STUDIES OF THE BLOOD AND MARROW PICTURE OF SHEEP.

A Thesis presented for the Degree of

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by

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INTRODUCTION.

During the last twenty-five years considerable advances have been made in the field of veterinary haematology, and it is now an important aid in clinical diagnosis of diseases encountered in domesticated animals. Its usefulness in this respect, however, is limited in certain species by the wide variations found in the normal range for some of the constituents of the blood picture. This is particularly true of the sheep. Because of the wide distribution about the mean found in both erythrocyte and leucocyte values, it follows that in interpreting the results of a blood examination only gross deviations from the normal can be considered as significant. There is evidence to suggest that helminth parasites have an effect on the variation found in the blood values (Mönnig, 1947 ; Morgan & Hawkins, 1949). Thus by defining the effect of the worm burden on the blood picture of sheep in normal health it should be possible to reduce the normal range for at least some of the properties in the blood picture. In view of the fact that it is well known that low nutrition enhances the effect of parasites, a due regard would have to be paid in any such studies to the nutritional level of the sheep under consideration. The difficulty in the interpretation of results applies to both erythrocyte and leucocyte findings, but from the clinical aspect it is probably in the diagnosis and classification of anaemia that the handicap is most important. In human haematology the changes in cell size and haemoglobin carrying capacity of the erythrocytes afford considerable assistance in the classification of anaemias, but/

but in the sheep both the mean corpuscular volume and the mean corpuscular haemoglobin concentration are subject to considerable variation even when erythrocyte levels do not indicate the presence of anaemia. In consequence, changes in these indices in anaemia in the sheep must be interpreted with caution. This is particularly true in the case of the mean corpuscular volume and as partly responsible no doubt for the fact that most of the anaemias of the sheep have been classified as normocytic. Nevertheless, in certain cases of anaemia it has been possible to show significant changes in the red cell indices. Thus for example, in worm infestations, where the blood changes are due to actual blood loss, the anaemia has been classified as macrocytic and hypochromic (Fourie, 1931; Holman, 1945a). Similar alteration in the erythrocyte indices has also been noted in the anaemia of advanced copper deficiency. (Marston, Lee & Macdonald, 1948). On the other hand, a microcytic anaemia has followed the repeated removal of small amounts of blood, but no alteration in the mean corpuscular haemoglobin concentration was seen (Coffin, 1944). Significant changes in the mean corpuscular volume and mean corpuscular haemoglobin concentration in anaemic sheep have been proved to be the exception rather than the rule, and it has not been possible to make use of alterations in these indices in the classification of ovine anaemia. It has therefore been found necessary to classify abnormal reductions in erythrocyte levels in the sheep according to the cause rather than on the effect seen in the peripheral blood. In the case of post-haemorrhagic and haemolytic anaemias, the presence of regenerative signs in the peripheral blood, together with/

with other symptoms characterising the disease, such as haemoglobinuria, have made this method of classification adequate. Unfortunately it has been necessary to group the commoner forms of anaemia such as those found in many forms of helminthiasis, and the anaemia which occurs in association with the trace element deficiencies, under the general heading of dyshaemopoietic anaemia. (Holman, 1945a). This covers all gradations of change from a simple reduction in the number of erythrocytes without the appearance of immature forms in the peripheral blood to the truly aplastic state. The only method by which these anaemias may be subdivided at present is on the basis of changes in the indices and by the evidence of marrow activity as shown by the presence or absence of regenerative forms in the blood. The limited value of the mean corpuscular volume and mean corpuscular haemoglobin concentration has already been discussed. In the case of signs of regeneration in the peripheral blood the position is little better. At present it is not now known exactly at what degree of anaemia immature forms may be first expected to appear, and it is therefore necessary to adopt the arbitrary rule that regenerative forms should not be expected in a blood film unless the erythrocyte count is less than half the mean for the species (Holman, 1950). This is an unfortunate situation in view of the fact that certain anaemias commonly occurring in the sheep take the form of a simple oligocythaemia without the appearance of immature forms in the peripheral blood. This type of anaemia has been frequently reported in association with some species of helminth parasite and

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even in apparently healthy sheep at pasture in the months of February, March and April (Holman, 1944a). Furthermore, it has been recently suggested that the anaemia in cobalt deficiency in Australia is associated with an aplastic condition of the marrow, (Marston, Lee & Macdonald, 1948), although no results of systematic examinations of the marrow have been presented to support the theory. The progress that has been made in the human field during the last fifty years has been largely due to the recognition of the fact that the peripheral blood is only part of a greater tissue and that before the importance of changes in the blood can be properly assessed they must be related to the changes occurring in the tissue as a whole. The study of serial samples of blood and bone marrow obtained by biopsy techniques, has greatly increased the knowledge of the pathology of the anaemias of man, and has thus increased the accuracy by which they may be diagnosed and the success with which they may be treated. So far no such approach has been attempted in the sheep and it is considered that the development of a technique for marrow biopsy in this species, which would permit a simultaneous examination of both blood and marrow, should lead to a better evaluation of the results of much of the valuable research which has been carried out in haematology in the sheep.

During the progress of the work on which this thesis is based a marrow biopsy technique was evolved, and this technique was used in a study of variations in the blood picture and their relation to the worm burden carried by apparently healthy sheep kept under natural conditions of grazing and management. The technique was also/

also utilised in an examination of the bone marrow in some disease conditions in which significant deviations from the normal blood picture had been demonstrated.

Historical.

The earliest description of the constituent cells of the blood of the sheep, and reference to standards for the normal blood picture are contained in Burnett's monograph, "The clinical Pathology of the Blood of Domesticated Animals", published in 1917. There is marked difference between the standards of the authorities quoted, the erythrocyte count, for example, ranging from eight to twelve million per cu.mm. while the limits of variation in leucocyte count range from four to eleven thousand per cu. mm. The figures are of interest in that they show the recognition from the first of the wide variation likely to occur in the blood picture of normal sheep, but as no information is given concerning the numbers of sheep sampled, or the conditions under which sampling took place, no explanation of the factors likely to cause this variation is possible.

The same general criticism appears justified in the case of the normal standards for the sheep suggested by Kleinberger (1927) in "Die Blutmorphologie der Laboratoriumstiere"; his figures were based on the examination of eleven healthy sheep and his range for erythrocyte counts varied from 9,600,000 to 16,300,000 per cu. mm.

A year later Kohanawa (1928) published the results of the examination of the blood picture of all the domestic animals. The figures/

figures in respect of sheep were based on the examination of seven ewes and five rams varying in age from four to eight years old. No variation due to sex was demonstrated, but again the range covered by the values for the thirteen animals was wide, erythrocytes varying from 8,500,000 to 11,600,000 and leucocytes from 8,900 to 12,800 per cu. mm. Kohanawa confirmed the findings of Burnett that in the blood of the sheep the lymphocytes exceeded the neutrophil leucocytes.

The results of the sampling of forty-eight sheep selected at random from those coming in for slaughter at Melbourne abattoir were published by Norris and Chamberlin in 1929. The authors describe the sheep as 'presumed in average health', but admit to parasites being present in varying degrees, and also draw attention to the high incidence of Gaseous Lymphadenitis in Australia, inferring that both these conditions may have an influence on the results. In calculating the averages from their results, these workers omitted counts which were 'unduly high' or 'unduly low', but even after this correction the calculated Standard Deviation about the mean for the erythrocyte count was ± 1.0 in the case of sheep of an average age of 4 years and ± 1.3 in the case of lambs from a few months to one year old. This gave a variation comparable to that reported by previous workers. The lambs were shown to have higher counts generally than adult sheep. The samples were collected from April to August inclusive, but no seasonal variation was observed. It is of interest to note that the counts obtained by these Australian workers differ from those reported in Europe - the/

the figures for red cells being higher and for leucocytes lower.

The first description of the blood of normal sheep to be published in Britain was by A. C. Fraser in 1930. His survey covered the blood picture of both cattle and sheep in health and disease. The major findings, as far as the latter species were concerned, were the higher counts of both red and white corpuscles in young as compared with adult animals; a tendency for lymphocytes to be predominant in the percentage formula of the white cells except in the very young, and relatively high eosinophil counts in adult animals. The discrepancy between Fraser's figures and those of previous workers in respect of the numbers of monocytes present was attributed by Fraser to the lack of uniformity among the various workers, in the criteria employed in differentiating these cells from large lymphocytes.

In 1931 Wirth published the first edition of his "Grundlagen einer klinischen Haemagologie der Haustiere". This text-book, a third edition of which was published in 1950, reviews from the normal and pathological aspects the previous work conducted on the blood picture of the domestic animals. It includes, as far as sheep are concerned, only the investigations into the normal range carried out by workers on the continent of Europe. Wirth draws special attention to the difficulty in making a statement of the normal blood picture of the sheep, owing to the marked individual variation, which he states is more marked than in the horse and cow.

Although the investigation was not concerned with the blood picture, the work of Hamersma (1934) at the Onderstepoort Laboratories in Pretoria, on the seasonal changes seen in some organic constituents/

constituents of the blood of sheep, is of interest in relation to the scope of this thesis. The examinations in this serial study, which extended over a period of twelve months, included an estimation of haemoglobin level. One hundred and sixty-two samples were collected from twenty-four sheep which varied in age from lambs to adult ewes. Throughout the experiment the sheep were kept on a standard diet and confined to a pen which gave protection from the weather but allowed ample exercise. Helminth control was by monthly dosing, and the helminth burden, as detected by worm egg count, was shown to be at no time more than a slight infestation. Although there was a wide individual variation between sheep, the results of the haemoglobin estimations showed no tendency for a seasonal variation to occur.

Further confirmation of the wide variation likely to be encountered in the blood picture of the normal sheep was furnished by Innes and Shearer (1940) in the course of an investigation of 'Swayback' in the counties of Leicester, Derby and Cambridge. They found for example, the erythrocyte count to vary from six to eleven million per cu. mm. These authors also reported the high erythrocyte counts described by previous workers as confined to very young lambs, to persist up to one year. Their most important finding from the point of view of normal variation was however the existence of an anaemia of a macrocytic hyper- or normochromic type in pregnant ewes. It is perhaps of importance to note that no attempt was made in their investigations to assess the possible influence of helminth burden on the blood picture of their sheep. A fall in packed cell volume has also been reported in gravid sheep by/

by Barcroft, Kennedy & Mason (1939), but Clark (Groenwald, Graf, Bekker, Malan and Clark, 1941) could not demonstrate any reduction in erythrocyte levels due to pregnancy.

The suggestion that the different localities in which sheep were sampled contributed in some measure to the variations found in their blood picture was not supported by the work of Allcroft in 1941. He found in a survey of the haemoglobin levels of sheep from widely separated districts of England and Wales, that the values obtained were very similar. Although the extreme range was wide, from the distribution of the values he concluded that the normal range could be taken as between 9.5 and 13.5 gm. per 100 ml.

The recognition in Australia and New Zealand of the existence of a complex of deficiency diseases associated mainly with pastures poor in copper and cobalt, initiated an extensive study of the blood pictures of animals on normal and deficient pasture. In 1942 Bennets and Beck published results of the regular haemoglobin estimation of wethers and pregnant and non-pregnant ewes between the ages of two and six years old. The sheep came from several different 'healthy' areas and the number of examinations, according to the authors, ran into 'some hundreds'. They found the haemoglobin and red cell count to show an extremely wide range, but could demonstrate no influence to be exerted on the values by age, sex, gestation or lactation. They concluded that as the normal range for haemoglobin was found to lie between 9.0 and 16.8 gms. per 100 ml., and the erythrocyte count varied from 7.6 to 13.3 million per cu. mm., it was impracticable to fix normal mean values for either of the properties for sheep, but that it was reasonable

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to accept haemoglobin figures below 8.0 gms. per 100 ml. as evidence of anaemia.

Thus, by 1944 no comprehensive survey had been carried out on the normal variations likely to occur in the blood picture of the sheep, and it was this aspect of the subject that formed the basis of Holman's first two papers in a series entitled 'Studies on the Haematology of the Sheep', which were published in 1944 and 1945. The first paper (1944a) describes the observations made on 171 sheep which were examined with the object of setting standards for healthy sheep to facilitate the interpretation of alterations due to disease. Holman found, after a short trial, that certain estimations could be discarded as having little practical value. These included the sedimentation rate, the counting of platelets, and the estimation of Price-Jones curves. He also found that certain modifications in the application of Schilling's nuclear index to sheep blood were desirable. These modifications consisted in placing the 'degenerative band forms' of Schilling, and other lobulated but non-segmented neutrophil leucocytes, among the adult group when making a nuclear index. The influence of the following factors on the variation in the blood picture were examined: age, sex, seasons, state of exertion, and breed.

It was not possible to demonstrate a significant effect in the case of sex, breed, or extreme exertion prior to sampling. For the investigation of the variation due to age, four sheep were bled each month for two years, from the age of 2 - 4 weeks. It was found that the red cell count was lower during the first two months of/

of life than that shown in the period over that age, but the larger size of the erythrocytes up to two months kept the haemoglobin concentration in the blood constant. The changes later in the period of the observation, Holman attributes to the effect of season rather than age. The presence of greater numbers of band form neutrophil leucocytes during the first few months of life, due to the myeloid activity associated with growth, confirmed the previous findings of Fraser, as did the low incidence of eosinophil leucocytes in young lambs. It was however in the seasonal variations that the most striking results were obtained. It was observed that the lowest values for erythrocyte count P.C.V. and haemoglobin level were obtained in the blood from sheep sampled in February and March. It was found that a significant number of the sheep sampled during these months showed haemoglobin values below 11.0 gm. per 100 ml. when compared with the number of sheep showing a haemoglobin level of less than 11.0 gm. and sampled at other times of the year. The distribution of the numbers of the sheep sampled, on the basis of a haemoglobin level of 11.0 gm. per 100 ml. was as follows.

	Hb. values above 11.0 gms.	Hb. values below 11.0 gms.
Sheep sampled in February & March	2	19
" " at other times of the year	99	15

To avoid the bias due to the seasonal factor, Holman in declaring the distribution of the principal constituents of the blood of sheep excluded the results of the examinations of samples collected, to quote Holman's own phrase, 'during the Spring and the month/

month immediately preceding or succeeding it.' No attempt was made to investigate the cause of this seasonal variation, but Holman postulated that this fall in the Spring was most probably due to inanition, since the rise in haemoglobin commences with the flush of early summer grass. Even after the exclusion of the low values occurring in the spring months the normal range for the erythrocyte properties remained very wide, as did the ranges for the other blood attributes.

Working on the assumption that this variation was largely due to the sheep to sheep variation and that in fact each sheep had a normal range peculiar to itself, Holman proceeded to investigate the individual variation likely to occur when observations are made on successive days on the same individual (Holman,1944b). From the results of these investigations he was able to state a value for each constituent of the blood, which represented the maximum admissible difference (M.A.D.) of two readings made on the same sheep at an interval of twenty-four hours; another value being calculated where the interval extended to one month. The difference between two readings made at these intervals should not exceed the value for the M.A.D. in more than 5 per cent. of the cases in the absence of some interfering factor. The usefulness of these standards in the interpretation of,say,the effects of experimental interference in the sheep is obvious; it is disappointing that they show the ranges covered, even by the individual sheep from day to day, to be wide. For example, if the general standards for sheep laid down by Holman are considered in respect to red cell count, it is found that with a mean of 11.5 million per cu. mm. and a S.D. of ± 1.8/

1.8, the possible range covered by normal sheep may vary from 7.9 to 15.1 million (i.e. Mean ± 2 X S.D.). Whereas in the case of the individual animal with a red cell count of 11.5 million per cu. mm. by the application of Holman's M.A.D. of 2.8 for erythrocyte count it is found that this count may rise as high as 14.3 or fall as low as 8.7 in the course of twenty-four hours and still be considered normal.

These wide normal variations between and within individual sheep must at present be accepted as representing a species characteristic, and mean that in the interpretation of results for clinical diagnosis only gross deviations from the normal can be considered as significant. The results of work carried out by other workers since 1944 have contributed nothing which might lead to the modification of this general conclusion, and have only confirmed the findings of previous investigators. This statement is true of the work of Becker and Smith (1950), who carried out haemoglobin and P.C.V. estimations as part of a general chemical and morphological study of normal sheep blood. To facilitate the analysis of their results these workers set up a factorial design involving three breeds of sheep, three age classes, two sexes, three periods of bleeding, and two types of feeding. The three breeds used in the experiment were Corriedale, Dorset and Hampshire. They were divided on the basis of age into mature sheep, yearlings, and lambs. The types of feeding employed were barn and pasture. The sheep confined to barns received hay, corn, and silage, and a grain mixture, nursing lambs having access to a creep fed concentrate mixture. During pasture feeding only a

small/

small amount of grain was fed to mature and yearling sheep, but lambs were heavily grain fed. The sampling of barn fed sheep was carried out in March and early April, while the samples from pasture fed sheep were collected in June and July. A statistical analysis of the results showed that neither breed, age, sex, nor type of feeding gave any significance of effect. There was general agreement between the ranges observed by these workers and those of Holman, and the mean value for haemoglobin was also close to that given by Holman. In Becker and Smith's sheep however the mean P.C.V. was higher than the standard suggested by Holman.

From an analysis of the findings of previous workers reported in the foregoing historical review, certain conclusions may be drawn. There is ample evidence that a wide variation is likely to be encountered in the blood picture of healthy sheep. The effect of age has not been shown to exert any influence on this variation except in early life. There is no evidence that breed or locality play any part in the variation. There is some doubt concerning the effect of pregnancy and it is suggested that no conclusion can be reached regarding this factor until further investigations, especially designed to study the influence of pregnancy, have been conducted. On the other hand, the effect of nutrition has been postulated and there is some indirect evidence to show that it may be responsible for the changes encountered in erythrocyte levels under certain conditions. That such an oligocythaemia may occur in herbivorous animals during winter and spring months is acknowledged by Holman (1950), and is regarded by him as evidence of deficient blood production due to dietary insufficiency. He

questions/

questions the accuracy of designating this change as an anaemia, however, and suggests the term, 'physiological anaemia' as a possible alternative, presumably on the grounds that the variation may be regarded as the normal adaptation of the animal to its environment. A further possible cause for oligocythaemia in general, mentioned by Holman, is heavy infestation with some types of intestinal parasite. The close relationship between low nutrition and heavy infestations of parasites is now well established. Since the original demonstration by Foster and Cort (1941[3]) of the influence of diet on the susceptibility to and degree of, parasitic infestation, many workers have confirmed this relationship. (Fraser & Robertson, 1933; Doll & Hull, 1944; Gordon, 1948; Ross & Gordon, 1936; Lawrence, Groenwald, Quin, Clark, Ortlepp, & Bosman, 1951; Stoll, 1940). Although there are many reports of blood changes associated with naturally occurring and experimentally induced parasitism (Fourie, 1931; Fraser, 1930; Stewart & Piercy, 1934; Holman & Pattison, 1941; Andrews, 1942), it must be remembered that the sheep in which Holman (1944a) demonstrated significant oligocythaemia in the spring months were apparently healthy sheep, and were not showing any symptoms of what might be called clinical parasitism. On the other hand, Holman made no mention of anthelmintic treatment of his sheep and it is therefore safe to assume that they were in fact all carrying some worm burden. That indeed a naturally acquired subclinical parasitism is able to affect the blood picture was suggested by the work of Hawkins and de Frietas (1947). In a report of observations carried out during a five year period on ewes and yearling lambs, kept at pasture, these workers/

workers showed that there was a decided decrease in nematode egg counts and an increase in haemoglobin levels during what they describe as the 'late winter' months and that in late February and March the position was reversed, so that the worm egg counts were higher and the haemoglobin levels lower than in the late winter months. Notwithstanding this evidence, if the supposition is to be upheld that the seasonal variation shown by Holman to occur in erythrocytic properties in Scottish sheep is associated with parasitism, it is necessary to demonstrate the relative increase in the worm burden at the time at which the lowest erythrocyte values were found. Such evidence was first forthcoming in the work of Morgan and Sloan (1947), who in a study of the seasonal variation in the worm egg output in Scottish hill sheep found a very marked peak in the output of helminth eggs in the spring, in all age groups.

