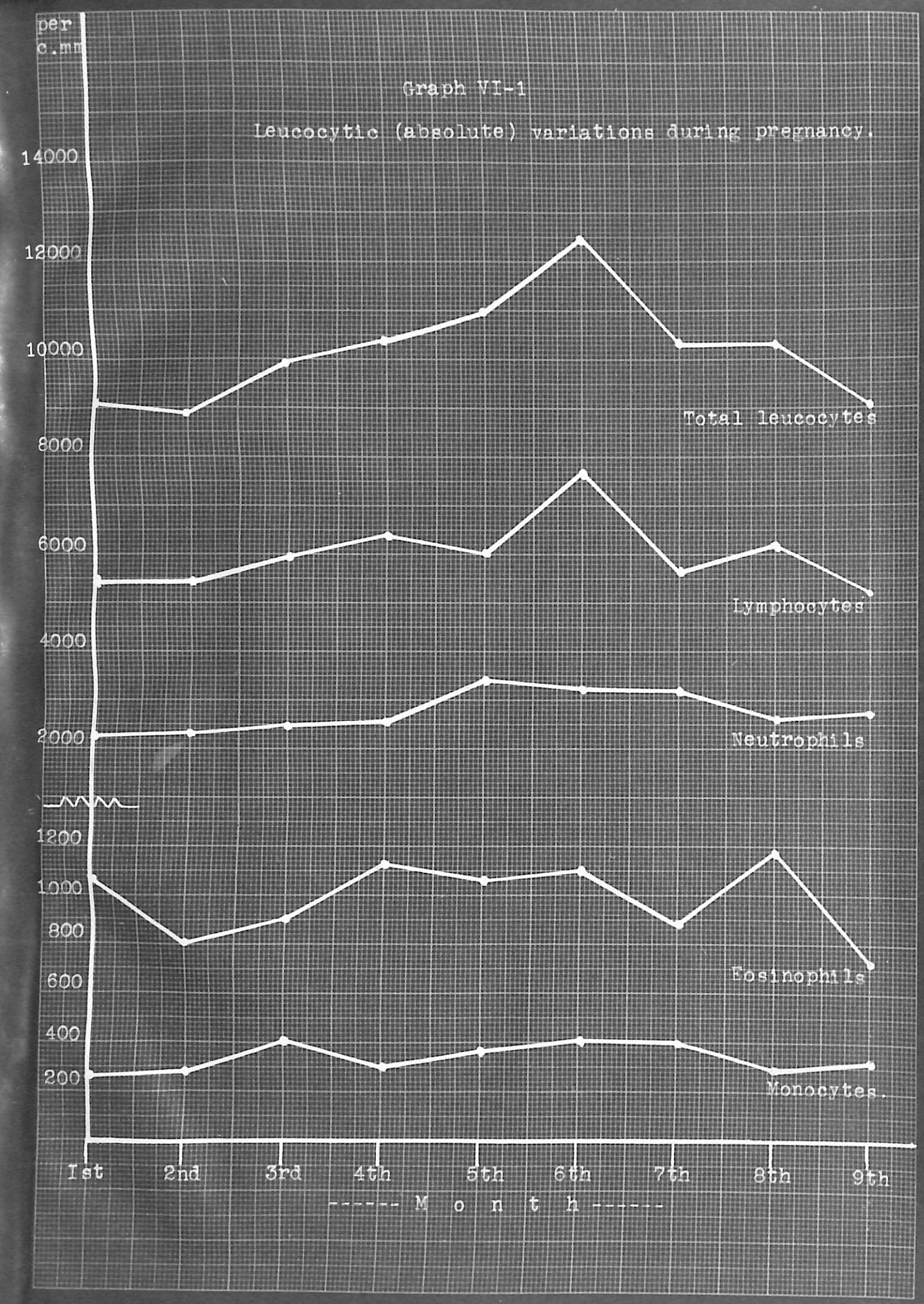
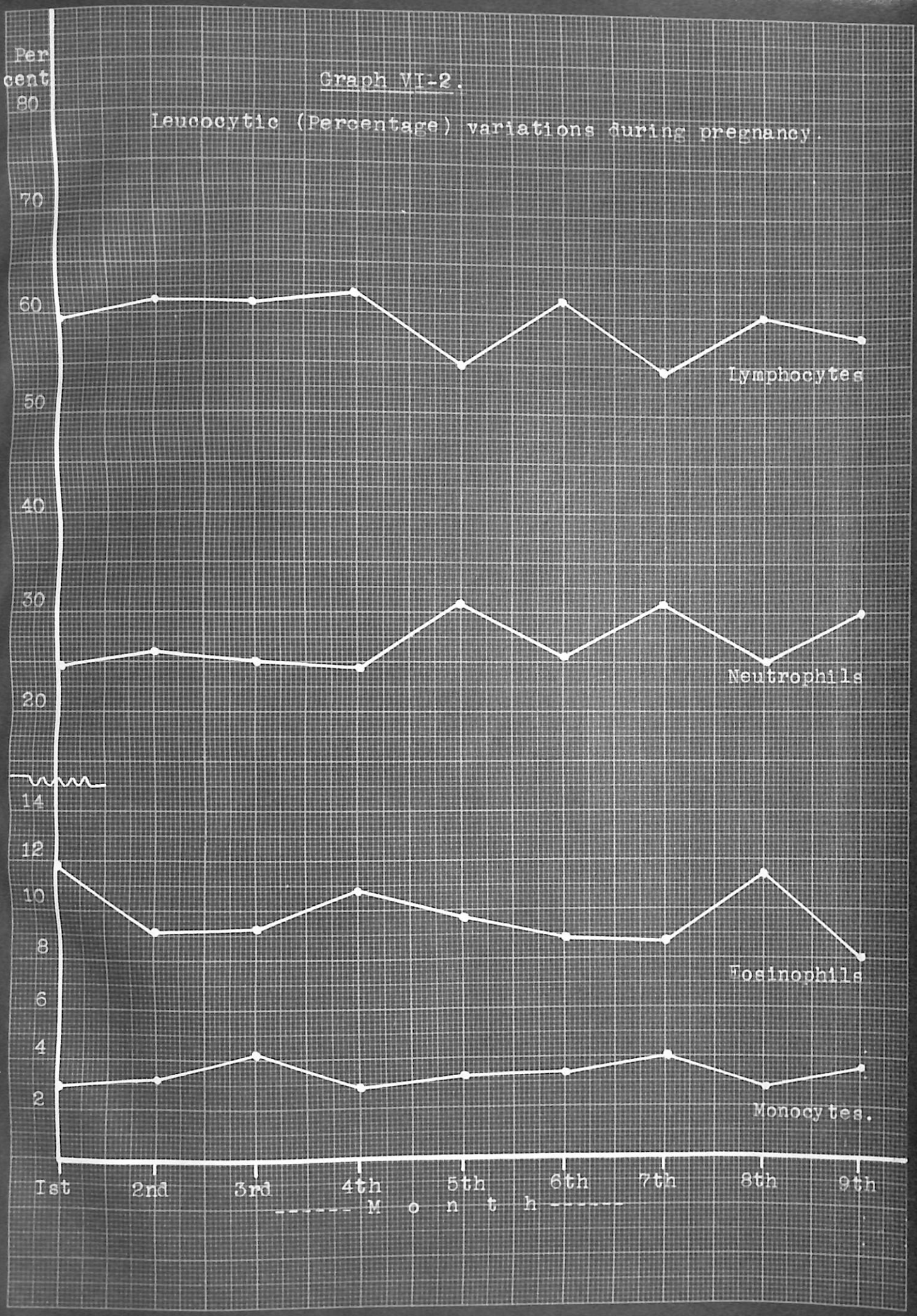