In view of the findings of Hawkins and de Frietas it appeared that a simultaneous study of the seasonal changes in blood and worm burden might result in a better appreciation of the nature of the low values found by Holman in the erythrocytic properties of Scottish sheep in the spring.

In 1949 an opportunity for such a simultaneous study to be carried out arose with the decision of Morgan and his co-workers to extend the scope of their previous observations on the seasonal variation in parasitism in hill sheep. Up to this time their results had been based on an estimate of the helminth burden by means of worm egg counts. Thus, although it seemed extremely likely that the rise in worm egg counts observed in the spring was at least in part due to a heavier worm burden at this time, until an absolute/

absolute rise in worm burden could be shown to occur at the same time as the rise in egg output, the true significance of their previous findings could not be appreciated. These workers therefore decided that in order to discover whether there was a relationship between the number of eggs passed in the faeces and the number of worms present in the alimentary canal, sheep would have to be slaughtered at different times throughout a whole year, so that a thorough quantitative and qualitative study could be made of their worm burden. It was in this investigation that the writer was invited to participate. During preliminary discussions it was found that unfortunately owing to the distance of the farm from the centre at which the examinations were to be made, it would only be possible in most cases to sample each sheep once, and this immediately prior to slaughter. In view of the wide variation known to exist in the blood picture of different sheep this was considered a disadvantage, but as the number of sheep available for sampling was likely to be fairly large, this undesirable feature was considered to be of less importance. On the other hand there were many features in the plan of investigation which made it eminently suitable for haematological observations. In the first place, this was a unique opportunity to relate such blood changes as might occur to actual worm burden, the contributing species of which would be known. Secondly, as each animal was to be slaughtered after sampling an inspection of carcase and viscera would be possible and should any pathological process likely to affect haematopoiesis be found, the blood findings could be interpreted accordingly. Thirdly, as prior to sampling the sheep would be under natural/

natural conditions of feeding and husbandry, the possible influence of artificial environment on the blood picture would be avoided. Fourthly, as a system of marking of individual sheep on the basis of year of birth was to be adopted, the exact age of each sheep would be available, and thus any effect on the blood picture due to age would be evident. Finally, the season of the year over which the most intensive study was planned, December to June, covered the period at which the greatest variation had been observed by Holman to occur in the blood picture of Scottish hill sheep.

It was therefore decided to accept the invitation to participate in the experiment from the haematological aspect.

A preliminary consideration of the results likely to be expected in view of Holman's findings on the one hand and those of Morgan et al on the other, showed that the time at which the main variation might be expected to occur would coincide to some extent with the period of the year when nutrition could be described as very low in hill sheep. Thus it might be difficult to separate the possible effects on the blood picture of low nutrition and helminth burden. It was not anticipated that the reduction in erythrocyte levels would be great enough to constitute a true anaemia, and therefore no assistance from the changes in the indices of M.C.V. or M.C.H.C. could be expected in any attempt to differentiate the effect of worm burden from low nutrition on the basis of characteristic changes in cell size, and haemoglobin concentration within the cells. It was therefore decided to carry out, in addition to peripheral blood examinations, a study of the marrow in at least a proportion of the sheep slaughtered, in the hope that from a consideration of the changes/

changes occurring in the whole tissue a better appreciation of the cause of the variation in the peripheral blood would be possible.

The concept of the blood as a tissue was first suggested by Professor A. E. Boycott in a presidential address, in 1929, to the Section of Pathology, of the Royal Society of Medicine, and is now the accepted approach by haematologists in human medicine, (Custer, 1941; Whitby & Britton, 1942; Wintrobe, 1946) Boycott proposed the name erythron as a comprehensive title for describing and emphasizing the unity of the tissue composed of circulating red cells and their precursors in the marrow. In extending the conception to include the leucocytes and platelets and their respective progenitive tissues, Kracke (1941) and others have suggested the terms 'leukon' and 'thrombon'. In the adult the leukon consists of three types of tissue: the granular series of the circulating leucocytes and the myeloid tissue of the bone marrow from which they are formed; the lymphoid series of leucocytes and the lymphatic tissue of the body from which they arise; and the scattered reticulo-endothelium and connective tissue which is responsible for the production of the monocytes and fixed phagocytes of the body. The thrombon includes the circulating platelets and the megakaryocytes of the bone marrow.

With the recent development of marrow biopsy methods for some species of the domestic animals (Hjarre, 1943; Holzel, 1939; Bloom & Meyer, 1944), a growing tendency is forecast for the approach in veterinary haematology to be widened to include in certain cases a simultaneous study of progenitive tissue and peripheral blood rather than of peripheral blood alone. It was therefore considered desirable in a study of the normal blood picture of sheep such as

was envisaged here, and in which a number of the factors likely to exert an influence would be measured, to include a simultaneous examination of the marrow with the object of defining the variation likely to occur in the tissue as a whole.

As each sheep examined in the investigation was to be slaughtered, it would have been possible to carry out marrow sampling at autopsy, but it was decided that as any future work on these lines would almost certainly have to be done on the living animal, the results of this investigation would be of more value if they were from the examination of material collected during life. As no previous record of bone marrow biopsy on the sheep could be found, this decision to sample marrow before the sheep were slaughtered involved the development of a technique for this purpose. A description of the details of the technique and of the material obtained by marrow biopsy on the sheep is given in Section II.

General Plan of Investigation.

In a paper entitled, "The seasonal variations in the worm burden of Scottish Hill Sheep", Morgan, Parnell & Rayski (1951) have described in detail the plan of the investigation in which the writer participated from the haematological aspect, and a summary of the general plan of investigation is presented here. A full description of the sheep used in the survey will be given in Section I. The experiment lasted from August 1948 to June 1950 and can be divided into two periods:

a. August 1948 - June 1949, and

b. December 1949 - June 1950.

a. August 1948 - June 1949.

Four main slaughterings were planned, each to involve approximately fifty sheep, among which would be representative members of each age group. The times of slaughter were based on the seasonal fluctuations which occur in the worm egg output of hill sheep. The first group was slaughtered in the last week of August, the second in January, the third in early April, and the fourth in the first week in June. A number of sheep were held in reserve to serve as replacements in the event of deaths occurring in the flock throughout the period of the experiment. These sheep were slaughtered as an extra group in July.

b. December 1949 - June 1950.

Two ewes were slaughtered in mid December and subsequently two ewes were slaughtered at fortnightly intervals from mid January until the end of June, making fourteen slaughterings in all and involving a total of 28 ewes.

The haematological investigations were designed in the following manner:

a. August 1948 - July 1949. At each of the slaughterings except the first (August 1948) blood samples were collected 3 to 4 hours before slaughtering with the object of comparing the blood picture in January, April, June and July.

b. December 1949 - June 1950. From all the 28 sheep s slaughtered during this period both blood and bone marrow were collected 3 - 4 hours before slaughter with a view to examining the changes in blood and bone marrow throughout the period.

The time between June and December 1949 was employed in devising/

devising a technique for bone marrow biopsy for the sheep, and describing the histology of the material so obtained. This was followed by a test of the value of the method of biopsy devised, in the detection of the effects on the marrow of an experimental interference such as bleeding. In addition, a controlled experiment was carried out, designed to show the effect of low nutrition on the blood and bone marrow of sheep in which the worm burden had been reduced to negligible proportions.

The results of these studies is presented in the following sections.

Section I. A study of the variations occurring in the blood picture of normal Scottish hill sheep from January to July, and the relation of this variation to changes in worm burden.

Section II. Bone marrow biopsy in the sheep and a description of the histology of the material obtained by this technique.

Section III. A study of the bone marrow response to bleeding in the sheep.

Section IV. An investigation of the variations in the blood and bone marrow associated with low nutrition in the sheep.

Section V. A study of the variation occurring in the blood picture and bone marrow of normal Scottish hill sheep from December to June, and the relation of this variation to changes in worm burden.

On the completion of these studies of the bone marrow of sheep, in normal health, the biopsy technique was utilised for the examination of the marrow in some disease conditions which showed pathological changes in the blood picture. The results of these investigations/

investigations are presented in Sections VI. and VII. as follows:

Section VI. A study of the blood and bone marrow of fifteen lambs in a debilitated state following an attack of the acute form of contagious pustular dermatitis.

Section VII. A study of the blood and bone marrow of six sheep suffering from cachexia.

SECTION I.

A Study of the Variation in the Blood Picture of Scottish Hill Sheep from January to July, and the Relation of this Variation to Changes in Worm Burden.

1. Description of sheep used in survey.

The 194 sheep from which blood was collected for the purpose of these observations were all of the Cheviot breed, and varied from a few weeks to seven years old. They were in normal health and had not been subjected to any anthelmintic treatment for twelve months prior to sampling. They were all born and reared on the Scottish Border sheep farm from which they came, and comprised most of the population of one heft.

On hill sheep farms in Scotland the flocks are divided into hefts, and each heft has the exclusive grazing of a well defined section of hill and does not stray from that part of the hill. Thus the sheep used in this investigation were all under identical conditions of environment and management, and the results of the examination of blood from a group selected at random could be assumed to represent the picture pertaining in the heft as a whole at the time at which sampling took place.

Sampling was carried out on four separate occasions, i.e., January 11th, April 5th, June 7th, and July 12th. The number of sheep examined on each of these dates varied as follows:- on January 11th, 44 sheep; on April 5th, 49 sheep; on June 7th, 64 sheep; and on July 12th, 37 sheep.

The age of each sheep in the heft was known by an identification mark denoting the year of birth, and the sheep sampled on each of the four occasions comprised representatives of all age groups from one to seven years old (estimated to the nearest year). In addition, the sheep sampled in June and July included lambs born during the previous April. As lambing took place in the heft between April 14th and April 28th, the sheep sampled in June and July were all non-gravid, whereas all the sheep which were over two years old and which were sampled in January and April were pregnant. In addition, of the seventeen sheep under two years old which were sampled in April, seven were pregnant.

It is of importance to realise that after blood samples had been collected the sheep were slaughtered in order to estimate the worm burden, thus only one blood sample was collected from each of the 194 sheep, and at each of the four samplings different individual sheep were involved.

For the purpose of reference in this investigation, the sheep sampled on the four separate occasions will be called Groups B, C, D, and R. as follows:-

Sheep examined on January 11th Group B.
" " " April 5th " C.
" " " June 7th " D.
" " " July 12th " R.

2. Methods.

i. Collection of blood. In every case 10 ml. of blood was collected from the jugular vein into bottles containing 12.0 mgm. Potassium oxalate and 8.0 mgm. Ammonium oxalate. (Wintrobe, 1946).

ii./

ii. Scope of examination. The blood collected from all sheep sampled on January 11th, Group B. was subjected to an estimation of packed cell volume and haemoglobin level, erythrocyte count, and total and differential leucocyte count.

In the case of the sheep sampled on April 5th, June 7th and July 12th (Groups C, D and R respectively) packed cell volume and haemoglobin estimations were carried out on all samples and total erythrocyte and leucocyte counts and differential leucocyte counts were performed on samples selected by the following method. From the results of the packed cell volume estimations, the samples were divided on the basis of ranges of value found, e.g., in the case of Group C. (April 5th), 18.0 - 28.0; 29.0 - 30.5; and 34.0 - 35.5. A representative number of samples having packed cell volume values within each of these ranges was then submitted to an erythrocyte count, and total and differential leucocyte count.

For ease in presentation the following abbreviations will be used:-

Erythrocyte count - R.B.C.

Leucocyte count - W.B.C.

Estimation of Packed Cell Volume - P.C.V.

Haemoglobin estimation - Hb.

Mean Corpuscular Volume - M.C.V.

Mean Corpuscular Haemoglobin Concentration - M.C.H.C.

Differential Leucocyte count - D.L.C.

iii. Techniques.

a. Erythrocytes. The P.C.V. was estimated by centrifuging oxalated blood in Wintrobe tubes at 3,000 revolutions per minute for fifty-five minutes, when a reading was taken; thereafter centrifuging/

centrifuging was continued for periods of five minutes until two identical readings were obtained. The time of spinning was chosen as closely approximating to that suggested by Holman (1950) for sheep's blood, which was sixty minutes. That more complete packing could have been obtained by longer periods of spinning is suggested by the unpublished results of subsequent work on P.C.V. estimation in the sheep conducted by the writer in collaboration with Mr E. W. Moodie. If longer periods of spinning had been adopted it would not have been found possible to carry out P.C.V. estimations on all the samples collected in the Groups (in one group they numbered 64) within the time limit of three hours after collection, laid down by Kracke. (1937). Haemoglobin estimation was by Sahli Haemoglobinometer, using N/10 Hydrochloric Acid for the conversion of the haemoglobin to acid haematin, and after allowing to stand for five minutes, matching the resulting colour in the liquid with a standard, by the addition of distilled water.

For the R.B.C., blood was diluted with normal saline to a dilution of 1/200 in a Thoma pipette, counting being carried out on the improved Neubauer slide. The M.C.V. and M.C.H.C. were calculated from the P.C.V. and R.B.C., and P.C.V. and Hb. respectively.

b. Leucocytes. The W.B.C. were made by diluting blood with 3% acetic acid to 1/20 in a Thoma pipette, the count being made on an improved Neubauer slide. The D.L.C. were carried out by the differentiation of at least 200 cells on films made from oxalated blood within three hours of collection, and stained by Wright's stain according to the method described by Coffin (1945). The neutrophil leucocytes were classified according to Holman's modification of Schilling's nuclear index. (Holman, 1944a; Schilling, 1929).

3. Results.

The examination of the blood of the 194 sheep sampled in this investigation resulted in the accumulation of a considerable volume of data which did not lend itself to easy presentation on the basis of results for each individual sheep. It was decided therefore to summarise the results in the form of tables of mean values. The observations from which these mean values were calculated appear in the Appendix, pages 1 - 17.

The results have been grouped in the following manner. All the sheep sampled on the same day have been regarded as constituting a Group. Thus, as sampling took place on four separate occasions there are four groups of sheep, and these groups have been designated B. C. D. and R. Group B. were sampled on January 11th, Group C. on April 5th, Group D. on June 7th, and Group R. on July 12th. Each of these groups contained representatives of the ages from 1 - 7 years old, and Groups D. and R. also included lambs which were born during the year in which sampling took place. The number of sheep of the different ages is shown in the Table below in respect of the four Groups B. C. D. and R.

Table I. The numbers and ages of sheep in Groups B. C. D. and R.

Group	Date of sampling	Ages of sheep to the nearest year								Total in Group
		Lambs	1	2	3	4	5	6	7	
B.	11-1-49	-	10	5	6	5	5	5	8	44
C.	5-4-49	-	10	5	8	7	6	4	9	49
D.	7-6-49	14	10	5	8	8	6	5	8	64
R.	12-7-49	20	6	2	2	2	2	1	2	37

Thus, in the summarising of the results, the sheep within the Groups B. C. D. and R. have been subdivided on the basis of their age into those under 1 year, those between 1 and 2 years old, those between 2 and 3 years old and so on.

By presenting the data in this manner it has been possible to examine the variations due to age on the one hand, and due to the different month in which sampling took place, on the other. Both these factors were found to exert an effect on certain of the properties of the blood picture and it was considered advisable to discuss these variations before comparing the results as a whole with those of previous workers.

Therefore the results are presented under two headings:-

A. An analysis of the variation observed in the blood picture of Scottish hill sheep between January and July.

B. The presentation of standards for the blood picture of Scottish hill sheep sampled between January and July.

A. The variations observed in the Blood Picture.

It was possible from the method by which the sheep were sampled, to measure the effects of two factors on the blood picture, They were -

a. The effect of age, and

b. The effect due to sampling at different times of the year, viz., January, April, June and July,

and an analysis of the variation observed under these two headings follows.

a. The Effect of Age. For the purposes of the consideration of the effect of age on the blood picture of the sheep sampled, the observations are divided into two parts as follows:-

i. The effect of age on the Erythrocyte picture.

ii. The effect of age on the Leucocyte picture.

i. The effect of age on the Erythrocyte picture.

The properties of the erythrocyte picture examined in this investigation consisted of P.C.V., Hb., and R.B.C. From the results of these examinations the indices of M.C.V. and M.C.H.C. were calculated.

The sheep in each of the Groups B. C. D. and R. were divided on the basis of their age, and for each age sub-group a mean value was calculated in respect of the erythrocyte properties and their indices. The results of these calculations are shown as Table II. for the erythrocyte properties and as Table III. for the indices.

Table II. shows the mean for P.C.V. and Hb. of the sheep in the various age groups within the Groups B. C. D. and R. and in the case of Group B. means for R.B.C. are also shown. In the case of the P.C.V./

30a.

Table II.

Packed Cell Volume and Haemoglobin.

Mean values for sheep of different ages in Groups B. C. D. & R.

Erythrocyte Count.

Mean values for sheep of different ages in Group B.

Erythrocyte Property	Group	Age in Years: Under 1	1 - 2	2 - 3	3 - 4	4 - 5	5 - 6	6 - 7	7 - 8
P.C.V. %	B.	38.10	39.10	41.17	40.00	42.50	41.10	41.48	-
	C.	25.60	24.00	31.00	37.71	30.00	29.25	29.33	-
	D.	29.70	30.35	31.20	31.31	33.19	34.08	30.80	32.25
	R.	31.23	32.00	32.25	32.25	31.00	34.50	30.00	33.50
Hb. gms/100ml.	B.	11.27	11.60	11.76	11.51	12.12	11.94	12.10	-
	C.	8.46	7.80	9.11	9.09	9.20	8.48	8.74	-
	D.	8.97	9.34	10.10	9.78	9.96	9.20	9.80	9.60
	R.	9.84	10.03	9.95	10.90	9.50	10.45	9.80	11.00
10^6 R.B.C./cu.mm.	B.	10.72	10.54	11.58	10.82	11.55	10.93	10.74	

P.C.V. it will be seen that there was some variation between the means for the sheep of different ages and that this was greatest in the case of the sheep sampled in April (Group C), where the means ranged from 24.0% to 37.7%. The most striking difference however was between sheep under two years of age and the sheep over two years of age. It will be seen that in all four Groups (B. C. D. and R.) the means showed a tendency to be lowest in the case of sheep under two years old. This was most marked in the case of the sheep sampled in April (Group C) and least marked in those sampled in July.

The mean values for red cell counts show the same tendency for the erythrocytes to be less numerous in the sheep under two years of age than is the case for the sheep over that age.

The haemoglobin levels for the younger sheep, viz., under two years old, are also lower than they are for the older sheep, but the difference is not as marked as it is in the case of the P.C. V. and R.B.C. Confirmation of this is to be found in the consideration of the index of M.C.H.C. which follows.

Table III. shows the means for M.C.H.C. of the sheep in the various age groups within the Groups B. C. D. and R. and the M.C.V. values for the sheep in Group B.

The mean corpuscular haemoglobin concentration did not appear to be influenced by age in the case of sheep sampled in July (Group R). In the other three Groups this index however was higher in the sheep under two years of age than it was in those over that age. This indicates that in the case of the sheep under two years old/

31a.

Table III.

Mean Corpuscular Haemoglobin Concentration.

Mean Values for different ages in Groups B. C. D. and R.

Mean Corpuscular Volume.

Mean Values for different ages in Group B.

Erythrocyte Index	Group	Age in Years							
		Under 1	1 - 2	2 - 3	3 - 4	4 - 5	5 - 6	6 - 7	7 - 8
M.C.H.C. %	B.	29.10	29.40	28.35	28.30	28.10	27.60	28.88	-
	C.	33.60	32.60	29.07	29.71	30.83	29.00	30.78	-
	D.	30.18	30.80	32.70	31.56	30.38	27.17	31.90	29.94
	R.	31.79	31.58	30.75	33.00	30.50	30.25	32.50	33.00
M.C.V. cu.μ.	B.	36.20	37.70	35.50	37.80	37.50	38.40	38.69	

old in Groups B. and C. the cells were carrying a greater proportion of haemoglobin than were the cells in the sheep over two years old. This fact shows that when the sheep under two years old are compared with those over that age, there is not the same degree of difference between Hb. levels as there is between P.C.V. and R.B.C. values.