per
c.mm
Graph VI-1
Leucocytic (absolute) variations during pregnancy.
14000
12000
10000
8000
6000
4000
2000
1200
1000
800
600
400
200
Total leucocytes
Lymphocytes
Neutrophils
Eosinophils
Monocytes.
Ist
2nd
3rd
4th
5th
6th
7th
8th
9th
------ M o n t h ------



Graph VI-2.

Leucocytic (Percentage) variations during pregnancy.

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Lymphocytes:

The lymphocyte percentages showed a higher trend from the beginning of pregnancy with the highest value of 61.7 % during the 4th month, and almost equal value during the 6th month with sudden drops during 5th and 7th months (table VI-1). The last month of pregnancy showed almost normal percentage of 57.8 % (table VI-1). The percentage of lymphocytes during the months of 1st, 2nd, 4th and 8th showed highly significant increases from the normal values (table VI-4), while there was a similar reduction in the 5th month (table VI-4).

The absolute lymphocyte values showed a slightly different trend. The number of lymphocytes rose gradually to a maximum of 7668 per c.mm. of blood during the 6th month, while as already stated above, the percentage values reached the maximum in the 4th month. The 7th month showed a drop followed by a rise in the 8th month. However, like relative values, the absolute values also reached the normal during the 9th month.The variation in the absolute values paralled those with the total leucocytic count (graph VI.1).

Monocytes:

The relative values of monocytes did not show much variation except a significantly lower value in the first month and a significantly increased value from the normal during the 3rd month of pregnancy. Near normal value prevailed at the end

of the term. The absolute monocytic values also showed similar variations compared to the normal values at the end of pregnancy.

Neutrophils:

The relative neutrophilic values in general showed the opposite tendancy to lymphocytic behaviour during the whole period of pregnancy (graph VI.2). The first four months of pregnancy showed rather low values, but reached the peak in the 5th month followed by a wavy variance upto the last month(TableVI.1). Again like other values, the last month of pregnancy exhibited normal relative values (table VI.1). On 't' test, it was found that the mean values were highly significantly lower than the normal values during the months of 1st, 2nd, 3rd, 4th, 6th and 8th, while the 5th and 7th months showed highly significant increase,(table VI.4). The number of neutrophils per c.mm. of blood gradually rose and reached their peak in the 5th month, after which it gradually decreased to the normal values by the 9th month (table VI.1).

Eosinophils:

The eosinophil percentage showed the highest value of 11.9% during the first month of pregnancy only. There were further rises during the 4th and 8th months. The lowest eosinophil percentage of 8.0 % was in the last month of pregnancy(tableVI.1). The 't' test analysis showed the increases of eosinophil percent

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compared to the normal values during the 1st, 4th and 8th months were highly significant; so also the falls from the normal value during the months of 6th, 7th and 9th (table VI.4). The absolute values, on the other hand, showed the highest values during the 8th month, but fell to the lowest during the last month of pregnancy (table VI.1). The earlier months showed in general slightly higher absolute values than normal (table VI.1).

Basophils:

The relative and absolute basophilic values rose gradually and reached the maximum during the 6th month after which there was a gradual fall to the normal by the 9th month of pregnancy (table VI.1).

DISCUSSION

Till now, only four detailed studies of the white blood cell picture during pregnancy by Morris (1944), Storkebaum(1951), Moberg (1953,1955) and Greatorex (1957) have been made.

From the available literature, it seemed that pregnancy had varying effects and influences on the white blood cells in cows. Various workers have reported dissimilar results.

Schultz (1905), Obentraut (1934) and Romano (1935) did not find any change in leucocytic picture during pregnancy in the cow; while Bungener (1927) and Szepeshelyi (1934) observed an increase in the total leucocytic count during normal pregnancy, especially so during the period immediately prior to

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parturition. Canham (1930) commented that pregnancy produced fluctuations in the white blood cell picture at varying times during pregnancy, but that the average remained fairly constant. Storch cited by Morris (1944) found no increase in leucocytes in pregnant cows and goats. Morris also cited Burnette (1917) who stated that in the latter period of pregnancy in women, there was usually a moderate increase in the number of leucocytes and that the tendancy was more marked in primeparae. Burnette found that in the leucocytosis during pregnancy, the relative values remained unchanged except eosinophils which might not be increased. Morris (1944) himself recorded varying results during pregnancy, with rises and falls throughout the pregnancy. Morris further recorded leucocytosis at the time , the cow was drying off finally from the previous lactation.

The trend of leucocytic variation during pregnancy in the present study concurred with the earlier findings by other workers. The rise of total leucocytes in the middle months of pregnancy of the present investigations might be due to the drying off from the previous lactations. Further it might also be partly due to the influence of oestrogen secretion from the placenta as shown by increased levels of the hormone in the urine of cows with advancing pregnancy (Cole and Cupps,1959; Pope et al.,1965). Moberg (1953,1955) obtained decreasing leucocytic count with increasing pregnancy except an initial increase. The present findings of increased leucocytic counts were similar to those found in women by Bethell et al.(1943). Davis

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and Hulit(1949), Andrews and Bonsnes (1951) and Greatorex(1957) also recorded a fall in the total count just before calving,i.e. in the 9th month, similar to the present findings.

The leucocytosis during the middle months of pregnancy was mainly due to an increase in the number of lymphocytes.The parallel behaviour of the two has been represented in the graph VI.1. It was also partly due to neutrophilia and eosinophilia indicating increased haemopoiesis in general during pregnancy due to the increased demands in connection with the fast growing foetus during the period.

The decrease in the percentage of lymphocytes during the middle months of pregnancy in the present investigations corresponded with the findings of Storkebaum (1951), Moberg (1953,1955) and others. Greatorex (1957) recorded a reduction in neutrophils and an increase in lymphocytes during the 2nd and 3rd months. The present findings of relative values were similar to these. Moberg noticed similar occurrences in the 4th and 5th months; the present results were also similar to these for the period mentioned. Morris (1944) also reported similar findings.