Inspection of Table III. containing the mean values for M.C.V. shows that age had no effect on the variations seen in this index.

ii. Effect of age on the Leucocyte picture.

In Table IV. the results of total and differential leucocyte counts for the sheep in Group B. are presented as mean values for the sheep of different ages. The variation in these measurements did not show any tendency to be influenced by the age of the sheep from which the blood came. The results of the W.B.C. and D.L.C. carried out on samples in Groups C. D. and R. are not included in Table IV. These data were omitted as in these Groups leucocyte counts were only made in respect of selected members of these Groups and therefore some of the age groups were only represented by one or two sheep.

Discussion of the effect of Age.

From an inspection of the Tables of Means in respect of P.C.V., Hb., R.B.C. and M.C.H.C. certain marked differences due to age were apparent. These were that the erythrocyte levels encountered in sheep under two years of age were almost invariably lower than those in the sheep over that age. It was also noted that in sheep sampled in January and April the M.C.H.C. was higher in the younger sheep. By reference to the observations given in the Appendix for individual sheep, it will be seen that there was considerable variation from

sheep/

Table IV.

Total Leucocyte Count and Differential Leucocyte Count for sheep of different ages in Group B.

Age in years	Under 1	1 - 2	2 - 3	3 - 4	4 - 5	5 - 6	6 - 7
Leucocyte Count 10^3/cu.mm.	7.67	6.42	7.77	6.56	7.26	7.30	6.36
Differential Leucocyte Count /cu.mm.							
Band neutrophils	35	50	29	46	17	31	41
Polymorphonuclear neutrophils	2274	2317	2367	2437	1880	2574	2591
Eosinophils	96	135	177	128	242	251	211
Lymphocytes	4881	3684	4781	2761	4894	4154	3238
Monocytes	586	233	418	164	222	300	281

Mean Values were not calculated in respect of Basophils, as the incidence of these cells was extremely low.

sheep to sheep even when they were of the same age. It was therefore decided to test the significance of the difference between the measurements for the sheep over two years old and under two years old. This was done by an Analysis of Variance using the method described by Snedecor (1946) for unequal subsample numbers. In this treatment of the results the observations made in Group R. were omitted because of the small numbers of sheep in some of the age groups, and as sheep between six and seven years old were only sampled in Group D. they also were not included in the analysis. The data for P.C.V., Hb., and M.C.H.C. for all the sheep sampled in January (Group B), April (Group C), and June (Group D with the omission of sheep between 6 and 7 years old) were submitted to this treatment. Full details of the calculations are given in the Appendix. Pages A.15 to A.17.

From the results of the Analysis of Variance for the P.C.V. and Hb. measurements it was shown that a variation significant at 1% and due to age existed. By a subdivision of ages this was shown to be due to sheep under two years old having lower P.C.V. and Hb. values than the sheep over that age, and this difference was also significant at 1%, meaning that it would only occur by chance once in every hundred obserfations. No other variation due to age could be shown to be significant.

From the results of the Analysis of Variance carried out on the calculated values for M.C.H.C. it was shown that the variation due to age was significant at 1% and this was due to the sheep under two years of age having higher values than the sheep over that age. Age also exerted a significant influence on the M.C.H.C. measurements among the sheep over two years old.

No previous reference can be found to the erythrocyte levels of sheep under two years of age being lower than those found in sheep over that age. Norris and Chamberlin (1929) on the contrary, reported that sheep up to one year old had higher erythrocyte levels than older sheep. The explanation for the discrepancy between their results and mine may lie in the fact that their lambs were in much better bodily condition than the sheep of this investigation, as sampling took place in Melbourne Abattoir and it can be assumed that their lambs were fat and ready for slaughter.

Holman (1944a), in his investigation of the effect of age on the blood picture of Scottish hill sheep, did not compare the values found in sheep under and over two years of age. His studies were based on the monthly examination of samples from four lambs. Sampling commenced when the lambs were under one month and continued until they reached two years of age. Holman attributed the changes he observed in the erythrocyte levels of these lambs to the effect of season rather than age. The fact that he found no difference between the erythrocyte levels of the lambs at one year old and at two years of age is confirmed by my results.

In considering the possible causes for the lower erythrocyte levels found in the sheep under two years old, the fact that young sheep are acknowledged to be more susceptible both to helminth infestation, and to the effects of helminthiasis may have some significance. It will be shown later that the worm burdens of sheep under two years old, at least in the case of sheep sampled in January and April, tended to be higher than the worm burdens found in sheep over two years old. This fact may in part account for the/

the difference in erythrocyte levels between the two age groups. The fact that the erythrocytes of the younger sheep had a higher percentage of Haemoglobin suggests a mechanism operating to keep the blood haemoglobin up, in the face of the lower erythrocyte levels seen in these sheep.

b. The effect of sampling at different times of the year.

From a comparison of the results obtained at the four different samplings, viz., January 11th (Group B), April 5th (Group C), June 7th (Group D) and July 12th (Group R), it was possible to study the effect of season on the blood picture during the first $6\frac{1}{2}$ months of the year. For this purpose the observations are divided into two parts as follows:-

i. The effect of season on the Erythrocyte picture.

ii. The effect of season on the Leucocyte picture.

i. The effect of season on the Erythrocyte picture.

In all four Groups, viz., B.C.D. and R. the erythrocyte level was measured by submitting the samples to packed cell volume and haemoglobin estimation, and from the results of these examinations the mean corpuscular haemoglobin concentration was calculated in respect of each sample examined. In order to compare the values for mean corpuscular volume found in the four Groups, samples in Groups C. D. and R. were selected on a basis already described on page 26, and subjected to a red cell count; the values for mean corpuscular volume calculated from R.B.C. and P.C.V. were then compared with those calculated for all the samples in Group B. The results of this comparison appear on page 11.

Table V. shows the mean values in respect of P.C.V., Hb., and M.C.H.C. for the four Groups B. C. D. and R.

Table V.

Mean Values in respect of Packed Cell Volume, Haemoglobin, and Mean Corpuscular Haemoglobin Concentration in Groups B. C. D. & R.

Groups		B.	C.	D.	R.
Sampled on:-		January 11th	April 5th	June 7th	July 12th
Mean Values for	P.C.V. %	40.30	28.71	31.27	31.70
	Hb. gms/100 ml.	11.72	8.73	9.49	10.00
	M.C.H.C. %	28.29	29.98	30.53	31.50

An inspection of the mean values for P.C.V. and Hb. in the above Table shows that the sheep sampled in January had the highest means for both P.C.V. and Hb. The lowest levels for both properties were found in the samples collected in April. The results of the examinations carried out in June showed a rise in both P.C.V. and Hb. levels, and the mean values for the samples collected in July were higher than those in June. Although the values in Group R. (July) were higher than those in Groups C. (April) and D. (June), they were still lower than those found in Group B. (January).

It will be seen from Table II. on Page 30[a] where the mean values for P.C.V. and Hb. are shown for the sheep at different ages, that the trends seen in Table V. and described above, are also/

also apparent within the age groups. For example in the case of the P.C.V. for sheep under one year old, the highest levels were found in January, the lowest in April, and there was a successive rise in June and July, but the value in July was still lower than that found in January. A further inspection of this Table shows that this pattern of change is found for both P.C.V. and Hb. in the case of almost all age groups.

A further inspection of Table V. shows that the changes noted above in P.C.V. and Hb. were accompanied by changes in the Mean corpuscular haemoglobin concentration. This index showed a progressive increase from January (Group B.) to July (Group R.)

Variation in Mean Corpuscular Volume between Groups.

It was not found possible to carry out R.B.C. on all the samples in Groups C. D. and R., and thus present M.C.V. figures for all the sheep in these Groups. Instead, the samples in these Groups were divided by reference to the range of P.C.V., and a number of samples in each range were submitted to R.B.C., and the M.C.V. for these samples calculated therefrom. The results are given in Table VI.. The mean and Standard Error (S.E.) for all samples examined in Group B. is included for the purpose of comparison. The S.E. was calculated according to the method described by Mainland (1938).

Table VI /

Table VI

Mean Values for Mean Corpuscular Volume

for selected samples in Groups C. D. and R.

Group	Range of P.C.V. for samples examined %	Number of Observations	M.C.V. Mean cu.μ	± S.E.
B.	31.0 - 51.8	44	37.31	0.440
C.	18.0 - 28.0	13	43.77	2.400
	29.0 - 30;5	6	42.00	0.489
	34.0 - 35.5	3	47.83	2.135
D.	27.5 - 28.5	4	42.63	2.357
	29.0 - 32.5	17	39.65	0.663
	33.6 - 36.0	3	38.50	0.953
R.	30.0 - 32.0	6	34.50	1.402
	29.0 - 35.0 Lambs	14	30.36	0.787

The variation as shown by the S.E. was wide, but there is a tendency for the M.C.V. to be highest in Group C. In Group D. the figures approximate more to those for Group B. while in Group R. the values are below Group B. The lowest M.C.V. figures are in the three month old lambs in Group R.

ii. The effect of season on the Leucocyte picture.

Total and differential leucocyte counts were only carried out in the case of Groups C. D. and R. on samples in which R.B.C. had been made. The results of these counts for leucocytes, together with those for Groups B. are shown in Table VII expressed as means. An inspection of these results shows that no great variation could be detected in the values of one Group as compared with another.

Table VII.

Total Leucocyte and Differential Leucocyte Count

expressed as mean values for sheep in Groups B. C. D. & R.

Group	N. Bands	N. Polys.	Eosins.	Bases.	Lymphos.	Monos.	Total Leucocyte count 10^3/cu.mm.
B. Mean	37	2442	172	0	4068	303	7.08
C. Mean	13	3049	51	9	3791	671	7.43
D. Mean	11	3767	185	10	5066	540	9.33
R. Mean	5	2500	141	0	4062	355	7.04

Discussion of the effect of season.

The Analysis of Variance which was carried out on the data from Groups B. C. and D. and to which reference was made on Page 33, in addition to showing the effect of age on the results, also showed that the difference in the P.C.V. and Hb. levels seen in the three Groups B. C. and D. were significant at 1% and would only occur by chance/

chance once in a hundred times. Thus, the sheep sampled in April and June had significantly lower erythrocyte levels (as judged by P.C.V. and Hb.) than those sampled in January. Furthermore, the levels in June were significantly higher than those found in April.

There was a tendency for the mean corpuscular volume to be increased in the sheep sampled in April. It is possible that as there was some degree of oligocythaemia in these sheep compared with those sampled in January, June and July, this increase in average cell volume was due to a stimulation of the marrow which in turn resulted in the appearance of abnormal numbers of immature cells in the form of macrocytes in the peripheral blood. To support this explanation it would be expected that the sheep showing the lowest erythrocyte levels in Group C. (April) would have the highest M.C.V. This was not found to be the case and, in fact, the highest M.C.V. values were in those sheep with the highest erythrocyte levels. However, in view of the fact that the numbers of observations in Group C. were limited and the range of M.C.V. found was wide, it is not possible to form a definite opinion on the significance of the increase in M.C.V. seen in the sheep sampled in April. Since the completion of these investigations Holman (1952) has demonstrated a negative correlation between size and number of erythrocytes of cows, sheep, goats and horses. In healthy members of these species he was able to show the existence of a compensatory mechanism by which a low erythrocyte count was accompanied by corpuscles above average size and vice versa. It is possible that the variations in the M.C.V. index in my sheep may be due to this phenomenon.

A progressive increase in the mean corpuscular haemoglobin concentration was seen throughout the period of the investigation, when the mean values for the observation in the Groups were considered. However, it was shown by the Analysis of Variance that the only statistically significant difference between the M.C.H.C. levels was between Groups B and C., and Groups B. and D. Thus, the values in the sheep in Group B. were lower than those in Groups C. and D., but the higher values seen in Group D. compared with Group C. could have equally well occurred by chance.

As the healthy corpuscle must be considered to be saturated with haemoglobin, it can be assumed that in the sheep sampled in April, June, and July the corpuscles were carrying their normal concentration of haemoglobin and that in those sheep examined in January the cells were not fully saturated with haemoglobin. As the erythrocyte counts were higher in January than at any time no explanation for this phenomenon can be given.

The Analysis of Variance carried out on the data from Groups B. C. and D. shows that the variation in the erythrocyte level as shown by P.C.V. and Hb. estimations is dependent on the time of year at which samples were taken. Thus the highest levels were found in the sheep sampled in January, the lowest in April, with the June sampling giving intermediate values.

These findings are in general agreement with the results of Holman's (1944a) studies on the seasonal variation in the erythrocyte levels of sheep at pasture. The significant fall in erythrocyte levels in his sheep occurred in February and March, and by April he found these levels had started to rise again. As no

examinations/

examinations were made in the case of my sheep in February and March it is not possible to compare my results with his for these months, but the fact that in my sheep the lowest erythrocyte values were in April and that the rise in these levels did not appear before June requires an explanation. It is suggested that this was because Holman's sheep were on 'low ground' pasture at the time of sampling and here the improvement in weather and pasture conditions occurred earlier than was the case on the hill grazings from which my sheep came. The possible relationship of nutrition to the variation seen in the erythrocyte level is postulated by Holman (1950), in a general discussion of the erythrocytic oligocythaemia which may occur in the blood of herbivorous animals during winter and spring months. He suggests that the condition may be due to deficient blood production caused by a lack in the diet of the substances necessary for erythropoiesis.

So far the possible association between seasonal changes in the blood picture and helminth burden have not been investigated in the British Isles, but in America, Hawkins and de Frietas (1947) have been able to show that rises in worm egg counts may be accompanied by inverse changes in erythrocyte levels.

It follows then that in a discussion of the possible causes for the variation found in the erythrocyte levels in the sheep in this investigation the two main factors for consideration are nutrition and parasitism. There is however another factor, the possible influence of which must be acknowledged in the case of adult Scottish hill sheep during the first three or four months of the year and this/

this is pregnancy. There is at present no record of an anaemia of pregnancy in Scottish hill sheep, but working on flocks in the counties of Leicestershire, Derbyshire and Cambridgeshire Innes and Shearer (1940) have recorded an anaemia of the macrocytic hyperchromic or normochromic type in pregnant ewes. Therefore before proceeding with the discussion of the possible influence of nutrition and parasitism on the erythrocyte levels in the sheep in this investigation, it is desirable to assess as far as possible the effect of pregnancy. This has been done by a comparison of the Hb. levels of pregnant and non-pregnant sheep in Group C.

Effect of Pregnancy.

It was found at post mortem examination that of the fifteen sheep between 1 and 2 years old in Group C. (April), seven were pregnant and eight were non-pregnant. The Hb. figures for these sheep divided on the basis of pregnancy appears below in Table VIII.

Table VIII.

Haemoglobin figures for pregnant and non-pregnant sheep under 2 years old in Group C.

Pregnant		Non-pregnant	
Sheep's Number	Hb. gms./100 ml.	Sheep's Number	Hb. gms./100 ml.
809	8.4	815	8.1
829	7.7	817	7.6
834	8.0	820	8.7
730	8.1	826	10.0
734	5.7	835	9.5
736	7.6	838	8.8
740	9.8	844	7.8
		728	7.8
Means	7.9		8.5

The mean figures for the pregnant sheep was found to be lower than that for the non-pregnant sheep but in a comparison of the Means test, according to the method of Mainland (1938) 't' was found to be 0.391 and 'p' to be 0.7 showing the difference between the two means to be insignificant. Thus, in the limited number of observations in which comparison can be made, pregnancy appears to exert no influence on Hb. level. This is in agreement with Clark (Groenwald, Graf, Bekker, Malan & Clark, (1941) who found that in the course of an investigation into Pregnancy Disease in Merino sheep, pregnant sheep sampled at the same stage of pregnancy as my sheep showed no difference in P.C.V. values from non-pregnant sheep kept under the same conditions. My findings also agree with those of Barcroft, Kennedy and Mason (1939), who recorded a fall in P.C.V. in sheep at about the fiftieth day of pregnancy, but noted no consistent change at the one hundred and fortieth day, which was about the stage at which the sheep in the present investigation were sampled.

It is concluded that no effect of pregnancy per se could be shown in the sheep in this investigation; however, pregnancy may well play an indirect contributory role in any influence malnutrition may have on erythrocyte levels.

Effect of Nutrition on Erythrocyte Levels.

There are a number of factors which make the late winter and early spring ~~spring~~ an especially critical time for hill sheep from the nutritional viewpoint. Chemical analysis of hill pastures shows their nutritive value to be extremely low at this time (Abrams, 1950). Even in a mild winter, the cold environment must make extra demands on heat production, and at a time when the amount/

amount of energy expended in foraging must be high. Superimposed on these increased demands at a time when food supplies are at a minimum, we have the fact that the sheep over two years old are pregnant. As the nutritive value and quantity of the grass will only start to increase at about the time of lambing, the last six weeks of pregnancy, during which as Wallace (1948) has shown, the demands of the foetus are particularly heavy, will occur at a time when the cumulative effects of the chronic malnutrition of winter are exerting a maximum effect. Under these conditions the fact that pronounced anaemia does not normally occur in hill sheep at this time of the year can only confirm the acknowledged high priority of claim on food intake exercised by the erythropoietic tissue. As yet there has been no attempt made to define the dietary factors concerned in erythropoiesis in the sheep. Cartwright (1947) in a comprehensive review of the subject concluded as follows: that certain vitamins, namely riboflavin, nicotinic acid, pyridoxine, folic acid, and the extrinsic factor, have each been shown to be essential for erythropoiesis in at least one species: that adequate protein intake is essential in view of the fact that the red cell stroma contains amino acids, and the globin fraction of the haemoglobin molecule contains all ten of the 'essential', and many of the non-essential amino acids; finally, that the three mineral elements which have been shown to be essential for normal erythropoiesis are copper, iron and cobalt.

There is no evidence to indicate the role of the vitamins in red cell formation in the sheep. Similarly it has not been possible to demonstrate so far the role of the amino acids in this connection in/

in sheep. Owen, Smith & Wright, (1943) have shown that in the developed ruminant, amino acids are synthesized by rumenal microflora from protein and non-protein fractions of ingested food, but it follows that factors which tend to alter the bacterial population of the alimentary tract may influence the nature of the amino acids synthesized. The fact may be of some importance in view of the findings of Orten and Orten (1945), who as the result of trying the administration of various amino acids to rats on a low protein diet, concluded that no one amino acid could be regarded as 'key' and that if any one essential amino acid was missing erythropoiesis would not proceed until it was supplied.

Although it may be safely assumed that iron is essential for blood formation in the sheep, examples of the effects of primary deficiency in this element are lacking. Holman and Pattison (1941) have recorded a hypochromic anaemia in rapidly developing lambs but the M.C.H.C. was not grossly abnormal.

It would appear at first sight that from the extensive observations on cobalt deficiency as a disease of sheep, the rôle of cobalt in blood formation in the sheep would have been decided. However, although it is true that anaemia has been reported as a fairly constant symptom of the disease as it occurs in Australia and New Zealand (Marston et al, 1948), and has been described in some outbreaks in Britain (Greig, Dryerre, Godden, Crichton & Ogg, 1933), Boddie (1947), Russell (1944) and others have drawn attention to two factors which make interpretations of blood findings difficult. The first is the fact that one of the early symptoms of the disease is loss of appetite, making it impossible to judge from the results of/

of haematological observations whether cobalt deficiency has a specific effect on haemoglobin levels or whether the anaemia, when it occurs, is merely secondary to inanition. The second factor is concerned with the severe parasitism which has been observed in many outbreaks (Stewart & Piercy, 1935; Patterson, 1938; Greig et al, 1933; Rowland & Harbour, 1941). In many of the accounts of the disease no attempt has been made to assess the importance of this second factor of helminthiasis and the results are correspondingly less conclusive. It may be that the more recent work of Gall et al (1949) may prove of help in understanding the role of cobalt in erythropoiesis. These workers suggest an indirect requirement for cobalt in the ruminant, the mineral's presence being necessary for the rumen bacteria to perform the function of assisting the animal to digest its food. Following the demonstration by Lester Smith (1948) that vitamin B_{12} contains approximately 4% cobalt, it has been well established that rumen contents are rich in B_{12}, where it is synthesized by the microflora (Zucker & Zucker, 1948; Abelson & Darby, 1949; Dyke et al, 1950) have also shown that considerable amounts of this vitamin are synthesized in the intestine of the sheep. Added interest in this vitamin in the sheep will no doubt follow the demonstration by Smith, Kock & Turk (1951) of the curative effect of large doses of this vitamin in cobalt-deficient sheep. It is concluded that there would appear to be considerable evidence that cobalt is in some way related to blood formation, but a definition of its exact role must await further investigation.