Greatorex (1957) found a rise in lymphocytic absolute values during the 2nd and 3rd months which decreased until the end of gestation except during the 5th to 7th months, when he recorded higher values. Moberg (1955) recorded the peak value during the 8th month which he found difficult to explain. In the present study, on the other hand, the lymphocytes gradually rose upto the 6th month during which the highest value was recorded.

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The 7th month showed a reduction with an increase again in the 8th month; the 9th month showing the normal value. The 8th month picture of this study though not the highest corresponded to a certain extent with that of Moberg's (1955).

Dougharty and Frank (1953) observed an increase in the large lymphocytes during the latter half of pregnancy and referred to such cells as 'stress lymphocytes' or 'monocytoid lymphocytes' by Winquist (1954). Moberg (1955) on the other hand , suggested that the increased weight of the foetus might cause the increased 'stress' lymphocytic numbers. Both the above arguments seemed to be far away from the proved facts about stress. All available experiments and literature have proved that 'stress' caused lymphocytopenia and not lymphocytosis. It is well known that progesterone is secreted from the corpus luteum and the placenta in increasing quantity with increasing pregnancy. Further, progesterone has been proved to be an intermediary in the biosynthesis of adrenal corticoid steroids(Hechter and Zaffaroni, 1951). It is present in the adrenal venous blood of cattle, sheep and pigs in amounts 10-100 times its concentration in arterial blood(Balfour et al.,1957).It seems then that the level of progesterone during pregnancy would be quite high. Moberg (1955) and others have proved that progesterone administration to oestrogen conditioned overiectomised heifers caused immediate increase in the lymphocytic value. It seemed reasonable and logical then, that lymphocytic values should correspond with the progesterone secretion and level. Moberg(1955) did

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not elaborate as to why the lymphocytes only showed the change due to the increased weight of the foetus and not the other leucocytic cells.

Lymphocytic picture and the opposing behaviour of neutrophils (graph VI-2) obtained in the present study seemed to follow the above pattern and reasoning.

Ferguson et al.(1941) found that monocytes during the pregnancy varied inversely with the eosinophils. Greatorex's (1957) observations were otherwise. The present investigations were rather similar to those of Ferguson et al.(graph VI.1).

The absolute neutrophilic values rose gradually from 1st month to a maximum at 6th month and then decreased gradually to a normal level by the 9th month. Moberg's (1955) observations showed the highest value during the 4th month; while Morris(1944) recorded the highest value in the 39th week, the reason being probably the stress due to the approaching parturition.The higher values during the middle months of pregnancy might have been due to the drying off of the cows from the previous lactations as suggested by Morris (1944). As already noted earlier, Greatorex (1957) showed lower percentages of neutrophils during the 2nd and 3rd months. The present observations for these months were similar in nature. The absolute values exhibited a more regular pattern than the percentage values in general.

In the present investigations, the eosinophil percentage reached the highest value during the 1st month only, and higher than normal values during the 4th and 8th months; and the

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absolute values during the 8th month. Morris (1944) also recorded high percentages during the 1st month, and again in the 4th month. Moberg (1955) recorded the highest eosinophilic value during the 2nd month with further rises in the 5th and 9th months of pregnancy. Greatorex (1957) recorded a decrease percentage for the 1st month. In the present work, the 2nd and 3rd months showed normal percentages, the 6th and 7th months slightly lower values, and the last month of pregnancy showed the lowest value. Moberg (1955) suggested that the eosinophilia during pregnancy might be the result of a direct antagonism between the anterior pituitary and the placenta, possibly in combination with the corpus luteum. Patel et al. (1960) reported higher eosinophilic percentages in pregnant cows. Ullery et al. (1965) also recorded higher relative eosinophilic concentration during gestation in sheep. Khajuria and Razdan (1966) recorded the same thing for heifers. Solimen and Selim (1966) found eosinophilia, lymphopenia, neutrophilia with leucocytosis in the buffaloes.

It could be concluded in general, that pregnancy produced varying influences on the white blood cell picture during different months. There was an increased activity of the haemopoietic system depending upon the various hormonal levels and influences of the corresponding months.

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WHITE BLOOD CELL PICTURE

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IN CONNECTION WITH PARTURITION

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THE WHITE BLOOD CELL PICTURE IN CONNECTION WITH PARTURITION IN COWS

MATERIALS

Blood samples from 6 cows were obtained, 21 days prior to and 21 days after parturition, for studying the white blood cell picture in connection with parturition. Blood samples were taken at the interval of 3 days before and after parturition, except from the 4 days previous to parturition and 3 days after parturition, when the blood samples were taken daily. In two cows, blood samples were taken within 4 hours before and after parturition; while in the remaining 4 cows, the blood samples could only be obtained 12 hrs. before and after 12 hrs. of parturition. The details of the cows were as follows:

	Age
(a) Cows below 5 years	2
(b) Cows between 5-10 yrs.	4

	Calvings
(a) Cows upto 2 calvings	2
(b) Cows with 2-5 " "	4

The methods of blood collection and examination have been mentioned in chapter " Materials and Methods ".

RESULTS

The data recorded were statistically analysed as per methods mentioned in chapters III and V. The results have been arranged in tables VII-1, VII-2, VII-3 and VII-4; while the mean values of the total and differential counts have been represented in graphs VII-1, VII-2 and VII-3.

Total count:

From table VII-1, it was observed that the total white cell count was normal at 18-21 days previous to parturition.It rose gradually to 13682 a day before parturition, but shot up to the maximum of 17666 about 4 hours before parturition(graph VII-1). The total count dropped down to 14454 about 4 hours after parturition and a day after it, the count had nearly reached the normal value. The near normal value prevailed during the 21 days following parturition except the small rise during the 2nd week.

The analysis of variance showed highly significant variation between cows and between intervals as well(table VII-2). The total count rose significantly on the 2nd day prepartus from its level of the 3rd day prepartus (table VII-3).The level further rose significantly on the day previous to parturition. The drop in the total count from the level of a day prepartus to the level of a day postpartus was highly significant (table VII-3). The rise in the total count from 6th to 9th day level

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after parturition was significant. The mean differences on days and intervals, pre- or postpartus were not significant (table VII-3). When compared to the normal values, the total leucocytic count from the 3rd day prepartus to 4 hrs. before parturition showed highly significant upward variation (table VII-4). The level of the total count 4 hrs. after parturition was also significantly more than normal, but the value on the day after postpartus when compared to the normal was not significant.

Lymphocytes:

The percentage of lymphocytes was normal 18-21 days previous to parturition (table VII-1). It decreased gradually to 48.8 % by 4 hrs. before parturition and further fell to 42.1 % by 4 hrs. after parturition (table VII-1). The next day to postpartus showed a rise in the percentage value with further recovery during the succeeding days. By the 6th day postpartus, the relative lymphocytic value had reached the normal and remained so afterwards to the end of the investigations.

The analysis of variance in table VII-2 showed that the cows and intervals differed highly significantly between themselves for relative lymphocytic values.

As the fall in the lymphocytic percentage values was gradual, the critical difference analysis (table VII-3) did not show any significant variations between adjuscent intervals before parturition; while during recovery to the normal values after parturition, the rise from the 2nd day level to the 3rd

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day level was highly significant. The percentage values when compared with the normal values showed highly significant decreases from the 3rd day previous to parturition upto parturition, and also during the first two days after parturition (table VII-4).

The recovery to near normal value was attained on the 3rd day postpartus.

The L/N ratio which was 2.5 at the start of the investigations, gradually decreased to 0.9, 4 hrs. before parturition, reached its lowest value of 0.6 by 4 hrs. after parturition (table VII-1). This showed the cross over of neutrophilic values over lymphocytic values,(graph VII-2 and VII-3) from 4 hrs. before to 4 hrs. after parturition. The ratio then gradually rose to its normal by the end of the investigations.

The absolute lymphocyte values showed a slightly opposite behaviour to their relative values. The value which was normal 5279, at the beginning rose gradually to 7950 at 4 hrs. before parturition (table VII-1). Then fell down suddenly to below normal value (table VII-1 and graph VII-3). The postpartus period in the beginning showed lower than normal values upto 9 days, after which the values recovered to normal.

Monocytes:

The monocyte percentages did not show any variation during the whole period of investigations (table VII-1), but remained rather below the normal values except for 3 days after

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parturition (table VII-1). The variance analysis showed significant difference between cows (table VII-2), while the intervals did not show any such variation. The absolute values showed a gradual rise upto parturition, after which the decrease was also gradual upto the end of investigations (table VII-1).

Neutrophils:

The relative value of neutrophils was normal 18-21 days before parturition (table VII-1). Percentage values rose gradually upto 4 hrs. before parturition. The rise was more marked in the last two days prior to parturition and 4 hrs.before parturition. The percentage further rose to a maximum of 50.8 % at 4 hrs. postpartus. The next day saw a sudden drop to 40.0 % with further reductions on the 2nd day postpartus. The value had come down to normal by the 3rd day postpartus and remained so till the end of investigations (table VII-1).

The analysis of variance (table VII-2) proved that the variance was highly significant between cows and between intervals. From table VII-3 of critical analysis, it could be observed that the increase between 21 and 18 days prepartus was significant; so also the increase between the 2nd day and the day just prior to parturition. The slight drop between the day prepartus and the day postpartus was not significant(table VII-3).But the decreases between the 1st and 2nd day postpartus and the 2nd and 3rd day postpartus were highly significant. The drop in the value between 15th and 18th days postpartus was

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also significant.

The 't' test (table VII-4), showed that the neutrophil percentage of the 3rd day prepartus was significantly more than the normal value; further the increase in the same value during the 2 days previous to parturition was highly significant. The high significant values prevailed on the 2 days postpartus and reached the normal on the 3rd day postpartus. The neutrophil percentage values crossed over the lymphocytic percentage values from 4 hrs. before parturition to 4 hrs. after parturition (graph VII-2 and VII-3).

The absolute neutrophil values showed more intensive changes. The neutrophilic numbers gradually rose from the level at 3 weeks prior to parturition to a maximum of 8126 at 4 hrs. prepartus (table VII-1). There were sudden spurts in the increases in the last two days before parturition. Actual parturition saw a sudden fall in the next 24 hrs. The 2nd day postpartus showed further decreases which reached the normal on the 3rd day and remained so till the end of investigations with minor variations (table VII-1).

Eosinophils:

The relative eosinophilic picture resembled that of the lymphocytic percentage. There was gradual reduction in the percentages (table VII-1). The reduction was more marked in the last two days prior to parturition. The percentage fell down suddenly 4 hrs. after parturition (table VII-1); likewise

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the recovery in the next 24 hrs. was also sudden and by the 2nd day, the value had reached a peak. The value then came down to normal during the next few days.

The variance between the cows and between the intervals was highly significant (table VII-2). The critical difference analysis showed that the decrease in the value between 2nd day and the day prepartus was significant (table VII-3).Likewise, the increase in the percentage value from the day prepartus to the day postpartus was also significant (table VII-3). The 't' test results showed that the decrease in the percentage of eosinophils from the normals on the last two days previous to parturition was highly significant (table VII-4). The percentage of 8.2 on the day after parturition was still significantly lower than the normal, but the value on the day after that had reached more than normal value.

The absolute values of eosinophils were slightly more than normal at 21 days previous to parturition and remained so till 2 days previous to parturition (table VII-1). There followed a sudden drop on the day prepartus, and an increase 4 hrs. before parturition. Another sudden drop followed parturition.The 1st day postpartus saw the normal eosinophilic value followed by a rise on the 2nd day postpartus. The normal value was reached in the next fortnight.