It had been shown in experimentally produced copper deficiency by Thomas and Wheeler (1932) and in naturally occurring copper deficiency/

deficiency by Bennetts and Beck (1942) and others that copper is necessary for normal haemoglobin formation and for red cell production in the sheep. It is of interest to note that Bennetts and Beck considered that these functions may be carried out in the sheep under conditions of copper deficiency, when blood and liver coppers are very low, provided it is not called upon to produce and rear progeny.

In a state of malnutrition such as Orr and Fraser (1932) have shown to exist in Scottish hill sheep in the late winter and early spring, the exact nature and relative importance of the dietary deficiencies is impossible to determine. It must be stated, however, that there is no evidence at present to show that malnutrition alone will produce the changes observed in the erythrocyte levels of the sheep in this investigation. For example, Clark, Graf, Bekker, Malan & Clark (1941) in the course of observations on the blood picture of Pregnancy Toxaemia, subjected sheep known to be worm-free, to semi-starvation for periods up to fifty days but were unable to show any alteration in the erythrocyte picture, as measured by Hb. estimation or P.C.V. readings.

The findings of Lawrence et al (1951), working in South Africa, are also of interest in this connection. Their investigation was designed to study the influence of the nutritional level on helminthiasis. The material consisted of 40 Merino lambs which were freed of worms by dosing. Half the lambs were fed on a high nutritional level and half on a poor nutritional level. Half of each of these groups was then artificially infected with Haemonchus contortus and Oesophagostomum columbianum. This resulted in the formation/

formation of four groups as follows:- Good nutrition with worms, good nutrition without worms, poor nutrition with worms, and poor nutrition without worms. Production measurements conducted throughout the experiment showed that the uninfected group on the poor diet maintained a very slow rate of gain, indicating that the rations supplied were little above maintenance requirements. Haemoglobin estimations were carried out at the end of the experiment and the mean values for the 4 groups are shown below.

Group.	Mean Hb. gms/100 ml. for group.
1. High nutrition, no worms.	9.6
2. " " worm infested.	8.9
3. Poor " no worms	9.8
4. " " worm infested.	7.6

The authors concluded that these figures suggested that the lower ration was sufficient to maintain the haemoglobin level at a normal value in the absence of worms.

It is concluded that although the lowest erythrocyte levels were recorded in the sheep sampled at a time of year when nutrition was on a low plane, there is no justification for the assumption that malnutrition alone was responsible for this reduction in red cells.

Effect of Helminth Burden.

A total and differential worm burden estimation was carried out in respect of all the sheep from which blood samples were taken. This work was carried out by Doctors Morgan, Parnell, and Rayski, and/

		ABOMASUM.			INTESTINE.									
Group	Mean and Range	Haemonchus contortus	Ostertagia circumcinota	Ostertagia spp.	Trichostrongylus axei.	Larval stages.	Trichostrongylus colubriformis.	Trichostrongylus vitrinus.	Cooperia curticei.	Bunostomum trigonocephalum.	Nematodirus.	Larval stages.	Oesophagostomum venulosum.	Chabertia ovina.	Means and ranges for worm burdens in respect of groups.
	Mean.	1	212	29	473	319	20	151	178	29	10	34	1	1	1,565
B.	Max.	25	2,500	350	3,800	2,750	250	2,200	1,750	230	1,750	550	21	32	7,828
	Min.	0	0	0	0	0	0	0	0	0	0	0	0	0	20
	Mean.	1	2,310	967	886	3,126		80	370	717	380	110	2	1	9,056
C.	Max.	10	8,900	3,350	5,550	18,450	1,350	3,400	3,900	209	4,650	1,300	295	103	25,174
	Min.	0	100	0	0	100	0	0	0	0	0	0	0	0	1,096
	Mean.	68	1,579	206	1,191	163	36	249	445	37	163	1	-	-	4,163
D.	Max.	674	12,350	2,500	3,650	3,750	450	4,500	3,300	179	3,150	50	87	276	21,168
	Min.	0	0	0	0	0	0	0	0	0	0	0	0	0	147
	Mean.	6	338	91	612	35	44	71	468	28	132	0	4	18	1,881
R.	Max.	79	2,900	1,250	1,800	250	750	750	2,950	78	1,800	0	27	166	4,029
	Min.	0	0	0	0	0	0	0	0	0	0	0	0	0	105

Table IX. Worm Burdens for sheep in Groups B. C. D. & R., shown as mean values and ranges.

and their results have been published in the Journal of Helminthology XXV. 3/4 (1951). It is with their permission that their findings are quoted here for correlation with the results of the examination of the blood.

Table IX. shows the means and ranges for the worm counts of the various species, in respect of the four slaughterings, viz., Groups B. C. D. and R.

Before discussing in detail the relationship of the worm burden to the blood picture in the sheep in this inve stigation, it is desirable to consider whether the worm burden found in these sheep was likely from its qualitative and quantitative character to be capable of influencing the blood picture.

Ross and Gordon (1936), Monnig (1947, latest edition), and others have emphasised the difficulty of declaring standards for the number of worms of any particular species necessary to cause serious or pathogenic effects, mainly because of the varying levels of nutrition obtaining in the hosts. Both the above named authorities have published numerical limits for worm burdens beyond which pathogenic effects may be expected, but in assessing the numerical significance of worm burdens in this experiment and the experiment recorded in Section V., the writer has made use of a scheme devised by Dr. D. O. Morgan in consultation with Dr. McL. Gordon. The details of this scheme, which has not yet been published, appear in Table X. It will be seen that points are allocated for the presence of a certain number of worms; the number required to score a point depends on the species and is governed by its pathogenicity, e.g., 50 Bunostomum trigonocephalum are equivalent to '1' point, whereas/

whereas 4,000 Nematodirus spp. must be present before the burden can count as '1'. To determine the significance of the burden as a whole, the points are totalled and by applying the key to the resulting figure an index of the severity of the infestation is given.

Table X.

Index for the assessment of pathogenicity of worm burden.

Species	Number of Parasites equivalent to value '1'
Haemonchus contortus	500
Ostertagia spp.	3,000
Trichostrongylus spp.	4,000
Cooperia curticei	
Strongyloides papillosus	
Nematodirus spp.	
Bunostomum trigonocephalum	50
Chabertia ovina	100
Oesophagostomum venulosum	200
Trichuris ovis	
Larvae	4,000

Key to pathogenicity of Infestation

A value of '1' probably would do harm in a young sheep.

" " " '2' " " " " " an adult sheep

" " " '3-5' " " lead to the death of young sheep.

" " " '5-10' " " " " " " " an adult sheep.

When the results of the worm burden examinations were analysed in the light of these standards it was found that in Group B. there was only one sheep in which the worm burden 'would probably lead to the death of the sheep'. In Group C. the infestation was found to be severe enough to 'probably do harm' in the case of fourteen sheep, and in eight more, 'probably lead to death'. Thus, in 44% of the sheep in this Group the worm burden reached pathogenic proportions. In Groups D. and R. the infestations were much lighter but were still above that seen in Group B. and in thirteen sheep the infestation was classified as 'probably doing harm'.

It is well recognised that infestations of some species of worm are more likely to be associated with blood changes than others, and it is now proposed to examine the nature of the worm burden found in this investigation from the aspect of potential pathogenicity for blood and blood forming tissue.

Abomasal Infestation.

Table IX. shows a notable absence of anything more than a slight infestation of Haemonchus contortus in the sheep in this experiment. This worm has been shown by Fourie (1931), Holman and Pattison (1941) and Andrews (1942) to be responsible for severe reductions in erythrocyte numbers, leading in heavy infestations to an anaemia which Fourie (1931) described as indistinguishable from the anaemia of chronic haemorrhage. The sheep in this investigation were Cheviots, and it is suggested that the low incidence of this parasite may be partly due to the breed resistance noted by Cameron (1935). Apart from Haemonchus contortus, the species found in the abomasa of these sheep were Ostertagia circumcinta, undifferentiated ostertagia, and/

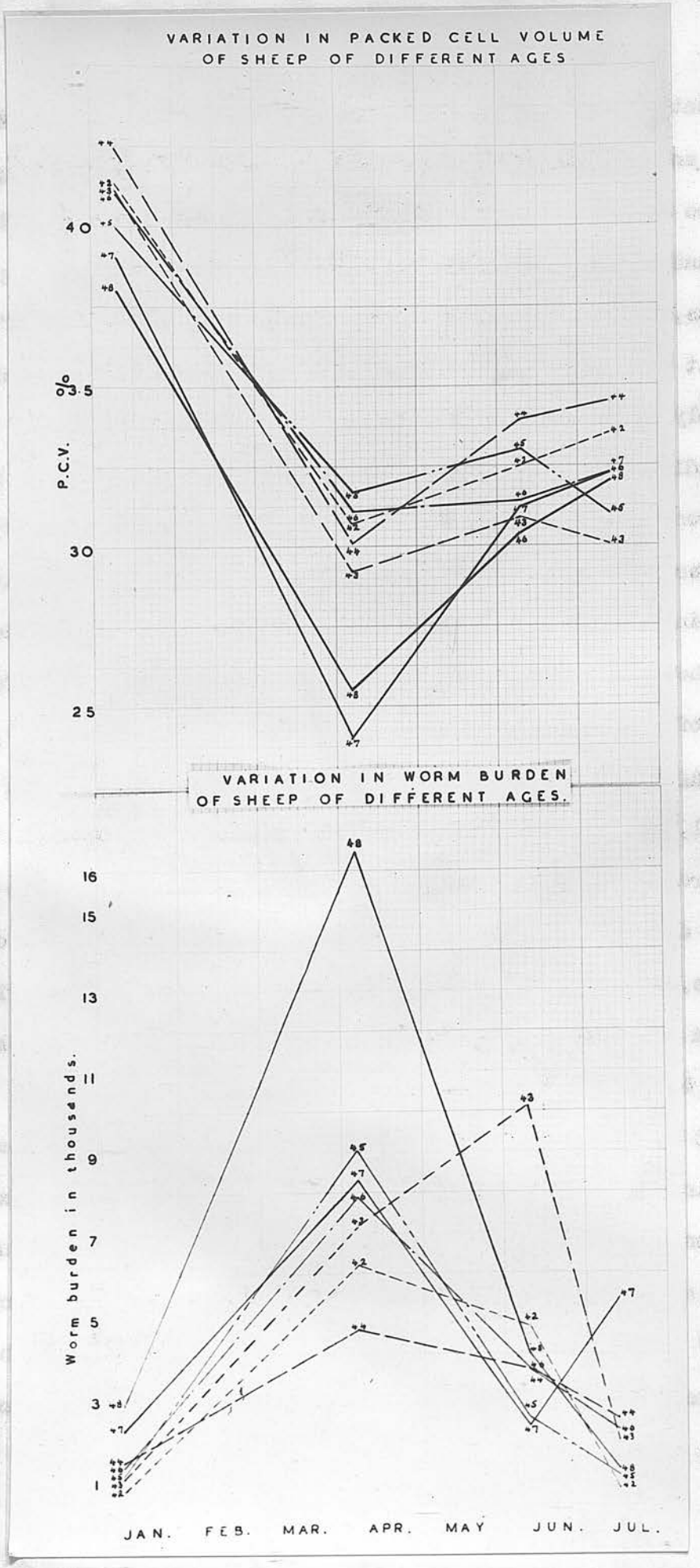
and Trichostrongylus axei. The infestations of ostertagie were heavier than those of trichostrongylus, but in Group C. both species approached in number the pathogenic ranges suggested by Ross and Gordon (1936) and by Mönnig (1947). Mönnig attributes pathogenic properties to both Ostertagia and Trichostrongylus, with the former more likely to produce anaemia than the latter. Todd et al (1951), reporting on the experimental infestation of six young sheep with O. circumcinta and O. trifurcata, describe the development of a hypochromic macrocytic anaemia as the result of a mild infestation. According to Morgan and Hawkins (1949), although Ostertagia cannot be regarded as so voracious a blood-sucker as Haemonchus, in pathogenic infestations some reduction in Hb. and R.B.C. may be seen. The opinions regarding the pathogenicity of T. axei are conflicting. Andrews, Kauffman and Davis (1944) did not observe any anaemia in sheep dying of experimental Trichostrongylosis, and Whitlock (1949) states that in Trichostrongylosis, anaemia is not marked except in cases of long standing. On the other hand, Gibson (1947), in a study of the pathogenic effects of T. axei on sheep maintained on a low plane of nutrition, describes the development of a marked oligocythaemia. Morgan and Hawkins describe the anaemia which may develop in Trichostrongylosis as one of a complex of symptoms, including gastric disturbance, weakness and emaciation, all of which are related to the liberation of a toxin by the worm.

Intestinal Infestation.

The species encountered in the small and large intestine of the sheep in this investigation included T. colubriformis, T. vitrinus, Cooperia curticei, Nematodirus spp. and much lighter infestations/

infestations of Bunostomum trigonocephalum, Oesophagostomum venulosum and Chabertia ovina. The pathogenicity of Trichostrongylus species has already been considered. Cooperia, it is stated by Ross and Gordon (1936), has not been found capable of causing any effects, although their presence may accentuate the effects of concurrent infestations of Trichostrongylus, Andrews (1938) considers probably by reducing the host's ability to utilise food. Nematodirus was found in very large numbers by Kauzal (1933) in fat sheep slaughtered in abattoirs, and Morgan and Hawkins (1949) and Ross and Gordon (1936) agree that when symptoms of parasitism occur in the presence of this worm it is usually in association with the Trichostrongylus species. Experimentally, pure infestations of Bunostomum trigonocephalum (Lucker & Neumayer, 1946), Oesophagostomum venulosum (Sarles, 1944), and Chabertia ovina (Kauzal, 1937) have all been shown to cause clinical symptoms in which reduction of erythrocytes was a feature. According to Morgan and Hawkins, Bunostomum is the most pathogenic of these species, as far as the production of anaemia is concerned. The anaemia produced is normocytic with a tendency towards hypochromia and is due to the blood-sucking propensities of the parasite.

It is considered that the worm burdens encountered in the sheep in this investigation were sufficiently heavy to have exerted pathogenic effects in the case of sheep sampled in April (Group C.) and to a lesser extent, in those sampled in June and July (Groups D. and R.) Furthermore, the worm burdens included species which are known to reduce erythrocyte levels as part of their pathogenic effect.


VARIATION IN PACKED CELL VOLUME
OF SHEEP OF DIFFERENT AGES
P.C.V. %
40
35
30
25
VARIATION IN WORM BURDEN
OF SHEEP OF DIFFERENT AGES.
Worm burden in thousands.
16
15
13
11
9
7
5
3
1
JAN.
FEB.
MAR.
APR.
MAY
JUN.
JUL.

Figs. 1 & 2

Relationship of worm burden to blood findings.

It has already been stated that the variation in the blood picture between Groups was restricted to the erythrocytic properties. For the purpose of studying the relation of these blood changes to the worm burden, the figure for P.C.V. has been taken as representing the erythrocyte level. In Figs. 1 and 2 are shown the changes in P.C.V. and worm burden respectively for the four Groups B, C, D, and R. The sheep within each Group were divided according to year of birth, and for each age mean values for P.C.V. and worm burdens have been calculated. Thus, the curves show the variations of both these measurements for the sheep of each age group throughout the experiment. From Fig. 2 it is seen that in April (Group C.) the rise in worm burden affected sheep of all ages, but was most marked in the youngest sheep. Similarly in April, although P.C.V. levels were lower in all ages, it was the younger sheep (viz., born 1947 and 1948) that suffered the most severe decrease in P.C.V. It is well known that young sheep have less resistance to helminth infestation and its effect, and therefore the fact that these were the sheep which showed the greatest decrease in erythrocyte levels suggests that the general fall in these levels was closely related to worm burden.

That the curves for P.C.V. and worm burden are not accurate reflections of each other is no doubt due to the individual variations in susceptibility both to infestation and to the effects of the parasites.

These findings in respect of the relationship of worm burden to erythrocyte level agree closely with the results of investigations/

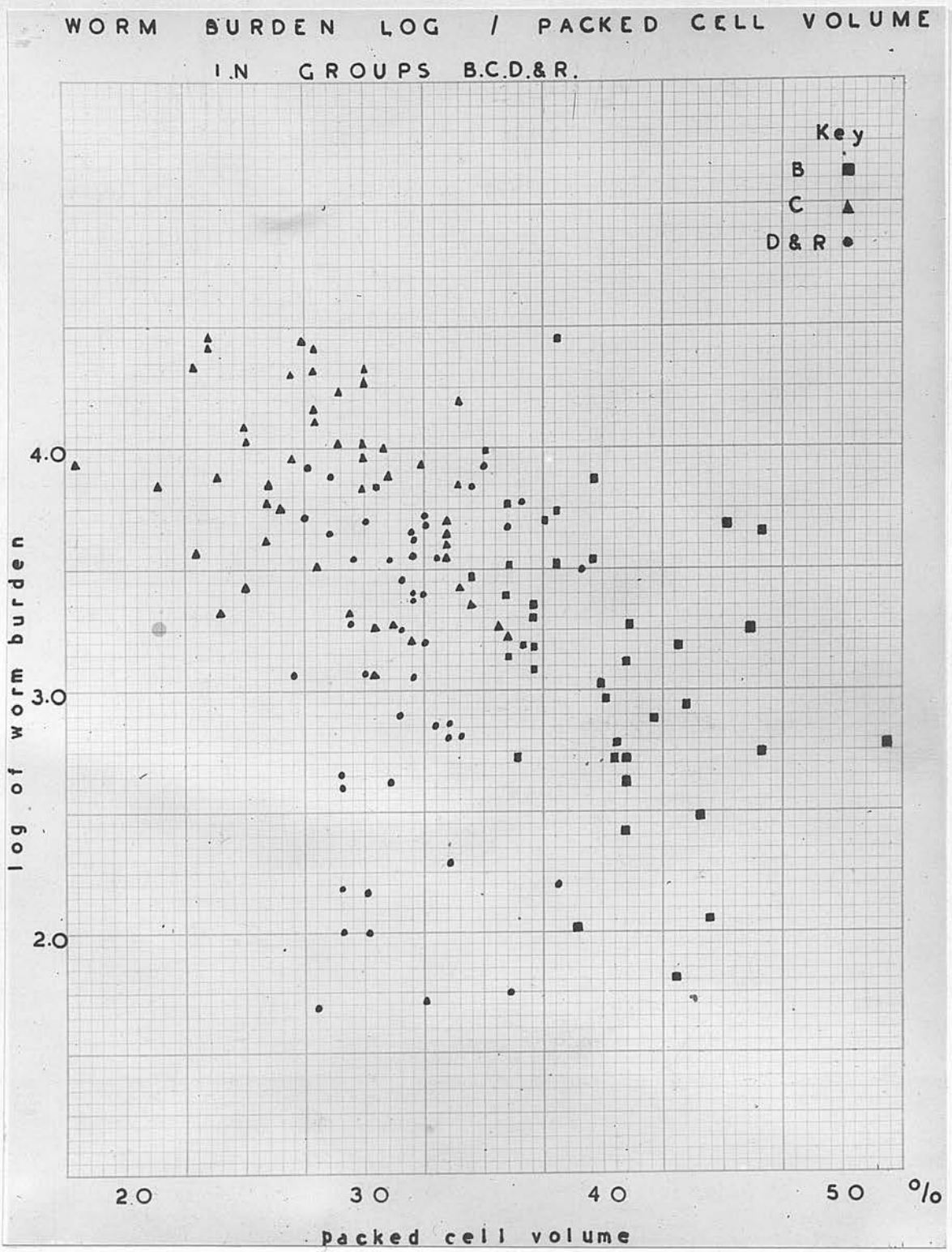

WORM BURDEN LOG / PACKED CELL VOLUME
IN GROUPS B.C.D.&R.
Key
B
C
D&R
4.0
3.0
2.0
log of worm burden
2.0
3.0
4.0
5.0
%
packed cell volume

Figure 3.

tions conducted in America by Hawkins and de Frietas (1947) and Hawkins, Cole, Kline, and Drudge (1944). Hawkins and de Frietas, working with ewes and yearling lambs over a period of five years, observed an increase in Hb. levels during 'late' winter months, at which time the worm burden as estimated by worm egg counts was low. In late February and March the position was reversed, so that worm egg counts rose and Hb. fell, and these workers assume that the rise in worm egg counts was due to the ingestion of infective larvae at this time of the year. As their sheep were kept on a standard diet and protected from severe weather, it is highly probable that the changes in Hb. were directly related to the change in helminth burden.