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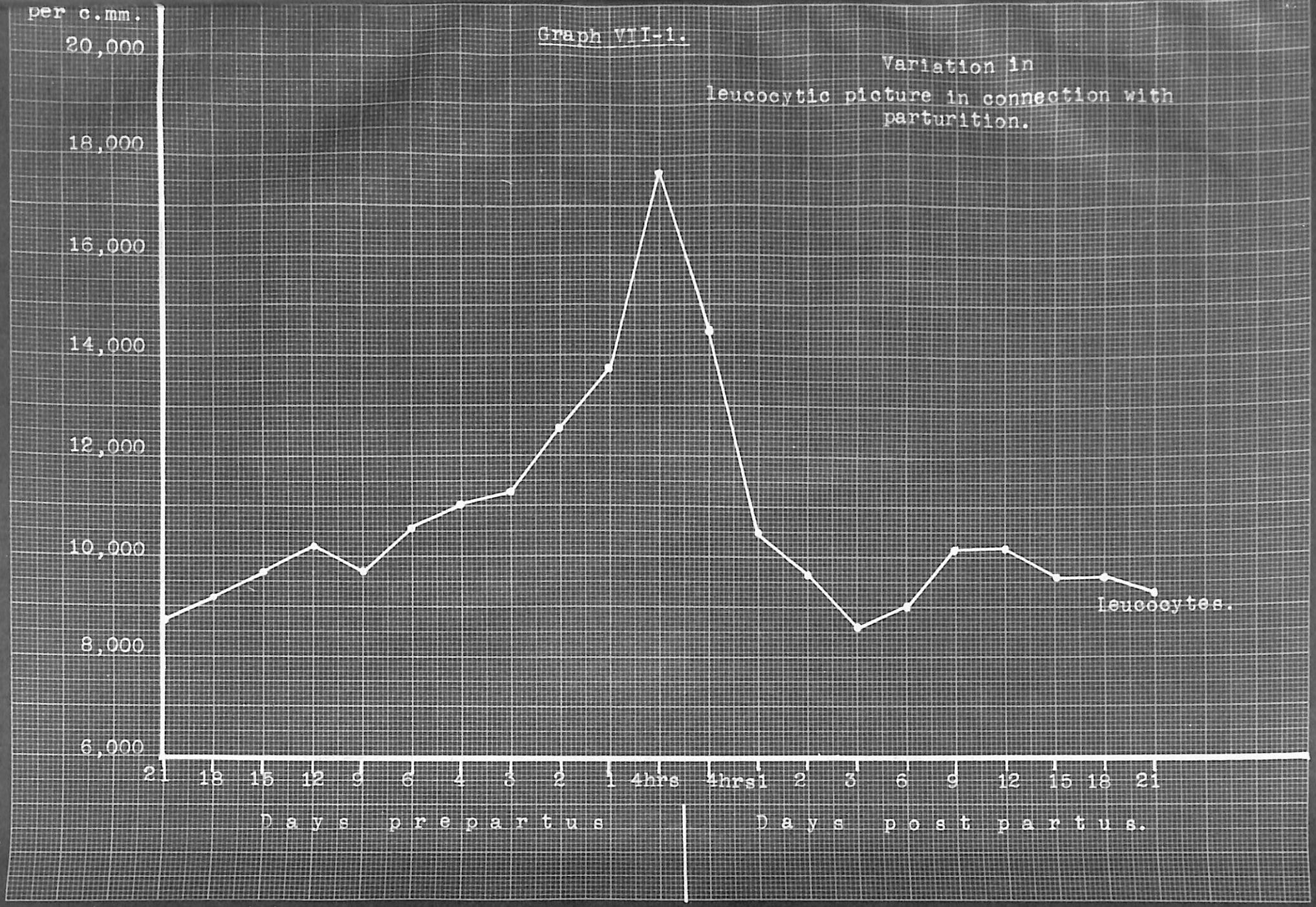
ition in Tharparkar cows.

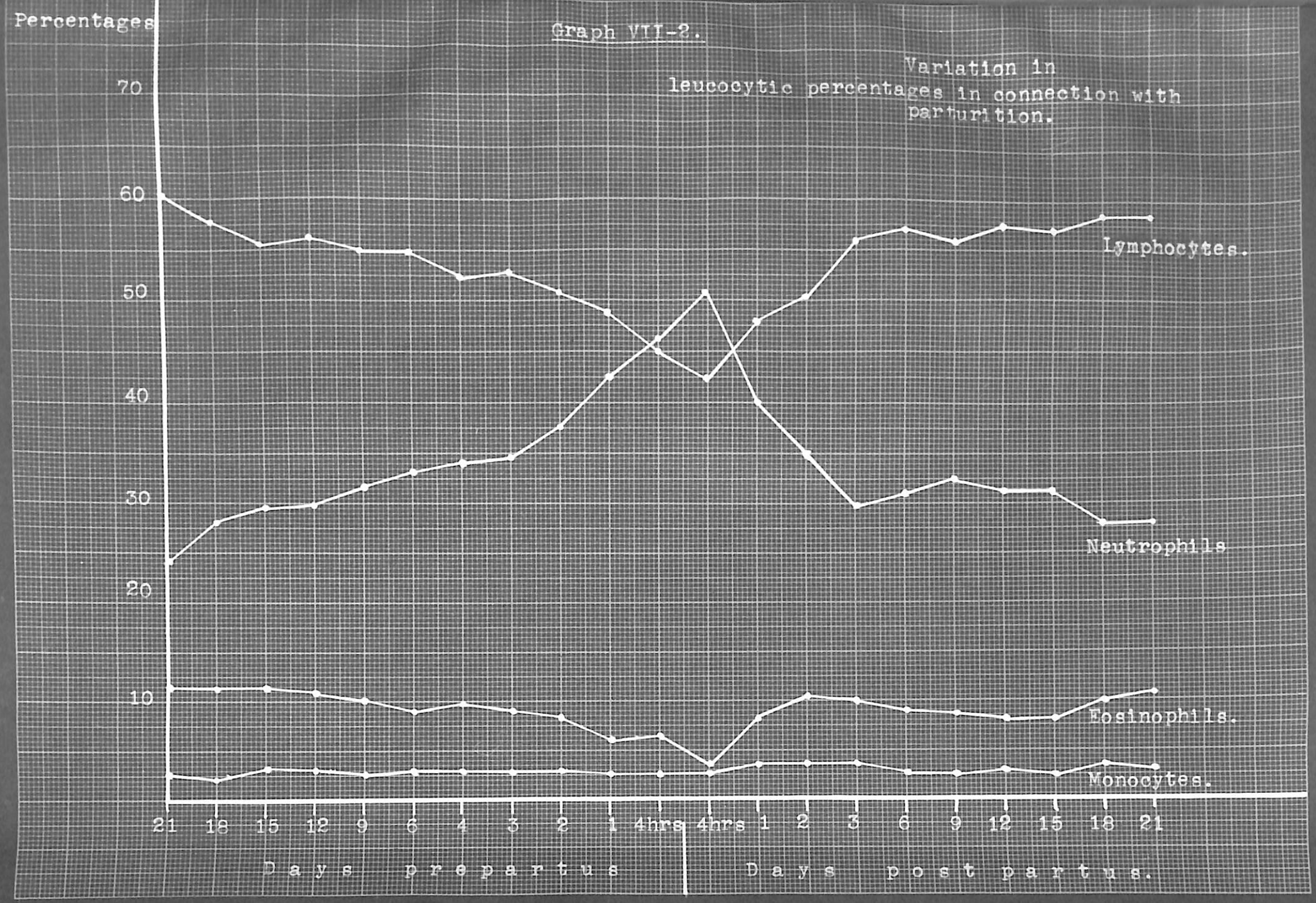
Leucocytes	Post - partus							
	3 days	6 days	9 days	12 days	15 days	18 days	21 days
1	17	18	19	20	21	22	23
Total count	32	6800	6622	7022	7044	6344	6032	6088
	88	9776	11676	12700	14844	12466	14210	12612
	73	8583	9006	10185	10183	9631	9633	9254
	25	1268	1786	2183	2903	2127	2899	2120
	93	528	774	909	1209	886	1208	808
	73	14.77	19.83	21.43	28.50	22.08	30.39	22.90
Lymphocytes	60	2822	3212	3792	4050	3616	3498	3409
	80	6275	6232	6567	8758	6545	8526	7693
	65	4815	5133	5673	5834	5499	5645	5413
	13	1625	1074	1012	1606	1721	1047	1427
	61	580	383	361	573	661	374	509
	82	33.74	20.92	17.83	27.52	27.65	18.54	26.36
Monocytes	14	236	155	140	145	157	151	91
	43	375	312	355	519	436	591	495
	35	292	234	234	285	212	337	268
	38	55	64	88	139	107	235	145
	31	23	26	36	57	45	97	50
	35	18.83	27.35	37.60	48.77	50.47	69.73	54.10
Neutrophils	56	1694	2100	1952	1880	1998	1810	1857
	50	3666	4028	5525	4329	4159	3520	2911
	5	2349	2810	3310	3208	3053	2668	2517