In the course of observations carried out on sheep at pasture Hawkins, Cole, Kline, and Drudge (1944) also demonstrated an inverse association between erythrocyte levels and worm egg counts.

In order to carry out a further examination of the relationship between the worm infestation and erythrocyte level, the estimated total worm burden was plotted against the value for P.C.V. for each sheep in the four Groups. The scatter diagram so constructed appears in Fig. 13. Both P.C.V. and Hb. were available for all sheep examined, but the former was chosen as likely to be the most reliable and free from subjective error. The figures for the worm burdens covered a very wide range and in order to facilitate their plotting in the diagrams, the log of the worm burden has been used in preference to the figure for the actual worm burden.

Study of the pattern for the individual Groups is made possible by the use of different symbols for each Group. Thus, it is seen

that/

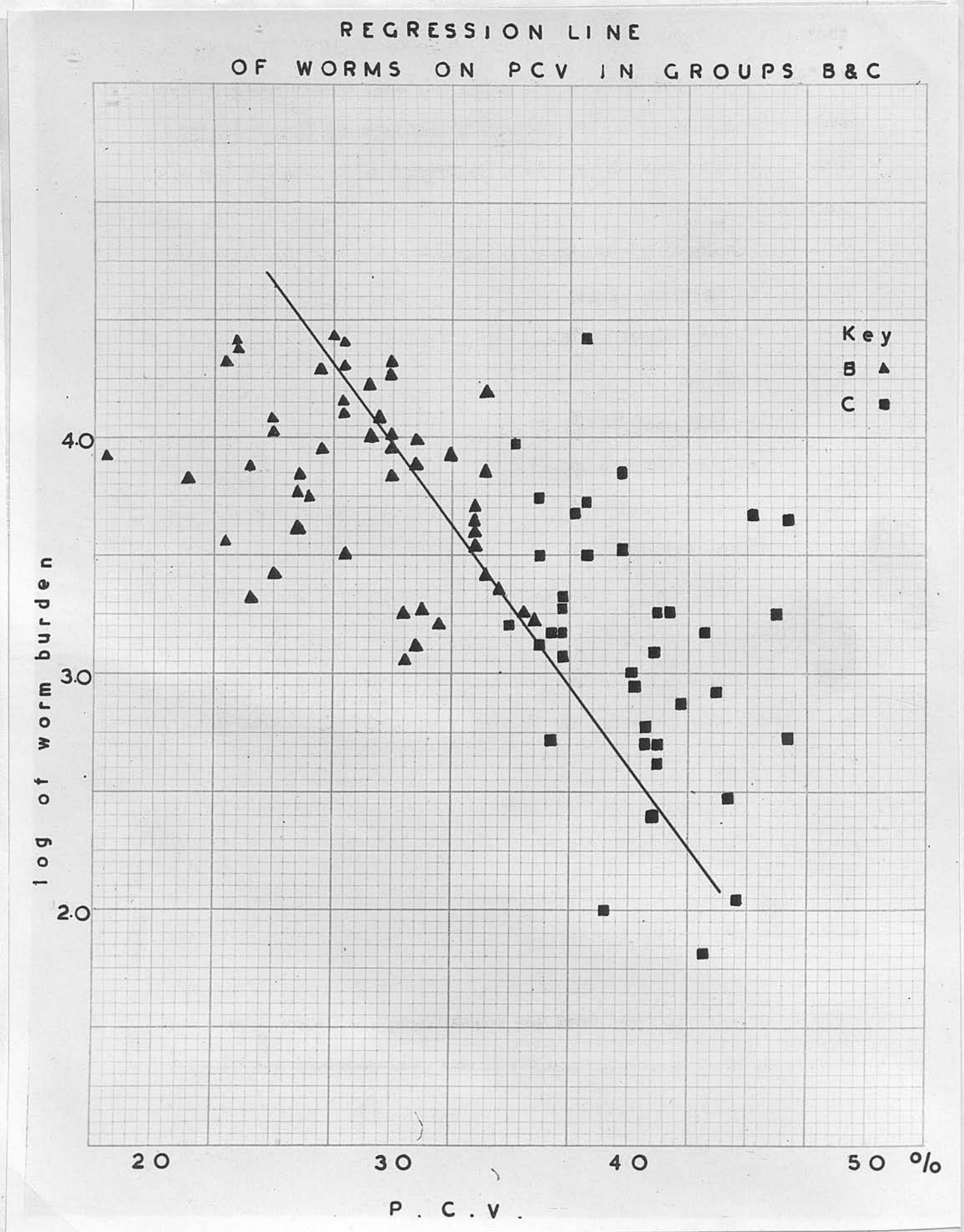

REGRESSION LINE
OF WORMS ON PCV IN GROUPS B&C
Key
B
C
4.0
3.0
2.0
log of worm burden
20
30
40
50 %
P . C . V .

Fig. 4.

that the worm burden is lowest and the P.C.V. highest in Group B; in Group C. the position is reversed, the worm burden being highest and the P.C.V. lowest; the pattern in the case of Groups D. and R. occupies an intermediate position in respect of bothP.C.V. and worm burden.

Consideration of the dots in individual Groups shows that although in no Group is the association clearly marked, it is best in Group C. less close in Group B., and non-existent in the case of Groups D. and R.

Worm Burden (log) and P.C.V. in Groups B. and C.

The scatter diagram appearing as Fig. 4. shows in respect of each sheep in Groups B. and C. the values for P.C.V. plotted against the log of the worm burden.

It was found that where worm burden (log) is represented by x, and P.C.V. by y, the regression line is given by $y = 60.75 - 7.70x$. Thus when P.C.V. = 25.0, worm burden (log) = 4.64; P.C.V. = 36.0, worm burden (log) = 3.21; and when P.C.V. = 40.0, worm burden (log) = 2.69.

The regression coefficient for worms (log) on P.C.V. = -7.7032 and is significant @ 1%, i.e. t = 8.57 and n = 91.

The coefficient of correlation was 0.6684 which is also significant @ 1%.

Conclusions in respect of relationship of P.C.V. to worm burden.

In view of the fact that the worms affecting these sheep have been shown to be both quantitatively and qualitatively capable of reducing erythrocyte levels, it is suggested that the significant correlation which has been shown to exist between worms and P.C.V. provides strong support for the conclusion that the low erythrocyte levels/

levels seen in sheep sampled in April were due to the higher worm burdens seen at this time.

It has been shown that in Groups D. and R. it was not possible to demonstrate the same degree of association between worm burden and P.C.V. as was seen in Groups B. and C. This is due, it is considered, to the fact that parasites exert their maximum effect on erythrocyte levels in the presence of malnutrition, such as would be likely to be in existence in April but not in June and July. It will be noted that there were sheep in Group D. which had a worm burden similar to those in Groups B. and C; there was also one sheep in Group D. which, according to Morgan's Index, would be classified as having a worm burden that would probably do harm. These parasitised sheep in Group D. showed widely different P.C.V. levels, and it is suggested that these differences were due to variation in individual resistance to the effects of the parasites shown by different sheep.

General Discussion of Seasonal Variation.

The explanation of the seasonal variation shown in the erythrocyte levels of the sheep in this investigation centres round the relationship of nutrition and parasitism to erythropoiesis. From a better understanding than exists at present of the nutritional requirements for normal erythropoiesis in the sheep, it might be possible to show that changes in pasture composition account for the seasonal variation noted in erythrocyte levels; however, at present there is no evidence to support the contention that a diet, the deficiency of which is entirely quantitative, can influence erythrocyte levels. On the other hand, Foster & Cort (1931)/

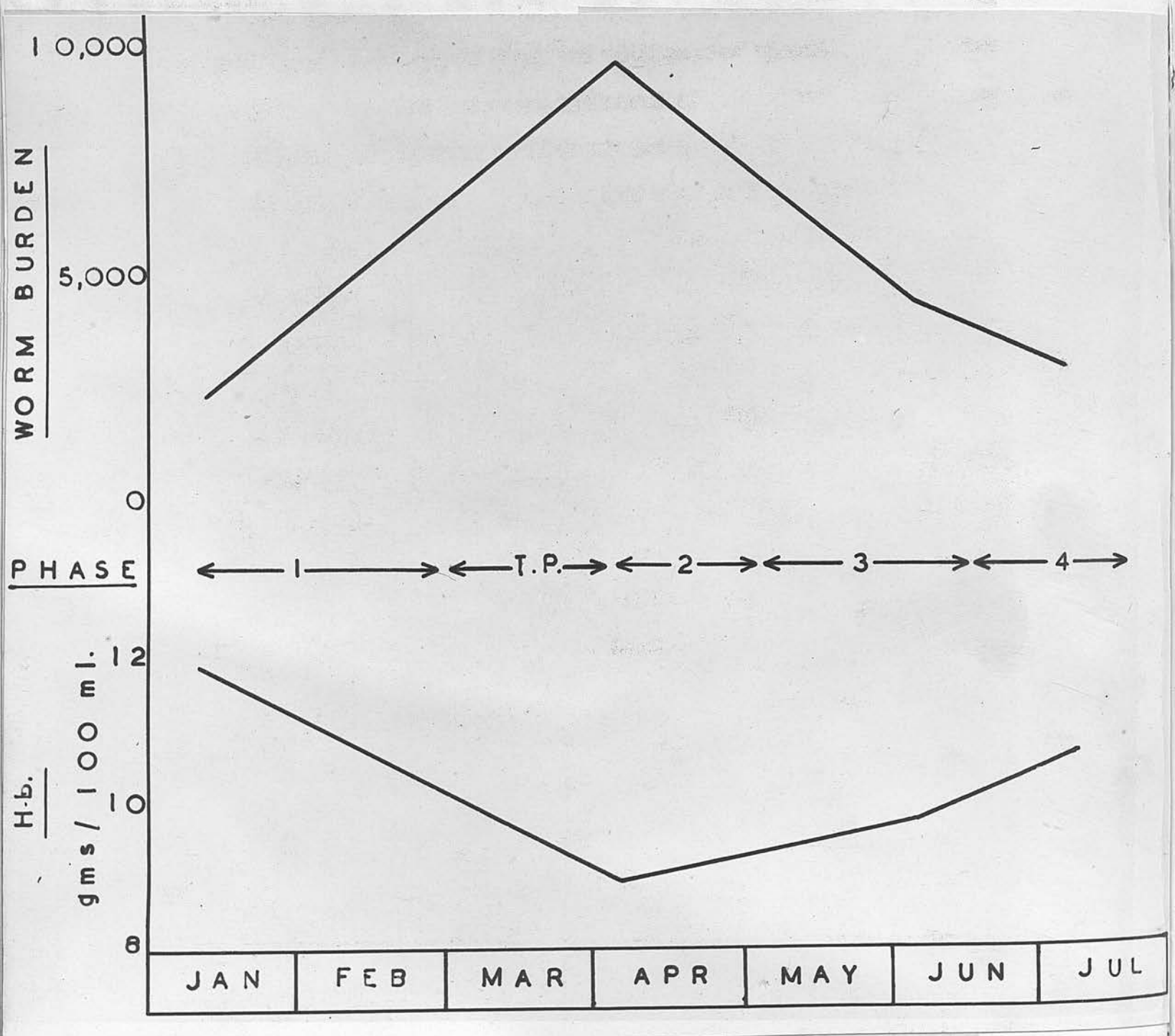


Figure 5. Showing changes in haemoglobin level and worm burden from January to July.

(1931), Fraser & Robertson (1933) and many others, have shown that low nutrition favours the development of helminth parasitism, and reference has already been made to the blood changes associated with parasitism in sheep. It is therefore suggested that the helminth burden was the causal factor for the fall in erythrocyte levels seen in my sheep, and it is on this basis that the following explanation of the pattern of variation which was observed is advanced.

Figure 5 illustrates the change in worm burden and erythrocyte level - the latter being represented by the haemoglobin values. Means have been calculated from all the measurements made in respect of worm burden and haemoglobin on the four occasions on which the sheep were sampled, i.e., January 11th, April 5th, June 7th, and July 12th. The points have been joined to form two lines representing changes in worm burden and haemoglobin level throughout the period covered by the observations. This period has been divided into four phases with a transitional period (T.P.) between Phases 1 and 2. In Phase 1 the worm burden was low and it may be assumed that as yet the nutritional level of the sheep had been unaffected by the winter conditions. The erythrocyte values were high.

During the transitional period the nutritional level was falling and Stoll (1940) has shown that one of the factors which may cause a breakdown of the host's resistance to parasitism is low nutrition, so that in this period a reduction in resistance to parasitism may be expected.

Phase 2 covers the period at which nutrition is at its lowest, and this together with the low resistance of the host, and the fact that weather conditions favoured helminth infectivity, all predisposed the/

the host to the intake of a new and heavy worm burden. The host was in no position to withstand the effect of this attack, among the effects of which was a reduction in erythrocyte level.

Phase 3 covered a period in which the nutritional level was rising due to the growth of young grass, there was also an increase in the host's resistance to both further increases in worm burden and to the effect of those parasites it already carried. Thus a rise in erythrocytes levels was seen in spite of the presence of a fairly heavy worm burden in some cases.

Phase 4 showed an extension of the tendencies seen in Phase 3 with the phenomenon of 'self cure' described by Ross & Gordon (1936) in operation. This was reflected in a rise in erythrocyte levels - a stimulated erythropoiesis making good the losses suffered in Phase 2.

It was anticipated that with the more frequent sampling envisaged in the observations to be carried out during the same period in the following year (Section V.) it would be possible to test the accuracy of this explanation for the variation in erythrocyte levels seen between January and July. Furthermore, by the simultaneous examination of marrow and blood it might be possible, by a study of the nature of the marrow changes, to separate the effect on the erythrocyte levels of nutrition on the one hand and parasites on the other. The results of the investigations described in Section V. show that these hopes were largely fulfilled.

General Conclusions.

1. The significant variations demonstrated in the peripheral blood were confined to erythrocytic properties.

2./

2. Sheep under two years of age had lower erythrocyte levels, as shown by P.C.V. and Hb. measurements, and higher M.C.H.C. indices than sheep over that age.

3. The erythrocyte levels, as shown by P.C.V. and Hb. measurements, for sheep sampled in January, April and June differed from each other significantly. The levels for January were highest, for April lowest, and those for June occupied an intermediate position.

4. The M.C.H.C. for sheep sampled in January was significantly lower than for sheep sampled in April and June.

5. A significant association between P.C.V. and worm burden was shown to exist in sheep sampled in January and April.

6. In sheep sampled in April, pregnancy could not be shown to cause a significant influence on Hb. levels.

B. Standards for the Blood Picture of Scottish Hill Sheep sampled from January to July.

From the results of the examination of the blood from the 194 sheep, standards are presented for Scottish hill sheep when sampled from January to July.

a. Erythrocytes.

In view of the variation which has been shown to be caused by age and season, separate standards have been calculated for sheep over two years old and for sheep under two years of age, and for both these age groups standard means have been worked out for the four occasions on which sampling was carried out, viz., January, April, June and July.

Tables XI. and XII. are drawn up on this basis and show mean values and S.E. calculated according to the Method of Mainland(1938). The standard of sheep under two years old appears in Table XI. and for over two years old in Table XII. Table XIII. shows the mean and S.E. for all sheep examined, irrespective of age or time of sampling.

b. Leucocytes. As no evidence of variation due to age or season of sampling could be demonstrated for leucocyte values, these are presented in the form of Means and S.E. for all sheep in which W.B.C. and F.L.C. were carried out. These figures appear in Table XIV, the D.L.C. being expressed in terms of absolute values.

Discussion of Normal Values.

To facilitate comparison of my results with those of previous workers Table XV. (erythrocytes) and Table XVI.(leucocytes) have been/

62a.

Table XI.

Erythrocyte Properties for Sheep under two years old sampled in January, April, June and July expressed as means values with standard error of mean. (S.E.)

Date of sampling		P.C.V. %	Hb. gms/100 ml.	R.B.C. 10^6/cu.mm.	M.C.V. Cu. μ	M.C.H.C. %
January 11th	Mean	38.43	11.38	10.66	36.70	29.20
(Group B.)	N.	15	15	15	15	15
	S.E.	0.620	0.193	0.252	0.733	0.463
April 5th	Mean	25.07	8.24			33.27
(Group C.)	N.	15	15			15
	S.E.	0.844	0.273			0.447
June 7th	Mean	30.02	9.13			30.44
(Group D.)	N.	24	24			24
	S.E.	0.792	0.217			0.271
July 12th	Mean	31.38	9.89			31.74
(Group R.)	N.	26	23			23
	S.E.	0.512	0.170			0.308
All Groups	Mean	31.11	9.62			31.14
	N.	80	77			77
	S.E.	0.580	0.160			0.237

N = number of observations.

62b.

Table XII.

Erythrocyte Properties for Sheep over two years old Sampled in January, April, June and July and expressed as means values with standard error of mean (S.E.)

Date of sampling		P.C.V. %	Hb. gms/100 ml.	R.B.C. 10^6/cu.mm.	M.C.V. cu. μ	M.C.H.C. %
January 11th	Mean	41.27	11.88	11.10	37.62	28.29
(Group B.)	N.	29	32	32	29	29
	S.E.	0.748	0.164	0.232	0.561	0.304
April 5th	Mean	30.32	8.95			29.98
(Group C.)	N.	34	33			33
	S.E.	0.546	0.300			0.393
June 7th	Mean	32.21	9.74			30.53
(Group D.)	N.	40	40			40
	S.E.	0.439	0.108			0.271
July 12th	Mean	32.45	10.25			31.45
(Group R.)	N.	11	10			10
	S.E.	0.455	0.198			0.482
All Groups	Mean	33.98	10.15			29.87
	N.	114	115			112
	S.E.	0.504	0.158			0.280

N = number of observations.

62c.

Table XIII.

Erythrocyte properties for sheep of ages ranging from approximately 2 months to 7 years and sampled during the period January to July.

	P.C.V.	Hb. gms/100 ml.	R.B.C. 10^6/cu.mm.	M.C.V. cu. μ	M.C.H.C. %
Mean	32.80	9.94	10.97	37.31	30.37
S.E.	0.39	0.10	0.19	0.44	0.20
Number of observations	194	192	47	44	189

Table XIV.

Leucocyte Counts for 121 sheep of ages ranging from approximately 2 months to 7 years and sampled during the period January to July.

	10^3/cu.mm. Total Leucocyte Count	LEUCOCYTES PER CU. MM. Neutrophil Bands	Polys.	Eosinos.	Basos.	Lymphos.	Monos.
Mean	7.62	20	2845	152	0.8	4241	431
S.E.	0.223	3.3	122.7	14.4	1.9	152.5	22.3

62d.

Table XV.

Erythrocytic Properties - showing previously published standards for normal sheep.

Authority.	Number of observations		P.C.V. %	Hb. gms/100 ml.	R.B.C. 10^6/cu.mm.	M.C.V. cu.μ	M.C.H.C. %	Month when sample
Allcroft (1941)	998	M.		11.5				-
		R.		9.5 - 13.5				
Becker & Smith (1950)	108	M.	37.9	12.41	11.9	32.0	32.0	Mch/
		S.E.	1.3	0.13	1.2	3.4	1.3	June/
Bennets & Beck (1942)	'Hundreds'	M.		9.0-- 16.8	7.6-13.3			-
Burnett (1917) *	-	R.		[illegible]	8.0-12.5			-
Coffin (1945)	-	R.	33-46	9.0-14.5	9.0-14.8	33.5-43.0		-
Dukes (1942) X	-	M.	31.0	11.18	8.12			-
Fraser (1930)	10	M.			10.345			-
		S.D.			1.57			
Holman (1944a)	98-114	M.	30.5	12.4	11.5	27.4	41.2	June
		S.D.	3.5	1.4	1.8	3.6	4.0	Decem
Innes & Shearer (1940)	48	R.	26-38		6.0-11.0	34 - 50		-
Kohanawa (1928)	12	M.			10.27			-
		R.			8.6-11.6			
Norris & Chamberlin (1929)	20	M.			11.6			April
		S.D.			1.3			Augu
Wirth (1950)	-	M.	32.5	12.0	8.0-13.0			-
Grunsell:								
All Ages	190	M.	32.8	9.94			30.37	Janu
	(approx.)	S.E.	0.39	0.10			2.20	to Ju
All Ages	45	M.	40.30	11.72	10.97	37.31	28.61	Janu
	(Approx.)	S.E.	0.573	0.125	0.190	0.440	0.233	only
Over 2 years	112	M.	33.98	10.15	11.10	37.62	28.87	Jan.
	(Approx.)	S.E.	0.50	0.16	0.232	0.561	0.28	x Jan
Under 2 years	78	M.	31.11	9.62	10.66	36.70	31.14	Jan/Ju
	(Approx.)	S.E.	0.58	0.16	0.252	0.633	0.24	x Jan.
X Quoting other authorities.								

M = Mean.

R. = Range.

S.D = Standard deviation.

S.E. = Standard Error.

been constructed showing the standards suggested by these authorities and in the case of Table XV. where it is known, the time of year when sampling took place is included. A summary of my results is included in the Tables.

a. Erythrocytes. Having demonstrated such marked variation due to season and age in the erythrocytic properties of my sheep, it is important when comparing my results with those of other authorities to have regard to the possible effects of these two factors. This is not possible in the case of most of the standards quoted, as no indication of the season of the year at which sampling took place is given by the author, and in these cases only my values for all sheep sampled can be used for comparison.

The most striking feature in a comparison of my results with those of other workers in respect of erythrocytic properties is that the mean figure for haemoglobin, calculated from the observation on all sheep irrespective of age or season, is lower than those of any other workers, and only if my highest levels are taken, i.e., from the January samplings (Table XII) of sheep over two years old (viz., 11.88 gm. per 100 ml) and compared with those of previous workers, is there any close agreement.

As only Holman (1944a) has published figures for Scottish hill sheep, it is with his results that mine may be most fairly compared. The main difference in the conditions under which sampling took place lies in the fact that his sheep were sampled from June to December and mine from January to July. Had his standards included the results of his examination of samples collected in the spring months, when he found his lowest erythrocyte levels, the means presented would/

would have been much lower, as he did not, it would be expected that his standards would be generally higher than mine. As far as Hb. and R.B.C. are concerned this is in fact the case, and it is only in the mean values for sheep over two years old, sampled in January (my highest levels) that there is any measure of agreement between our results. There is marked discrepancy however between my values for P.C.V. and those of Holman in that his are lower than mine, a fact that is reflected in the difference between our M.C.V. and M.C.H.C. standards. The explanation for this probably lies in the techniques of estimation. Holman's P.C.V. was carried out in small haematocrit tubes which were centrifuged for one hour at an unspecified speed, and it may be that a greater degree of packing was achieved by this method than was obtained in the Wintrobe tubes which were used in P.C.V. estimations in my investigations and which were spun for approximately 70 minutes at a speed of 3,000 r.p.m. If Holman's studies and the results of my observations are taken as complementary, it may be concluded that the erythrocyte values of Scottish hill sheep are generally lower from January to June than from June to December.

Innes and Shearer (1940), in the course of their investigations into 'Swayback' in the Midlands and Eastern counties of England, examined blood from normal sheep of all ages. As the result of these examinations they presented certain standards for the normal sheep. In comparing my results for sheep of all ages, sampled from January to July, with those of Innes and Shearer it will be seen that my mean value for P.C.V. lies in the middle of their range, and the fact that my mean for R.B.C. is nearer the maximum for their range need/

need cause no surprise as my mean value for this property was calculated from results obtained in January, when my sheep showed their highest erythrocyte levels.

Allcroft (1941) from the result of nearly 1,000 haemoglobin estimations gave 11.5 gm. per 100 ml. as the mean value for normal sheep. This figure is in close agreement with the calculated mean of 11.72 gm. per 100 ml. for the sheep sampled in January.

Inasmuch as Becker and Smith (1950) sampled their sheep between March and July, their results are comparable with mine for this period, but the fact that their sheep received supplementary feeding whereas mine were on natural hill pasture may account for their higher R.B.C. and Hb. values. Their high standard for P.C.V. is considered to be due to, at least in part, incomplete packing of cells as in their technique for P.C.V. estimation, although spinning continued for two hours the speed was only 1,900 revolutions per minute. In collaboration with E.W. Moodie (1951) the writer investigated the effect of speed of centrifugation on P.C.V. in sheep's blood, and found that it took four times as long at 2,000 revolutions per minute to get the same degree of packing of the cells as was achieved at 3,000 r.p.m.

My standard of R.B.C. falls midway between the maximum and minimum values suggested by Bennets and Beck (1942) as the result of a large number of examinations.

Detailed discussion of the standards presented by the other authorities quoted in Table XV. is not considered to be practicable as most are based on small or unspecified numbers of examinations made under unknown conditions. However, it will be seen that where values for erythrocyte counts are given the means do not differ greatly/

Table XVI.

Leucocytes - showing previously published standards for normal sheep.

Authority		Total 10^3 /cu.mm.	LEUCOCYTES IN THOUSANDS PER CU. MM. or % Neutrophil Bands	Neutrophil Polys.	Eosinos.	Basos.	Lymphos.	Monos.
Holman	Mean	9.2		24.0	4.2	0.5	67.3	2.3 %
	S.D.	3.1		8.7	4.5	0.5	9.6	2.7
Fraser	Mean	8.0		39.1	4.9	0.7	52.0	3.2 %
	S.D.	1.9		7.7	6.1	0.94	7.9	0.95
	Mean			3111	392	57	4155	245
	S.D.			949	537	78	1249	75
Norris & al	Mean	5.3		52.0	1.0		39.0	6.0 %
	S.D.	0.4		5.0			5.0	
Wirth	Mean			33.5	8.0	0.5	s 1 50 5	3.0 %
	Range	8.0-10		20-45	2-15	0-1	45-60 2-10	1 - 5
Kohanawa	Mean	10.45		31.8	8.7	0.02	57.7	1.8 %
	Range	8.95-12.8						
Dukes, Kuhl, Welsch, Fritsch	Mean	7.44		42.0	4.0	1	48.0	6.0 %
Burnett (Woltmann)	Mean	8.0		37.0	1.7	0.3	53.0	8.0 %
	Range			30 - 55	0.2 - 8	0 - 2	40 - 60	3 - 11 %
Coffin	Range	4.0-10.0		1.0-4.50	.05-.7	0 - 0.22	2.5-17.00	0.05-.8 Thou.
Innes & Shearer	Range	5.0-14.0						
Burnett(Bethe)	Mean	4.14						
Burnett(Filtner)	Range	5.3-11.9						
Burnett(Storch)	Mean	9.42						
Grunsell	Mean	7.62	20	2845	152	0.8	4241	431
	S.E.	0.223	3.3	122.7	14.4	1.9	152.5	22.3

s = small. l = large.

greatly from my standards calculated for the observations made in January.

In view of the importance of the indices M.C.V. and M.C.H.C. in the classification of anaemia, it appears extremely desirable that a standard technique for P.C.V. estimation in the sheep should be adopted. Most authorities agree on the wide variation likely to be found in M.C.V. in the sheep even with the same method of P.C.V. estimation, but when in addition to this normal variation different techniques of P.C.V. estimations are in use, comparison of results by different workers becomes impossible.

b. Leucocytes. The standards laid down by other authorities for leucocyte levels in sheep are contained in Table XVI. There is little to be gained from a comparison of values for W.B.C. and instead it is proposed to compare the standards laid down for D.C.L. From this Table it will be seen that with the exception of Fraser (1930) the D.L.C. is expressed as a percentage of the W.B.C. In order to compare the findings of these workers with my own, their percentage figures for D.L.C. have been converted into absolute values by reference to the W.B.C. mean, and the results of these calculations appear in Table XVII.

In the case of the results of most of the workers quoted, this calculation has been made by using the mean values for W.B.C. and D.L.C. The exceptions to this rule were in the case of Wirth (1950) and Coffin (1945). To calculate an absolute mean figure for the D.L.C. from Wirth's results the mean D.L.C. has been used, with the figure midway between the two limits of the range given for W.B.C. e.g., for neutrophil leucocytes, 33.5% of 9,000 i.e., midway between 8,000 and 10,000. Coffin's standards for D.L.C.

are/

are given as ranges, and for the figures quoted in Table XVII. a value midway between the two limits has been taken.

From Table XVII. it is seen that my value for neutrophil leucocytes is in fairly close agreement with Holman's figure, but below that given by the other authorities. This also applies to my figure for eosinophils. It is possible that this latter discrepancy may be accounted for by the low worm burden found in the sheep to which this value applied. The most striking feature in the Table is the wide variation in the mean values for lymphocytes. It is possible that this may be a feature of the sheep's blood and account for the widely different standards given by the various authorities for W.B.C. If in future in the statement of standards for D.L.C., absolute figures are used in preference to percentages, it may be possible to verify this point. The variation in the standards for monocytes may be due to a difference in criteria used by different workers for the classification of the large mononuclear appearing in the blood of sheep which has the characters of both monocyte and large lymphocyte.

Table XVII.

	Neutrophils	Eosinophils	Lymphocytes	Monocytes.
Grunsell	2,845	152	4,241	431
Holman	2,208	386	6,192	212
Fraser	3,111	392	4,155	245
Norris et al.	2,756	530	1,643	318
Wirth	3,115	720	4,950	270
Kohanawa	3,323	909	6,030	188
Dubos	3,125	297	3,571	446
Woltmann/Burnett	2,960	298	4,240	640
Coffin	2,750	425	12,250	400

Conclusions.

There was a sufficient measure of agreement between my results and those of previous workers to justify the conclusion that the sheep used in this investigation were representative of the species as a whole. It is however important to draw attention to the fact that as far as the erythrocytic properties are concerned the greatest measure of agreement between my results and those of others was evident when the values obtained in January in this investigation were used for comparison. This indicates that the lower erythrocyte levels found in April, June and July constitute a change which is probably pathological in nature.

SECTION II.

Bone Marrow Biopsy in the Sheep, and a description of the Histology of the Material obtained by this Technique.

Leitner (1949) in tracing the early observations of Naegeli and Schilling on the rôle of the bone marrow in the production of the cells of the peripheral blood, draws attention to the limitations imposed by the examination of material at autopsy. These limitations are due to the rapidity with which degenerative changes occur in the marrow cells. This led to the earliest attempts at bone marrow biopsy by Pianese (1905) and others. However, the techniques evolved were not suited to clinical investigation and it was not until Arinkin (1929) devised his sternal puncture method that bone marrow biopsy was established as a useful aid to diagnosis. Since 1929 various methods of biopsy have been evolved for the study of human bone marrow and these have been reviewed by a number of workers, notably Dacie and White (1949) and Osgood and Seaman (1944). In discussing the advantages of bioptic marrow sampling, Jones (1938) has laid stress on the value of a series of marrow samples for comparison with changes in the peripheral blood in the course of a disease, and has drawn attention once again to the avoidance of post-mortem changes in the cells when material is obtained in this way. In veterinary medicine this latter consideration is of great importance in view of the lapse of time which almost always occurs, particularly in the case of farm animals, between the death of the subject and autopsy. The value of bone marrow biopsy in diagnosis and in the study of functional pathology of diseases associated with blood changes in the domestic animals has been recognised and the/

the results of the examination of marrow obtained during life are available for the horse, cow, pig, and dog. In a search of the literature however no reference could be found to the bioptic sampling of marrow in the sheep, apart from an allusion to the iliac canal as a possible site in this species (Bloom, 1945).

The first bone marrow biopsies to be carried out on the horse were by Hjarre and Berthelsen (1938). These workers, using material obtained by sternal puncture, described the changes in the marrow in Infectious Equine Anaemia, correlating them with the reactions demonstrated in the peripheral blood. This was followed in 1943 by the publication by Hjarre of the results of the examination of material obtained by sternal puncture from the normal horse, cow, pig, and dog, ranges for the constituent cells of the marrow being presented. The success of sternal puncture as a means of obtaining marrow from the living bovine was also demonstrated by Holzel (1939) who from the examination of the material from fifty normal cattle, described the morphology of the cells of the marrow, and constructed myelograms showing the relative percentages of the cells present. Using the same technique, Marcato, in 1941, published the results of sampling twenty-seven cattle of all ages.

Marrow biopsy by means of sternal puncture presents certain practical difficulties in the larger domestic animals. In the first place, the depth of the tissue overlying the sternum necessitates the use of a long needle or trochar and canula, and the length of the needle must provide for a penetration of at least 20 cm. Secondly, even with the use of a local anaesthetic the operation will require a very effective method of restraint on the movement of the animal/

animal if the procedure is to be without danger either to the operator or the subject. These objections are met to some degree by an alternative technique described by Calhoun (1946) for marrow biopsy in the horse and cow. Samples of marrow were successfully obtained from seven horses and fourteen cattle by drilling the shaft of the eleventh rib at a level just below the Longissimus dorsi muscles, marrow being aspirated through a needle trochar of the same external diameter as the drill. The mean values for the Myeloid/Erythroid ratio calculated for the normal horse by Hjarre and Calhoun show some difference, the former worker's figure being 0.5: 1 and the latter 1.64: 1. The different nomenclature used in the construction of the myelograms by the two investigators makes comparison of their figures for the relative incidence of the individual cells virtually impossible. This same difficulty arises when attempts are made to compare the myelograms constructed for normal cattle by Hjarre, Holzel, Marcato and Calhoun. However, general agreement exists between the last three named workers as to the higher incidence in bovine marrow of the eosinophil series when compared with the normal values for the human; Hjarre did not differentiate these cells from the other members of the granulocytic series. In the case of the Myeloid/Erythroid (M/E) ratio Hjarre drew attention to the low figure found in the marrow of cattle as compared with man, suggesting a mean of 0.7 : 1 in contrast, for example, to the normal human mean given by Wintrobe (1946) of 4 or 5 : 1. The mean values or ranges for the M/E ratio for normal cattle calculated by Holzel, Marcato and Calhoun were 1.7 : 1, 0.47 : 1 to 0.96 : 1 and 0.67 : 1 respectively, thus confirming the difference between/

between cattle and human marrow reported by Hjarre. The morphology of the individual cells in the bovine marrow as described by Holzel and Marcato is essentially similar to that given for the human counterparts. Holzel found however a pale zone separating the nucleus and cytoplasm to be a constant feature in the erythroid cells classified by him as macroblasts. These cells as judged by the description given of their nuclear structure and staining affinity of cytoplasm represent the intermediate normoblasts of Israels (1948) or basophilic erythroblasts of Maximow and Bloom (1938) and Wintrobe (1946); no record of this zone occurring as a constant feature in the human cell can be found, moreover, it is not described by Marcato in his account of the marrow of cattle.

The successful use of sternal marrow puncture reported by Hjarre in 1943, to which reference has already been made, was preceded in 1940 by the description of a technique using the ileum as the site by Ho, Chu, & Yuan (1940). A gauge 17 needle of $2\frac{1}{2}$ inches in length was used and the site for the puncture was the longitudinal midline of the ileum about 1 cm. below the crest. From samples obtained, using Ho's technique, Meyer and Bloom (1943) described the morphology and percentage incidence of the cells of the marrow of ten healthy dogs (Bloom & Meyer, 1944). Figures for the total white cells per cu. mm. of marrow blood were also given.

An alternative method for serial marrow biopsy in the dog was described by Mulligan (1941). This technique, which is designed for the quantitive study of marrow, consists of the resection of 2.5 cm. of rib. The portion of rib so removed is squeezed and the marrow exudate emulsified with homologous plasma - smears being made from the resultant suspension. The remainder of the rib

segment/

segment is then available for sectioning. Details of the results of the examination of material collected in this way were given by the author in respect of 35 adult dogs and 4 puppies.

From a general consideration of the investigations already carried out on the use of marrow biopsy in the domestic animals certain conclusions may be drawn. The usefulness of the sternum as a site for marrow biopsy in animals has been confirmed. The presence of cellular red marrow in this bone at all ages in the human has been demonstrated by Custer and Ahlfeldt (1932). Their findings appear to have been confirmed in respect to cattle (Marcato, 1941) and dogs (Meyer and Bloom, 1943), and although Custer and Ahlfeldt (1932) found the vertebra to contain a higher percentage of active marrow, the fact that the sternum is more readily accessible, at least in the smaller domestic animals, suggests that the sternum is the site of choice. Marked species variation is apparent from an examination of the respective myelograms presented, and before any clinical use can be made of marrow biopsy, it is necessary for the normal range for the species in question to be determined. Comparison of the results of different investigators is hampered by the lack of a standard nomenclature, and the adoption of a more widely recognised system is required. In view of the extent of the information available in the human species, a system generally used in human haematology would seem preferable. Because of the wide numerical variation to be expected in the cells of normal peripheral blood in most species of domestic animals, the inclusion of erythrocyte and leucocyte counts carried out at the time of marrow biopsy would seem desirable, if only in the hope that by correlating the

blood/

blood and marrow pictures it may be possible to understand the variations seen in the normal blood picture. Finally, where possible, the most detailed information concerning breed, age, sex, (with details of pregnancy where applicable), and state of nutrition should be available in respect of each individual examined.

Reference has already been made to the absence of any previous account of marrow biopsy in the sheep and until 1951, when the writer published the results of the examination of the marrow obtained by sternal puncture from ten normal sheep (Grunsell, 1951), British veterinary literature contained no description of the application of the technique to the domesticated animals. Furthermore, no comprehensive account of the cells of the marrow of the adult sheep could be found, although the role of the bone marrow in haematogenesis in foetal sheep has been described by Goodall (1907).

In the succeeding pages in this Section a full description of the technique of marrow biopsy in the sheep by sternal puncture is presented, together with an account of the morphology of the material obtained using this method. The object of including this full description of the cells encountered in the marrow was to ensure that as the standards presented in this thesis may form a basis of comparison for the results of future investigators, no confusion should occur on the question of nomenclature.

Technique of Sternal Puncture as a Means of Marrow Biopsy in the Sheep.

Anatomy.

From a dissection of the sternum of an adult Cheviot ewe the following anatomical description of the bone was made.

The sternum of the sheep is a median segmental bone consisting of seven sternebrae united to each other by cartilage. The anterior extremity of the manubrium is formed by the first sternebra which is elongated and narrow with a free tuberous extremity. It is curved upwards and is palpable. The posterior extremity or xiphisternum is formed by the last sternebra; it is elongated, narrow and flattened and has a well-defined xiphoid cartilage, and may also be palpated. The body is composed of five remaining sternebrae, which are distinctly flattened and increased in width towards the posterior extremity. The ventral surface of the body is superficial and between the first and second sternebrae there is usually a well-defined prominence. The lateral surfaces of the body are narrow and bear at the junction of adjacent sternebrae, concave facets for articulation with the cartilages of the sternal ribs.

The technique adopted for the operation of sternal puncture followed closely that described by Israels (1948) and others for the human.

Equipment.

The equipment for bioptic sampling of marrow from the sternum was as follows:-

1. 2% Procaine solution,
2. 2 ml. Syringe, needles - bore 1 mm. length 4 cm.
3. 1 ml. Syringe.
4. Salah needles - bore 1.5 mm. length 4 cm.
5. 3.8% Sodium citrate solution.
6. Watch glasses.
7. Fine pointed forceps,
8. Slides - clean and grease free.

The results given in this section are from the examination of marrow from the 3rd sternebra, but it was found that puncture of the 2nd, 4th, and 5th sternebrae was equally satisfactory.