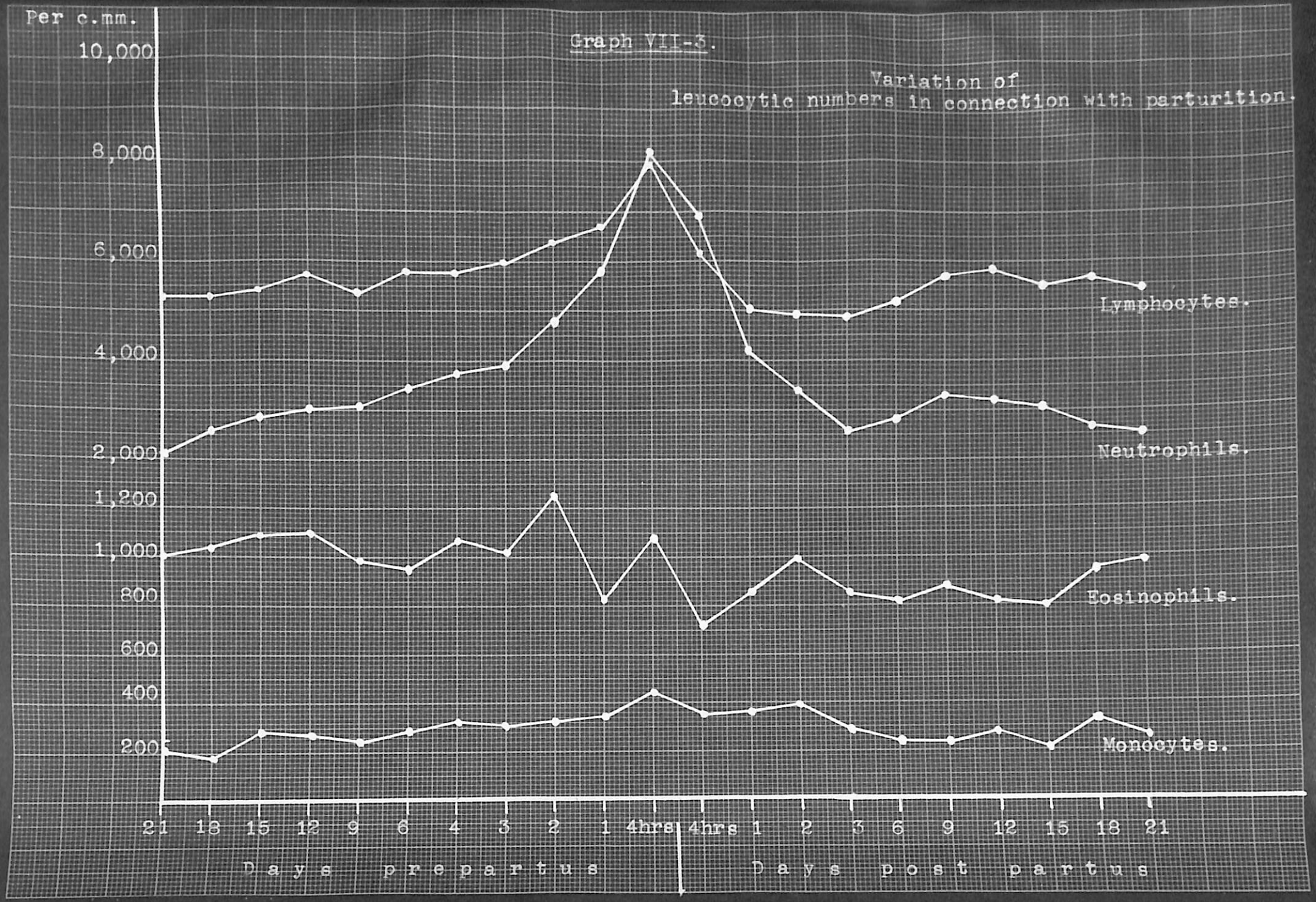
TABLE VII-2

Analysis of variance of white blood cell picture during parturition in Tharparkar cows

Source of variation	D.F.	Total count		For percentages									
				Lymphocyte		Monocyte		Neutrophil		Eosinophil		Basophil	
		M.S.	F.	M.S.	F.	M.S.	F.	M.S.	F.	M.S.	F.	M.S.	F.
Between cows	5	109555002	36.89**	125.91	15.46**	5.90	2.42**	188.06	15.46**	46.11	12.8**	3.74	1.41 NS
Between intervals	18	10006807	3.46**	24.53	3.01**	3.47	1.42 NS	43.03	3.53**	11.95	3.3**	2.78	1.04 NS
Error	90	2969414		8.14		2.43		12.16		3.60		2.65	
Total	113												


per c.mm.
20,000
18,000
16,000
14,000
12,000
10,000
8,000
6,000
Graph VII-1.
Variation in
leucocytic picture in connection with
parturition.
leucocytes.
21 18 15 12 9 6 4 3 2 1 4hrs 4hrs1 2 3 6 9 12 15 18 21
Days prepartus
Days post partus.


Percentages
Graph VII-2.
Variation in
leucocytic percentages in connection with
parturition.
70
60
50
40
30
20
10
Lymphocytes.
Neutrophils
Eosinophils.
Monocytes.
21 18 15 12 9 6 4 3 2 1 4hrs 4hrs 1 2 3 6 9 12 15 18 21
Days prepartus
Days post partus.


Per c.mm.
Graph VII-3.
Variation of
leucocytic numbers in connection with parturition.
10,000
8,000
6,000
4,000
2,000
1,200
1,000
800
600
400
200
Lymphocytes.
Neutrophils.
Eosinophils.
Monocytes.
21 18 15 12 9 6 4 3 2 1 4hrs 4hrs 1 2 3 6 9 12 15 18 21
Days preparturus
Days post parturus

Basophils:

Basophil percentages showed a decreasing tendancy with approaching parturition, the lowest being 4 hrs. before parturition. The recovery took place on the 1st day postpartus. The absolute values also showed a similar trend. No basophils could be recorded 4 hrs. prior to parturition.

DISCUSSION

The gradual increase in the total leucocyte count culminating in parturition observed in the present investigations have been recorded also by many authors in cows and buffaloes (Krisch,1923; Bungener, 1927; Canham,1930; Szepeshelyi,1934; Garm, 1950, 1951; Storkebaum, 1951; Moberg,1953,1955; Holmann, 1955; Straub, 1959; Solimen and Selim, 1966).

A number of workers have recorded a high leucocytic value at the time of parturition in other species also(Sturgis and Bethell,1943 in women; Burnett and Traum cited by Morris, 1944 in bitch; Roy et al.,1965 in sheep and goats, Ullery,1965 in sheep).

Ferguson et al.(1941) did not mention about the leucocytic picture before parturition, but found a decrease after parturition. Paterson (1957) on the other hand recorded sharp decreases in the total count at about parturition. Agarwala, (1963) found almost a constant total count in connection with parturition. Greatorex (1957) recorded actually a reduction in

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50 % of the cows studied.

The leucocytosis in the present investigations was mainly due to an absolute neutrophilia and to a certain extent to lymphocytophilia also. The increase in the total count at 4 hrs. before parturition was nearly 100 % more than the normal value, while the increase in the neutrophils was more than 300% and that of the lymphocytes was only by about 50 %. Other authors (Krish, Storkebaum, Garm, Kerr et al., Moberg etc. cited above) have also recorded that the leucocytosis at parturition was mainly due to high neutrophilia. Staub (1959) and Agarwala (1963) recorded that the leucocytosis was also due to an increase in lymphocytic numbers.

Ferguson et al. (1941), Moberg (1953,1955) have recorded a decrease in the total leucocytes soon after parturition and in the next 24 hrs. In the present study also, the total leucocytes recorded a fall during similar period.

The present study showed significant reductions in the relative lymphocytic value with approaching parturition and this reduction continued even 4 hrs. after parturition; while the absolute values showed a rise upto parturition only. There was a sudden fall soon after parturition. Morris (1944) also recorded a low percentage for lymphocytes before parturition. Garm (1950,1951), Merryl and Smith (1954) stated similar findings for the period just before parturition. Moberg (1953,1955) spoke only of absolute values, in which he recorded a decrease during actual labour. Holmann (1955) stated that the fall in

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lymphocytic values continued to fall in value even at 12 hrs. after calving. Paterson (1957) did not observe any changes in lymphocytic values during parturition in sheep. Straub (1959) and Agarwala (1963) recorded an increase in the numbers of lymphocytes in connection with parturition. The findings of Roy et al.(1965) in sheep and Solimen and Selim (1966) in she buffaloes were also similar.

The absolute monocytic value behaved in opposite direction to that of eosinophils in general. Similar behaviour has been recorded by Ferguson et al.(1941) and Straub (1959).Moberg (1955) stated that the monocytes showed normal variations.

All the authors (Krish, Morris, Kerr et al., Storkebaum, Garm, Moberg, Merryl and Smith, Holmann, Greatorex,and Agarwala cited earlier) have recorded neutrophilia of varying extent in connection with parturition. Paterson (1957), and Roy et al.(1965), in sheep and goats and Solimen and Selim(1966) in she buffaloes recorded similar findings. The cross over of neutrophils over lymphocytes recorded in the present work has also been recorded by others including Kerr et al.(1949), Merryl and Smith (1954) and Moberg (1953,1955). In the present study, the major increases in the relative and absolute values of the neutrophils which were 2-3 times the normal, took place during the 2 days previous to parturition. Moberg (1953,1955) also found similar sudden spurts with his cows also. The fall in the neutrophilic values of the parturition followed similar patterns reported by Morris (1944), Garm (1950,1951),etc.

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Moberg (1953,1955) with his extensive studies also recorded similar reductions in the neutrophilic values.

Ferguson et al.(1941) could not record any changes in the neutrophils after parturition.

Holmann (1955) reported that the neutrophilia persisted for some hours after parturition and then subsided.

Like lymphocytopenia, there was also gradual eosinopenia in connection with parturition, the major changes occurring during the last two days before parturition. Moberg (1953, 1955) recorded a gradual decrease in the absolute eosinophilic values from the 21 days previous to parturition to a minimum at parturition, similar to the present findings. Eosinopenia has also been recorded by Ferguson (1941), Kerr et al.(1949), Garm (1950,1951), Shaw (1952), Shaw et al.(1952), Merryl and Smith (1954), Straub (1959), Otsuka and Nakajami (1961) and Agarwala (1963) - all in cows. Roy et al.(1965) in sheep and goats, Ullery et al.(1965) and Solimen and Selim (1966) in she buffaloes also recorded similar findings.

Holmann could not detect any change in the eosinophilic count in connection with parturition.

While the relative eosinophils showed a continuous drop before parturition, the absolute numbers showed small rises and falls before parturition. Moberg (1955) observed an isolated peak the day postpartus; while in the present study, though the 1st day postpartus showed an increase over the value prevailing at 4 hrs. after parturition, the peak was observed

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on the 2nd day postpartus.

In the present study, the relative and absolute values of basophils showed some regular pattern in their behaviour in connection with parturition. Moberg (1955) commented that the basophils presented but normal variations.

It is well known that during parturition, various hormones act together in a well integrated mechanism (with various other stimuli) culminating in parturition. Some of these hormones produce "stress" effects bringing about changes in the leucocytic morphology. Kerr et al.(1951) were of the opinion that in the period before the initiation of parturition, there seemed to be some degree of cortisone effect which varied with the individual animal and this would partly account for the different responses shown in the total leucocytic counts.

The progesterone content of human placenta is reported to be higher before labour than after (Cole and Cupps,1959). The same authors stated that at 280 days pregnancy in the cow, the content of oestrogen was 6636 R.U./24 hours - the maximum - and was reduced at full term. Hays and VanDemark (1953) proved the release of oxytocin on stimulating cervix and vulva of cows. Bell and Morris (1934) proved that, oxytocic power in blood increases during the last week before parturition. Oxytocin in connection with oestrogen enhances the activity of uterine muscles (Knaus,1934; Robson,1947 and Reynolds, 1949). Apart from the above hormones, a fourth one "Relaxine" is also

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involved during parturition (Hisaw, 1926; and Hisaw and Zarrow, 1950). Siegrist (1956) and Paterson (1957) have proved that the blood level of 17 - hydroxycorticosteroids increased before parturition.