The sheep was restrained in a sitting position by an assistant, who stood behind the sheep to support it and held a foreleg in each hand. The third sternebra was located by following the third rib down to its articulation with the sternum. The wool was clipped from the part and the skin cleansed with spirit. The skin, subcutaneous tissue, and periosteum of the 3rd sternebra was then infiltrated with 1 - 1.5 ml. Procaine 2%.

The Salah needle and syringe to be used for aspiration were sterile and dry. Immediately before the puncture was made, both syringe and needle were washed out with 3.8% Sod. citrate solution, to prevent clotting occurring in syringe or needle during the operation (Davidson, Davis & Innes, 1943).

With the stilet in situ and the needle held at right angles to the body of the sternum, a puncture was made as near the centre of the 3rd sternebra as possible. The needle was pushed through the skin and subcutaneous tissue until it could be felt and heard against the bone. Penetration of the bone to a depth of approximately 0.5 cm./

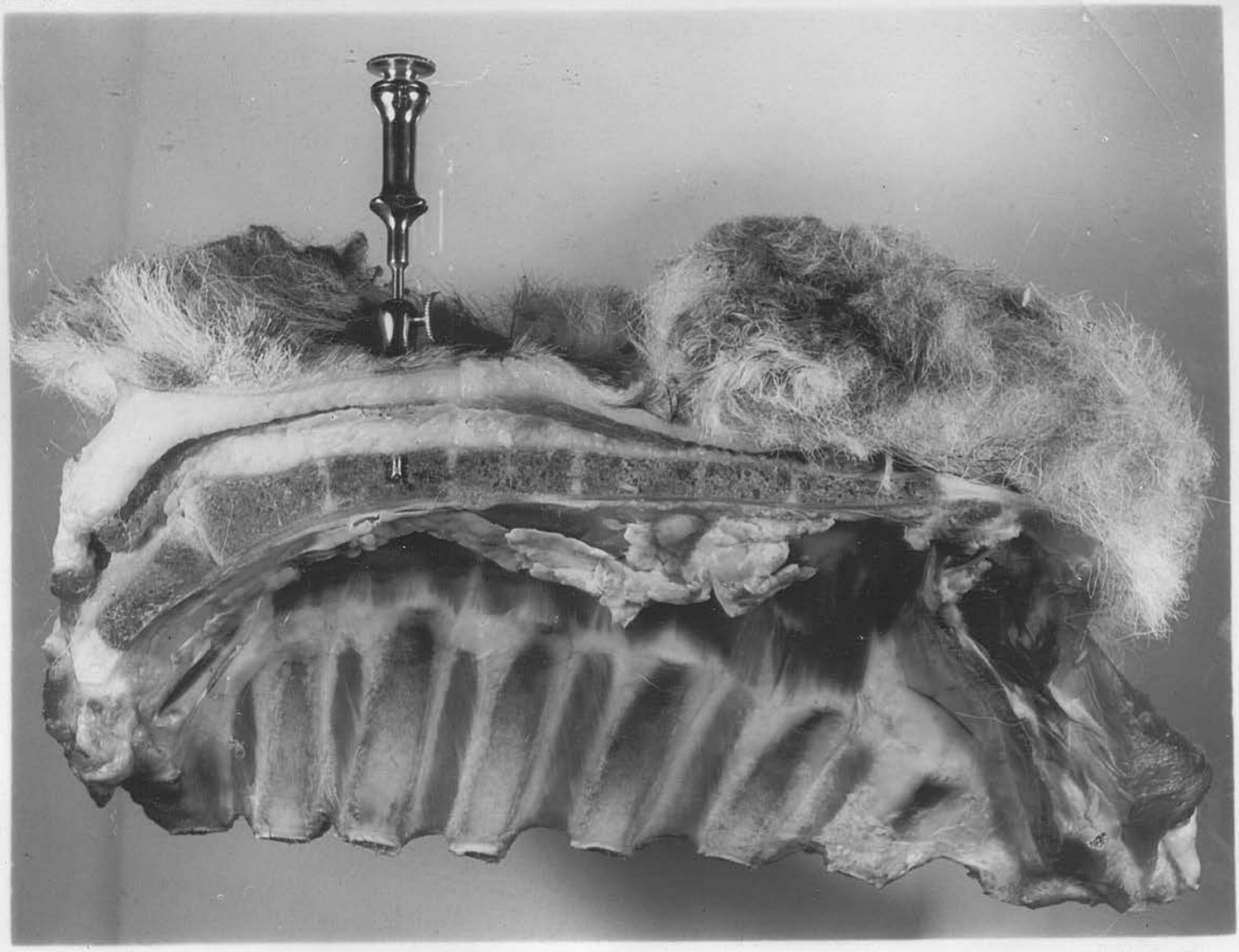


Fig. 6. Longitudinal section of sheep's sternum with Salah needle in position for marrow aspiration. The movable guard shown here was used in the earlier stages of the development of the technique to restrict sternal penetration to approximately 0.5 cm.

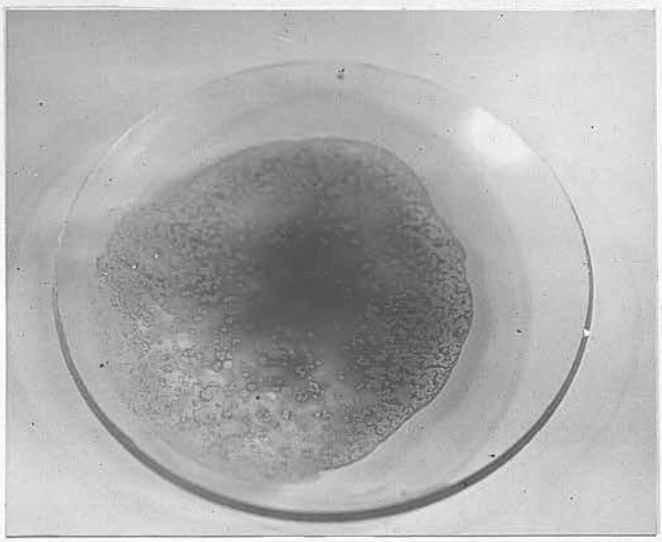


Fig. 7. Watch glass showing marrow flecks adhering to the glass after the removal of marrow blood.

0.5 cm. was achieved by a screwing movement, and in most cases a sudden reduction in resistance could be felt as the narrow cavity was entered. (Fig. 6). The depth of penetration measured from the skin varied from 2.5 - 3.8 cm. The stilet was withdrawn and the syringe attached to the needle. Gentle suction was applied until approximately 0.3 ml. of marrow fluid had been withdrawn. The syringe was next detached and the contents expelled on to a watch-glass; when the blood was poured off, the marrow flecks could be seen remaining adherent to the watch-glass. The flecks varied from 20 or 30 to several hundreds in number, and measured from 0.5 to 4 mm. in size. (Fig.7).

Marrow spreads were prepared at once according to the technique described by Davidson et al. This consisted in transferring four or five flecks by means of fine pointed forceps to one end of a slide. These flecks were then crushed and spread by means of another slide held at right angles to the first. (Fig. 8). In order to damage the cells as little as possible, it was found very important to exert only enough pressure to make a good spread of the tissue. Considerable practice was required before satisfactory preparations could be made. At least six spreads were made from each aspirate.

No after treatment was necessary, apart from the removal by means of a swab of a small amount of blood which exuded from the hole following the withdrawal of the needle.

Staining.

The spreads were air-dried and defatted in chloroform for 30 seconds, and staining was then as follows:-

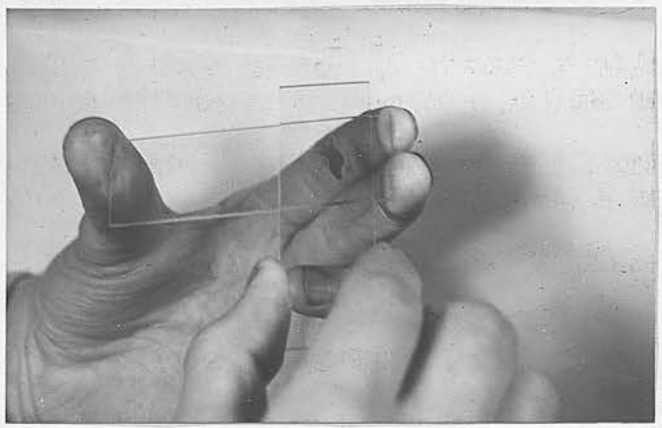


Fig. 8. Preparation of marrow spread. The fleck is shown in the process of being crushed. The spread will be made by moving the upper slide to the left, thus spreading the crushed fleck.

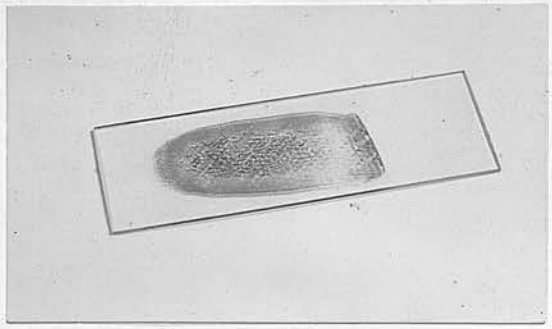


Fig. 9. Stained marrow spread, showing crushed fleck in the centre surrounded by marrow blood.

1. Undiluted Leishman (Revector)..... 2 minutes

2. Dilute Leishman - 1 part Leishman to 2 parts distilled water..... 6 minutes.

3. Dilute Leishman removed by tilting slide - no washing .....

4. Dilute Giemsa (freshly prepared) - 1 part Giemsa to 9 parts distilled water ..... 8 minutes.

5. Washed and differentiated with distilled water ... 2 minutes.

Using this technique, marrow spreads (Fig. 9.) were obtained from six adult sheep, which were sampled at the local abattoir prior to slaughter. Post-mortem examination of these animals showed them to have no gross pathological lesions, the organs and viscera being passed for human consumption.

Scheme of Nomenclature for Marrow Cells (Israëls, 1948)

Haemocytoblast: the large, basophil precursor of erythroblasts, granuloblasts, lymphocytes and monocytes.

A. Erythroblasts: includes all differentiated nucleated red cell types.

a. Pro-erythroblast: earliest differentiated erythroblast-precursor of the normoblasts.

b. Normoblasts: differentiated on nuclear structure into:-

i. Early normoblast.

ii. Intermediate normoblast.

iii. Late normoblast.

B. Granuloblasts: includes all cells of granulocyte series found in the marrow.

a. Myeloblast: most primitive member of the granulocyte series.

b/

b. Promyelocyte: earliest granulated cell of the series, granules not yet differentiated.

c. Myelocyte: early differentiated granulocyte, appears in three forms - with neutrophil, eosinophil, and basophil granules.

d. Metamyelocyte: intermediate form between myelocyte and cells with lobulated nuclei. Cytoplasm may contain either neutrophil, eosinophil, or basophil granules. Subdivided into:-

i. Juvenile metamyelocyte: nucleus indented but showing no aggregation of chromatin.

ii. Band or stab form metamyelocyte: nucleus in the form of an S, U, or W, and with aggregation of nuclear chromatin.

e. Polymorphonuclear leucocyte: granulocytes with segmented nuclei and cytoplasm containing neutrophil, eosinophil, or basophil granules.

C. Plasma cells: includes plasmablasts, immature and mature plasma cells, nucleus usually eccentric in position and having characteristic disposition of chromatin in nucleus.

D. Megakaryocytes: including megakaryoblasts, immature and mature megakaryocytes - precursors of platelets.

E. Reticulum cells: wandering macrophages with primitive nuclear structure and ill-defined outline.

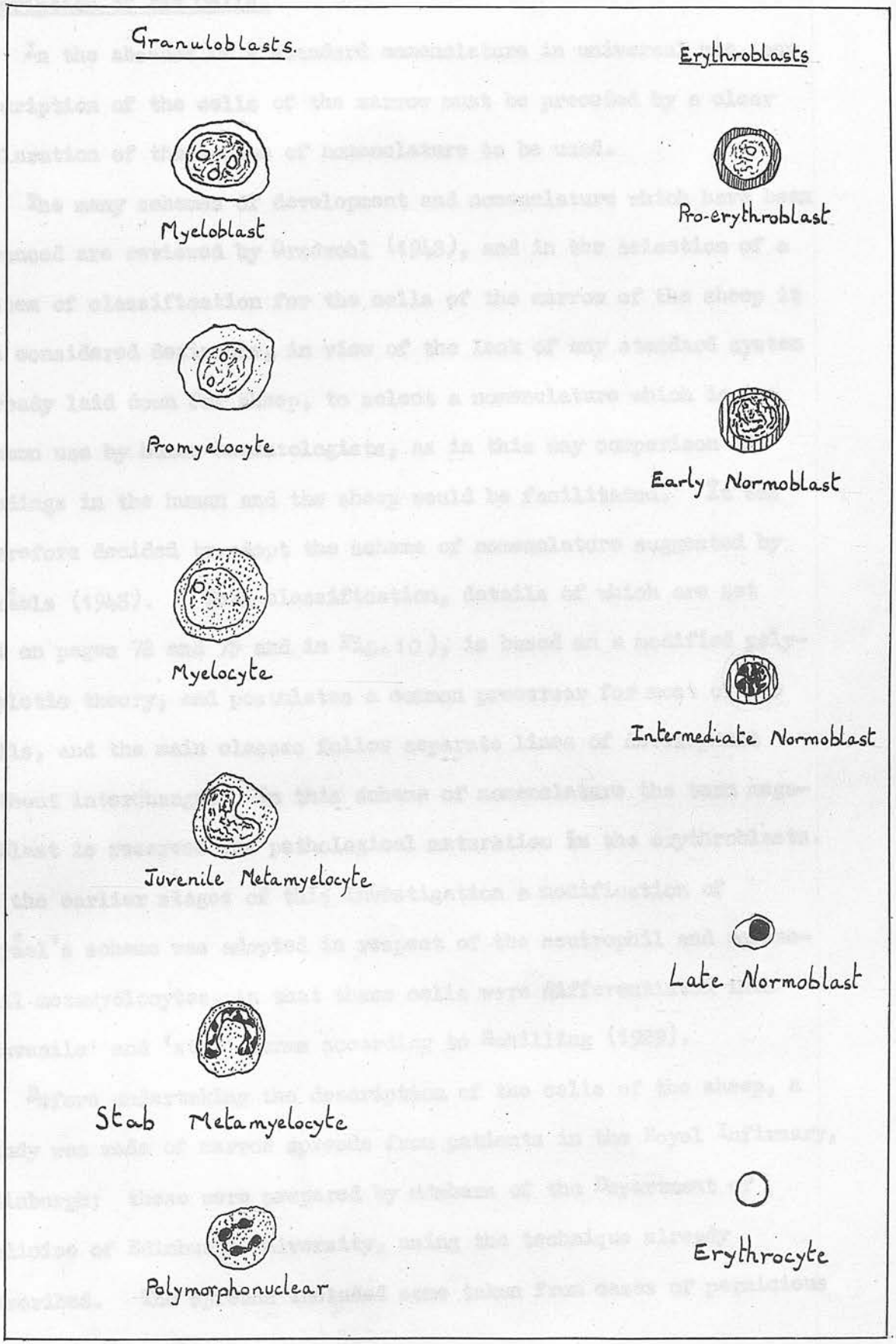


Fig. 10. Diagram showing scheme of nomenclature adopted in respect of Granuloblasts and Erythroblasts.

Description of the Cells.

In the absence of a standard nomenclature in universal use, any description of the cells of the marrow must be preceded by a clear declaration of the scheme of nomenclature to be used.

The many schemes of development and nomenclature which have been advanced are reviewed by Gradwohl (1948), and in the selection of a system of classification for the cells of the marrow of the sheep it was considered desirable, in view of the lack of any standard system already laid down for sheep, to select a nomenclature which is in common use by human haematologists, as in this way comparison of findings in the human and the sheep would be facilitated. It was therefore decided to adopt the scheme of nomenclature suggested by Israëls (1948). This classification, details of which are set out on pages 78 and 79 and in Fig. 10), is based on a modified polyphyletic theory, and postulates a common precursor for most of the cells, and the main classes follow separate lines of development without interchange. In this scheme of nomenclature the term megaloblast is reserved for pathological maturation in the erythroblasts. In the earlier stages of this investigation a modification of Israël's scheme was adopted in respect of the neutrophil and eosinophil metamyelocytes, in that these cells were differentiated into 'juvenile' and 'stab' forms according to Schilling (1929).

Before undertaking the description of the cells of the sheep, a study was made of marrow spreads from patients in the Royal Infirmary, Edinburgh; these were prepared by members of the Department of Medicine of Edinburgh University, using the technique already described. The spreads included some taken from cases of pernicious anaemia/

anaemia in relapse, and from this material experience of the morphology of the cells classified as megaloblasts was obtained.

The description of the cell morphology which follows was based on the examination of spreads prepared from the six normal sheep to which reference has already been made (see page 78). In the case of the myeloblast, promyelocyte, proerythroblast, plasma cell and reticulum cell, at least 100 cells were examined, while in the case of the other cells of the marrow, the description is from the examination of at least 1000 cells.

Cell Measurement.

Haemocytoblasts, pro-erythroblasts and all cells younger than metamyelocytes in the Granuloblast series, were measured by micrometer eyepiece. In the case of the remainder the images were projected in a dark room by a prism on to a sheet of paper and measured by a ruled scale. This latter procedure was adopted in the case of the more easily recognisable cells for its greater accuracy and because it caused less eye strain than measuring by micrometer eyepiece.

The assumption that the marrow cells of the sheep would resemble their human counterparts in general morphology was based on the findings of previous workers (Holzel, 1939; Hjarre, 1942, Marcato, 1941) on the marrow of the domestic animals, and particularly those dealing with cattle, the anatomy and physiology of which are acknowledged to be very similar to the sheep. It was found that the classification adopted for the human species was applicable to the cells found in the marrow preparations from the sheep; and no cells were seen which it was not possible to classify on this basis.

Haemocytoblast. Few of these cells were encountered. They were recognised as having an open pink network of chromatin in the nucleus, which contained up to five nucleoli. The nucleus occupied the greater part of the cell and was irregular in shape. The ragged cell outline enclosed a pale grey-blue cytoplasm devoid of any inclusions. The size of the cell varied from 20.0 μ to 45.0 μ. with a mean of 33.0 μ.

Myeloblast. The nucleus was composed of a reticulum, which tended to be more aggregated and slightly more basophilic than in the case of the Haemocytoblast. Nucleoli were always visible and up to five in number. The nucleus was irregularly round in shape, varying somewhat in the extent of the cell it occupied. In about one-third of the cells examined, the nucleus was surrounded by a pale area marking it off from the pale blue cytoplasm in which no inclusions were visible. The size varied from 18.3 μ to 22 μ with a mean measurement of 19.18 μ.

Promyelocyte. The morphology often resembled the myeloblast closely, although there was a tendency for it to be larger than its precursor. Nucleoli were visible. Differentiation of this cell from the myeloblast was possible by the presence of fine red granules which could be seen dusted all over the nucleus and cytoplasm. The size varied from 16.6 μ to 28.2 μ with a mean measurement of 19.60 μ.