All the above hormones were expected to produce considerable stress during parturition and influencing the white blood cell picture during the period. Moberg (1955) and others have already proved the effect of oestrogen and progesterone on the circulating leucocytes in the blood. This aspect has already been discussed in earlier chapters. Selye (1946), Spiers and Meyer (1951), Archer (1957) and many others have proved that the stress caused by the pituitary-adrenal complex produced considerable leucocytosis, mainly due to neutrophilia, accompanied by eosinopenia and lymphocytopenia.

The considerable increase in total count as a result of absolute neutrophilia at about parturition and accompanied by eosinopenia and lymphocytopenia in the present study at about parturition could be interpreted as a result of the stress caused by the increased activity of the pituitary-adrenal complex. The changes might also be partly due to the other hormones secreted at the time of parturition and acting either directly on the haemopoietic system or indirectly influencing the hypothalumus and hypophysis through the neuro-humoral pathway.

The eosinophilic peak on the 2nd day postpartus might be due to a temporary exaustion of the pituitary-adrenal complex. The disappearance of the eosinophils from the circulating blood

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during the effects of stress at parturition might be due to the destruction of them by the glucocorticoids as suggested by Godlowski (1952) and/or the withdrawal of them to the storage place in spleen as suggested by Spain and Thalhimer (1951). The disappearance of eosinophils might also be due to the increased utilisation of these cells which presumably contain substances useful for humoral (Serological) defences of the body (Selye,1946). Further, the bonemarrow is the main site of production of eosinophils (Cunningham et al.,cited by Archer, 1960) and Winquist (1954) had shown that the eosinophils in the bone marrow increased simultaneously with a marked drop in the blood eosinophils following treatment with ACTH. This, Winquist interpreted as due to a partial inhibition of the migration of the mature eosinophils from the bone marrow into the circulating blood.

That during parturition, there was considerable neutrophilic production and release was proved by the findings of Winquist (1956) who detected a "shift to the left" in the neutrophilic granulocytes indicating increased production in the bone marrow of parturient cows. It was probable that the absolute neutrophilia might be helping in the defence against infection through phagocytosis in the body in general and the genital organs in particular. The increased production of neutrophils in connection with parturition might further gain support from the findings of Selye (1946) who found that haemorrhage caused neutrophilia, and haemorrhage to a certain

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extent was inevitable in any parturition.

The sudden decrease in the neutrophils after parturition might be due to their large scale migration into the genital tissues as proved by the findings of Gier et al.(1962) who detected large number of neutrophils in the sluffing in the uterus after parturition in cows.

The slight increase in the total count, the absolute and percentage values of neutrophils and the decrease in the same values of eosinophils during the second week postpartus might be due to the involution of the uterus as suggested by Krisch (1923).

It could be concluded from the discussion that the various hormones connected with parturition with their highest influences at about parturition, acting through the neuro-humoral pathway brought about increased activities in the pituitary-adrenal complex producing stress of medium intensity and the stress so caused in turn brought about considerable and significant changes in the total and differential leucocytic values in the circulating blood.

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S U M M E R Y

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S U M M A R Y

The purpose of the present investigations was to study the influences of oestrous cycle, pregnancy and parturition in heifers and cows on the white blood cell picture and the changes brought about thereby in it.

The literature concerning the white blood cell morphology in normal cattle, during oestrous cycle, pregnancy and parturition was reviewed.

The Tharparkar heifers and cows belonged to the Government Cattle Farm, Patna. Blood samples were always collected in the morning avoiding excitement to the animals. Double oxalates were used as anticoagulants. Wright's stain was used for staining the blood smears. The total and differential counts were made adopting standard techniques. The data were statistically analysed to know their significance.

Normal white blood cell picture:

Blood samples from 40 cows were studied to fix the normal white blood cell picture in adult cows. The values for the differential counts were: total count, 9120 ± 1356; lymphocytes, 5212 ± 1172; monocytes, 346 ± 183; neutrophils, 2682 ± 844; eosinophils, 839 ± 136 and basophils, 36 ± 53. The percentage values were: lymphocytes, 57.2 ± 0.51; monocytes, 3.8 ± 0.30; neutrophils, 29.4 ± 0.32; eosinophils, 9.2 ± 0.35

and basophils, 0.4 ± 0.12.

Blood samples from 20 heifers were studied and the results compared with those of adult cows. It was found that there were significant differences in the total and differential counts except absolute eosinophilic values. Basophilic values were not compared. It might be concluded from the results that the age of the animals had definite influence on the leucocytic picture; Gestation and lactation might cause permanent changes.

White blood cell changes during oestrous cycle:

Changes in the white blood cell picture of 12 cows in their 20 oestrous cycles and of another 10 cows during oestrus only were studied. There were discernible changes in the leucocytic values of the follicular and luteal phases. The total count showed somewhat higher values in the follicular phase than the luteal phase. The lymphocytes - both percentage and absolute showed significant variations between cows and between intervals. The neutrophils showed higher values during the follicular phase. The L/N ratio was lowest on the day of ovulation and remained at a higher level during the luteal phase. The absolute monocytic values showed an opposite behaviour to that of eosinophils.

The influences of hormones in conjunction with pituitary adrenal complex on the white blood cell picture were also discussed.

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White blood cell picture during pregnancy:

Blood samples of 9 heifers and 64 cows in various months of pregnancy were studied. Only the total leucocytic count showed highly significant variations between months. The 6th month recorded the highest total count, the 2nd month the lowest. Significant variations were recorded between 5th, 6th and 7th months and between 8th and 9th months. The lymphocyte percentages were highest during the 4th month, while the absolute values reached their highest during the 6th month. The neutrophil percentage did not show any definite trend while the neutrophil absolute values rose gradually upto the 6th month and then decreased gradually by end of the term. The relative neutrophil values showed an opposite tendancy to that of lymphocytes. The percentage of eosinophils were highest during the 1st month and reached another peak during the 8th month. The 9th month recorded the lowest relative and absolute values.

The effects of hormones during pregnancy leading to the changes in the leucocytic picture were discussed.

Leucocytes in connection with parturition:

Variations in the leucocytic picture of 6 cows were studied, 3 weeks before and 3 weeks after parturition.

It was found that parturition had considerable influence on the white blood cells. "Cows" and "intervals" showed

highly significant variations between themselves with the total counts, and with relative and absolute values of lymphocytes, neutrophils and eosinophils. Total count showed a 100 % increase at parturition and this was mainly due to a 300 % increase in neutrophilic numbers. The L/N ratio gradually decreased with approaching parturition which actually saw a cross over of lymphocytes and neutrophils. The eosinophils percentage showed also a slowly decreasing tendancy with approaching parturition. The value was the lowest soon after parturition and then recovered in the next few days afterwards.

The secretion of various hormones during parturition and their influences on the leucocytic picture were discussed. It seemed that parturition caused sufficient stress to produce considerable changes in the leucocytic picture.

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B I B L I O G R A P H Y

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