Neutrophil Myelocyte. The nucleus was observed to occupy from one-half to three-quarters of the cell and was usually situated to one side of the cell, being surrounded by a narrow rim of cytoplasm for about half its diameter, and having a wider area of cytoplasm opposite/

opposite the remainder of the diameter. The structure of the nucleus was often obscured by the granulation, but those parts of the nucleus which could be seen showed it to be finely reticular and containing up to five nucleoli. In some cells a slight indentation of the nucleus was discernable, and this always occurred in the part of the outline of the nucleus opposite the wider rim of cytoplasm. The cytoplasm, like the nucleus, was largely obscured by the granulation, but it was often visible near the edge of the cell, when it was seen to be pale blue in colour. The granules in this cell were of two types; there were azure granules which could be seen plainly in the cytoplasm and over the surface of the nucleus, but they were not so numerous as the other type of granule, which appeared as a colourless outline, through which it appeared possible to make out the pale blue underlying cytoplasm, or pink nucleus, depending on the situation of the granule in the cell. The staining affinity of these granules requires more detailed consideration. Ehrlich (1879) in his earliest description of the specific granules of the granulocytes, used a 'triple stain' and if the granules were stained by neither acid nor basic components, but only showed affinity for the neutral stain they were classified as neutrophil. According to Cowdry (1938) this phenomenon is only evident in man and some of the higher apes, whilst in most animals there is a tendency for these granules to stain with the acid dyes, when they are termed pseudo-eosinophilic. Bunting (1938) has stated that with the eosin-methylene blue stains the granules showing an affinity for the neutral dye appear as purple or lilac coloured structures. This is not the case in the granules under consideration in the sheep where, as has been stated/

stated, neither acid nor basic components colour the granules, which are left as colourless structures only rendered visible by the defraction of the light round their margins. Because they exhibit neither basophilic nor acidophilic affinity the term neutrophil, in the strictest sense of the word, appears to define most logically their staining property. These neutrophilic granules were very numerous, almost completely obscuring the nucleus. In cells in which there was any tendency to indentation of the nucleus, it was frequently found possible to see a well marked cytocentrum appearing as a clear zone in the cytoplasm opposite the nuclear indentation.

Neutrophil Metamyelocyte.

a. 'Juvenile' Metamyelocyte. In the younger ('juvenile') metamyelocyte the nucleus was frankly indented, giving the nucleus a kidney shape. There was little or no change in the disposition of the chromatin of the nucleus as seen in the myelocyte, but nucleoli were never present, and the nuclear outline was much more clearly visible, because the granules encroached much less on the nucleus, being more confined to the cytoplasm. The cytoplasm contained numerous neutrophil granules, and occasional azure granules, the latter being much less frequent than in the myelocyte. Where visible, the cytoplasm was seen to be sky blue in colour.

b. 'Stab' form metamyelocyte. The term 'stab' form was used for those cells in which the nucleus showed aggregations of chromatin, and was in the form of a narrow band. This was often in the shape of a horse-shoe or letter "S", and in some cases was twisted upon itself. The staining was strongly basophilic, being purplish red/

red in colour. The cytoplasm in these cells was colourless and the granules were only faintly visible. It appeared that maturation considerable shrinkage of the granules had occurred and they were indistinguishable from the granules seen in the adult cells encountered in the peripheral blood. The size of the neutrophil metamyelocytes varied from 10.0μ to 23.0μ with a mean of 14.63μ. (These figures include both Juvenile and Stab forms).

Lobulated Neutrophil Granulocyte. The morphology of these cells was identical with that of the lobulated neutrophil granulocytes seen in the peripheral blood. The chromatin was aggregated into clumps, which were dark purple in colour. (Fraser, 1930; Holman, 1944a) and others). In some cells there was advanced segmentation of the nucleus with four or five lobes being present.

Eosinophil Myelocyte. The nucleus in these cells occupied one-half to three-quarters of the cell and, like the neutrophil myelocyte, was only very rarely centrally situated, being usually pushed to one side. The disposition of the chromatin in the nucleus was similar to that observed in the neutrophil myelocyte. Nucleoli were seen in the nuclei of some of these cells, but the presence of granules covering the nucleus made their detection in most cells difficult. It seems likely that nucleoli were in fact present in a high proportion of the eosinophil myelocytes, as where the preparation of the spread had caused partial rupture of the cell and the granules were stripped off the nucleus, four or five nucleoli were almost always plainly visible. The cytoplasm varied in colour from slatey blue to pale blue, the latter being more common. The granules of these cells were brightly stained, and it was possible in some cells to distinguish/

distinguish four types of granule. The largest and predominating granule was a bright golden colour and in some cells as many as 135 such granules were counted. Much less numerous were the azure granules, which were of two sizes; the larger type occurring singly and being almost equal in size to the golden granules already described; the smaller sized azure granules showed a tendency to be grouped together. The colour of these granules was, of course, bright red. The third form of granulation seen in these cells was pale blue in staining reaction, and equal in size to the eosinophil golden granule. It was much less frequent in occurrence than the golden forms but was always present. The fourth type of granule, which, unlike the foregoing, was not a constant feature, showed an affinity for the basic component of the stain, and appeared dark purple in colour. These basophil granules, when present, were never numerous and only five or six at the most were visible in any one cell. The cytocentrum, which was such a frequent feature of the neutrophil myelocyte, was never seen in its eosinophil counterpart. The size of these cells varied from 11.0μ to 40.0μ with a mean measurement of 20.57μ.

Eosinophil Metamyelocyte. As in the case of the neutrophil metamyelocyte, it was possible to divide this stage of maturation into juvenile and stab forms. The nuclear structure was the same as the neutrophil counterpart, and the cytoplasm, in the case of the juvenile form, retained its basophilic staining property. In the case of the stab form this pale blue colouration was lost, the cytoplasm forming a colourless background for the bright eosinophil granules. In both stages of maturation only two forms of granule were/

were present. These were the golden granule and the larger azure granule, the former being much more numerous than the latter. The size of the eosinophil metamyelocyte ranged from 7.8μ - 15μ with a mean of ~~10.84μ~~ 15·87μ. WRONG. See Lynnee (1951) p.22.

Lobulated Eosinophil Granulocyte. The only difference observed in this cell as compared with the stab form consisted in the lobulation of the nucleus, which was segmented into as many as five lobes in some instances.

Basophil Granulocytes. The stages of maturation already described for the neutrophil and eosinophil series were only differentiated with difficulty in the basophil granulocytes. This difficulty was due to the fact that in almost all cells, nucleus and cytoplasm were almost completely obscured by the dark purple granules. In the few cells in which nucleus and cytoplasm were visible, both appeared to agree in staining affinity and structure with their neutrophil and eosinophil counterparts. Nucleoli were not observed in the basophil myelocytes. The granulation was exclusively basophilic and in the myelocytes two sizes of granule were recognisable.

Proerythroblasts. The proerythroblast, which is the earliest precursor of the erythrocyte, was seen to be a round cell which was composed largely of a darkly basophilic nucleus centrally placed in the cell. The chromatin was disposed in coarse purple strands, and the nucleus characteristically contained one to four nucleoli. The cytoplasm was seen as a dark blue narrow rim round the nucleus, and because of the strongly basophilic affinity of nucleus and cytoplasm it was often difficult to make out clearly the line of demarcation/

demarcation betwixt nucleus and cytoplasm. The cell was easily distinguishable from the stem cell haemocytoblast by being smaller and more regularly round, and more generally basophilic. In some cases however it was difficult to decide if the cell under observation was a proerythroblast or a myeloblast. This fact caused no surprise when consideration was given to the fact that the two cells represented comparable stages in the maturation process of their respective series. This difficulty was the exception rather than the rule and usually differentiation was made possible on the following characters. The proerythroblast tended to be smaller in size, with the nucleus occupying more of the cell. The reticulum of the nuclear chromatin in the proerythroblast was coarser and more basophilic than was the case in the myeloblast. The lighter blue of the cytoplasm of the myeloblast made the nuclear membrane in the cell more plainly visible than in the proerythroblast.

The size of this cell varied from 11.6μ to 19.9μ with a mean of 15.60 μ .

Early Normoblast. The nucleus in the early normoblast was seen to occupy most of the cell, being centrally placed. The chromatin was disposed in distinct whorls, and was much less basophilic than was the nuclear chromatin of its precursor, making the colour more pink than reddish purple. No nucleoli were ever seen. The nucleus was always clearly marked off from the cytoplasm and in half the cells examined a clear zone in the cytoplasm was noted surrounding the nucleus. The cytoplasm consisted of a narrow ring varying from deeply basophilic to polychromatophilic in staining affinity. The size varied from 9.1μ to 19.0 μ with a mean measurement of 13.40 μ.

Intermediate Normoblast. As in the case of its precursors, the nucleus occupied roughly the centre of the cell, the outline of which was difficult to determine in some cells. As has been described, the identification of erythroblasts, at the intermediate normoblast stage of maturation, was based merely on the evidence of any aggregation of chromatin in the nucleus. This resulted in great variation in the nuclear structure observed. Thus in the earlier members of the group the general morphology of the cells resembled the early normoblast, whilst in the later members the nuclear structure, although still reticular and therefore belonging to the intermediate stage, could also be described as somewhat pyknotic and forecasting in this respect the nuclear appearance of the late normoblast. The nuclear pattern consisted of a dark purple reticulum of thick bands of chromatin, sometimes in whorls, sometimes disposed like the spokes of a wheel. There appeared to be great depth to the nuclear structure and through the dark purple reticulum a light pink background could be seen in many cells. The pale area of cytoplasm surrounding the nucleus, described as occurring in some early normoblasts, was seen in approximately 25% of the intermediate normoblasts examined. In staining affinity the cytoplasm showed variation from lightly basophilic to polychromatophilic, the latter being more common than the former. A study of the staining affinity of the cytoplasm in one hundred intermediate normoblasts showed that 25% of the cells had basophilic cytoplasm, the remaining 75% being polychromatophilic. It was also found that the intermediate normoblasts showing the more juvenile structure almost all had the basophilic cytoplasm, whereas in the older cells the/

the polychromatophilic cytoplasm was much more common. Thus, the colour of the cytoplasm appeared to bear some relationship to the age of the cell. In no case was a cell, which by its nuclear structure was classified as an intermediate normoblast, found to have a fully haemoglobinated or orthochromatic cytoplasm. There was a marked variation in the range in size seen in this cell, being from 5.8μ to 17.0μ with a mean of 9.19μ.

Late Normoblast. Only cells having completely pyknotic nuclei were classified as late normoblasts. No structure was visible in the nucleus of these cells, and in many cases no cytoplasm could be seen. Where the cytoplasm was visible, there was a tendency for the cell membrane to be ragged, and the cytoplasm was almost invariably polychromatophilic in staining affinity. Fully haemoglobinated cytoplasm was only very rarely seen and then in cells in which the nucleus was reduced in size to about 1μ. The mean size of these cells was 5.27μ with a range of 4.0μ to 9.0μ.

Erythrocytes in the marrow showing punctate basophilia and polychromasia.

Fully haemoglobinated erythrocytes, being equal in size to erythrocytes in the peripheral blood, were frequently seen to show punctate basophilia. Much less frequently polychromatophilic erythrocytes were observed, and here the cells were larger than the adult erythrocytes and appeared more fragile in that rupture and curling of the cell membrane was common.

Plasma Cells. The plasma cells were recognised as darkly basophilic cells with a round eccentric nucleus. The chromatin of the nucleus was clumped, often being cartwheel in disposition, and deeply basophilic in affinity. Nucleoli were only seen in nuclei having/

having a more primitive structure of loosely reticular chromatin, which was not stained as deeply as in the more mature cell. The nucleus in these immature cells was often less eccentric in position. In all stages of maturity, the nucleus occupied one-third to one-half of the cell, the remainder in the case of mature cells being composed of a royal blue cytoplasm in which vacuoles were often visible. In the less mature cells the cytoplasm was slatey blue in colour. With very few exceptions, all plasma cells contained a pale unstained zone in the cytoplasm usually lying against the nuclear membrane, giving the impression of a cone of light, the origin of which was below the nucleus.

Reticulum Cells. These polymorphic cells always showed a well defined nucleus, and even when the preparation of the spread had caused considerable disruption of the cytoplasm, the nucleus remained intact. The nucleus was round or oval in shape, having a fine reticulum of pink chromatin, often showing nucleoli. The cytoplasm was seen to be smoky grey in colour and often contained fat droplets and green staining pigment. On numerous occasions several darkly pyknotic structures, which were indistinguishable from the nuclei of late normoblasts, were seen as cytoplasmic inclusions. In one cell 24 such inclusions were noted.

Megakaryocytes. These cells, by reason of their greater size as compared with the other cells of the marrow, were easily seen under the 16 mm. objective. The nuclei were always deeply basophilic and the chromatin was disposed in streaks and clumps. In the majority of the cells the nucleus was lobulated. The degree of lobulation varied from a faintly visible division of the nucleus/

nucleus to a stage when the lobes were frankly separate and distinct from each other. Occasional cells were seen in which there was no division of the nucleus into lobes. These nuclei and those in which lobulation was just starting, were surrounded by a zone of homogeneous dark blue cytoplasm, which separated the nucleus from the wider area of cytoplasm which was speckled and presented a granulated pink appearance. In cells in which the lobulation of the nucleus was distinct, the cytoplasm was very extensive, being consistently granular and pink in colour. In these cells it was common to see all types of marrow cell 'caught up' in the cytoplasm. The size showed great variation ranging from 31.0 μ to 107 μ.

Lymphocytes and Monocytes. It was not possible to identify any cells as immature lymphocytes or monocytes, although the mature forms of both series were observed. Their morphology was that of the lymphocytes and monocytes of the peripheral blood as described by Fraser (1930), Holman (1944a) and others.

Haematogones. A number of small cells were noted in which the nucleus was flat and structureless and appeared as a round red structure. Often no cytoplasm could be seen, but when present it formed a narrow pale blue ring round the nucleus. These cells were classified as haematogones.

Discussion.

There has been no previous systematic description of the cells of the marrow of the sheep, with which the results of this examination could be compared; however, in view of the fact that Fraser (1930) found the morphology of the leucocytes in the peripheral blood in sheep and cattle to differ only in minutae, it was considered/

sidered justifiable to compare the description of marrow cells in the sheep as given above with that of cattle made by previous investigators. The only accounts of the morphology of bovine marrow cells available are by Holzel (1939) and Marcato (1941).

Granulocytes. On the subject of the nuclear structure of the granulocytes close agreement was found between the findings here presented and those of Holzel and Marcato, and it was on the question of granulation that differences were noted. Holzel describes the myelocyte as having lost the azure granulation seen in its precursor, the promyelocyte, which does not agree with the findings of Marcato, who, in agreement with the account given above, found azure granules to persist in the myelocyte, and only to be lost at the metamyelocyte stage. This difference may possibly be accounted for by the fact that Holzel included as myelocytes cells in which there was indentation of the nucleus, which under the nomenclature adopted in this thesis would be classified as metamyelocytes. The staining affinity of the neutrophil granule described for the sheep confirms the findings of both Holzel and Marcato for cattle; they declare the granule to be truly neutrophilic and staining with neither acid nor basic components of the stain. While Marcato observed two types of granule in the eosinophil myelocyte, an azure and an eosinophilic granule, Holzel found only the latter, and neither of these authorities was able to differentiate either the pale blue granule or the basophilic granule which have been described above, in this cell in the sheep. The presence of basophilic granules among those which stain with acid dyes in eosinophil myelocytes is well known, according to Ringoen (1938), and has been associated with the ripeness of the granules./

granules. In a description of the eosinophils of the bone marrow and haemoly mph node of the sheep, Ringoen states that he believes that the eosinophilic granule undergoes an evolutionary process, during which the staining passes from basophilic to polychromatophilic and then to eosinophilic. As has been stated above, the basophilic and pale blue granules were only observed in the eosinophil myelocytes and were never seen at the metamyelocyte stage, a fact which may support Ringoen's theory.

Erythroblasts. Both Holzel and Marcato classify the maturation stage of erythroblasts on the degree of haemoglobination of the cytoplasm, while in this report age was judged on nuclear structure. This made the comparison of results difficult. However, both workers describe a normoblast having a densely pyknotic nucleus, which would be a late normoblast according to Israel's classification, with a completely haemoglobinated cytoplasm. This degree of haemoglobination was never observed in the late normoblast of the sheep's marrow. That this difference may be a species characteristic, and not merely due to staining technique, was supported by the fact that in my preparations the adult non-nucleated erythrocytes of the marrow blood were pink in colour and showed no tendency to overstaining with the basic component of the stain.

The clear area seen separating the nucleus and cytoplasm in some early and intermediate normoblasts, in the sheep, was described by Holzel as occurring in the corresponding cells in cattle, as a constant feature. Jones (1948) has shown, using phase contrast ± techniques that this clear zone is not a true hyaloplasm, but due

to/

to the unstained negative images of mitachondria.

Dacie and White (1949) have stressed the point that progressive diminution in size, and pyknosis of the nucleus of the erythroblast is a more reliable guide to cell maturity than is the state of ripening of the cytoplasm. This method of cell ageing seems particularly well suited to the erythroblasts of the sheep, where the degree of cytoplasmic ripening varies so widely from cell to cell, irrespective of age as shown by the nuclear structure. Furthermore, the cytoplasm of the more mature erythroblasts appears very fragile, so that in spread preparations it may be very scanty or completely absent.

Punctate Basophilia and Polychromasia. The presence of erythrocytes showing punctate basophilia in the marrow is not recorded by Holzel and Marcato as occurring in cattle. Key (1924) and Sperahsky and Sklianskaja (1928) have denied the existence of stippled cells in normal bone marrow. On the other hand, Naegli (1904) reported the converse. Although the exact nature of the basophilic material is as yet undetermined, according to Wintrobe (1946) the most commonly accepted view is that it represents the remains of the spongioplasm of the erythroblast, and is not the result of nuclear degeneration. Polychromasia is believed to be related to punctate basophilia (Wintrobe, 1946) and according to Farmer and Maizels (1939) the relative incidence of the two forms is dependent on the duration of staining and fixation. Furthermore, Cooke (1929) has shown that punctate basophilia, polychromasia, and reticulation can be produced in any immature cell, the three forms being merely degrees of the same phenomenon. It appears therefore that/

that from the demonstration of punctate basophilia in some of the erythrocytes in the marrow spreads examined, no conclusions can be made regarding the method by which the nucleus of the erythroblast in the sheep is lost. On the other hand, the incidence of punctate basophilia in the erythrocytes of the peripheral blood of normal adult sheep is extremely low, and Fraser (1930) was unable to find any such cells in lambs over three days old. It must therefore be assumed that the presence of punctate basophilia indicates a state of immaturity, through which the erythrocyte passes before liberation to the peripheral blood. Support for this assumption is found in the records of punctate basophilia occurring in the red cells of the peripheral blood as part of the response of the erythropoietic tissue to simple blood loss. (Wirth, 1938; Fourie, 1931).

Other Marrow Cells. The description of the adult plasma cell recorded above agrees with that made by Marcato and Holzel for the cells in the bovine marrow, except that no mention is made by Holzel of the perinuclear halo so commonly seen in plasma cells in sheep marrow. Neither authority describes the immature forms, but this may be due to their relative infrequence in normal marrows (Israels, 1948).

No mention is made by Marcato of the reticulum cell in bovine marrow, but Holzel describes the cell as occasionally present. His general description of the cell agrees with mine, except that he compares the cell's size to that of the megakaryocyte. Although Holzel gives no measurement figures for his cells, from the measurements recorded for the megakaryocyte in the sheep in this study, it would appear that the reticulum cell in the marrow

of/

of cattle is very much larger than that in sheep. The frequent finding of inclusions in the cytoplasm of these cells in the sheep confirms the accepted view that these cells are actively phagocytic. The fact that round pyknotic structures, closely resembling the nuclei of late normoblasts, were commonly seen in the cytoplasm suggests that at least in some cases the nucleus of the late normoblast may be lost by extrusion; alternatively it may be that these nuclei represent the remnants of degenerate late normoblasts which have never reached maturity. In a number of reticulum cells the pyknotic inclusions were seen to be changing in colour from black to dark green, and it may be that the green pigment so frequently seen in these cells originated from the fragmentation of disintegrated nuclei of late normoblasts.

Megakaryocytes. The descriptions given by Holzel (1939) and Marcato (1941) of the megakaryocyte in bovine marrow agree closely with my own for the sheep. Holzel was able to see nucleoli in the cells showing no lobulation in their nuclei, but in my examinations no nucleoli were seen. Holzel, in agreement with my findings, noted other marrow cells appearing as inclusions in the cytoplasm of the megakaryocytes. He concluded that either they had been actively phagocytised by the megakaryocyte, or that the other marrow cells were superimposed on the megakaryocyte. There is some difference of opinion as to whether the megakaryocyte is actively phagocytic or not. According to Rosenthal (1938), some workers claim to have shown that it is, but others attribute the appearance of cells in the cytoplasm of the megakaryocyte to the viscous property of the cell which causes other cells to adhere to

it/

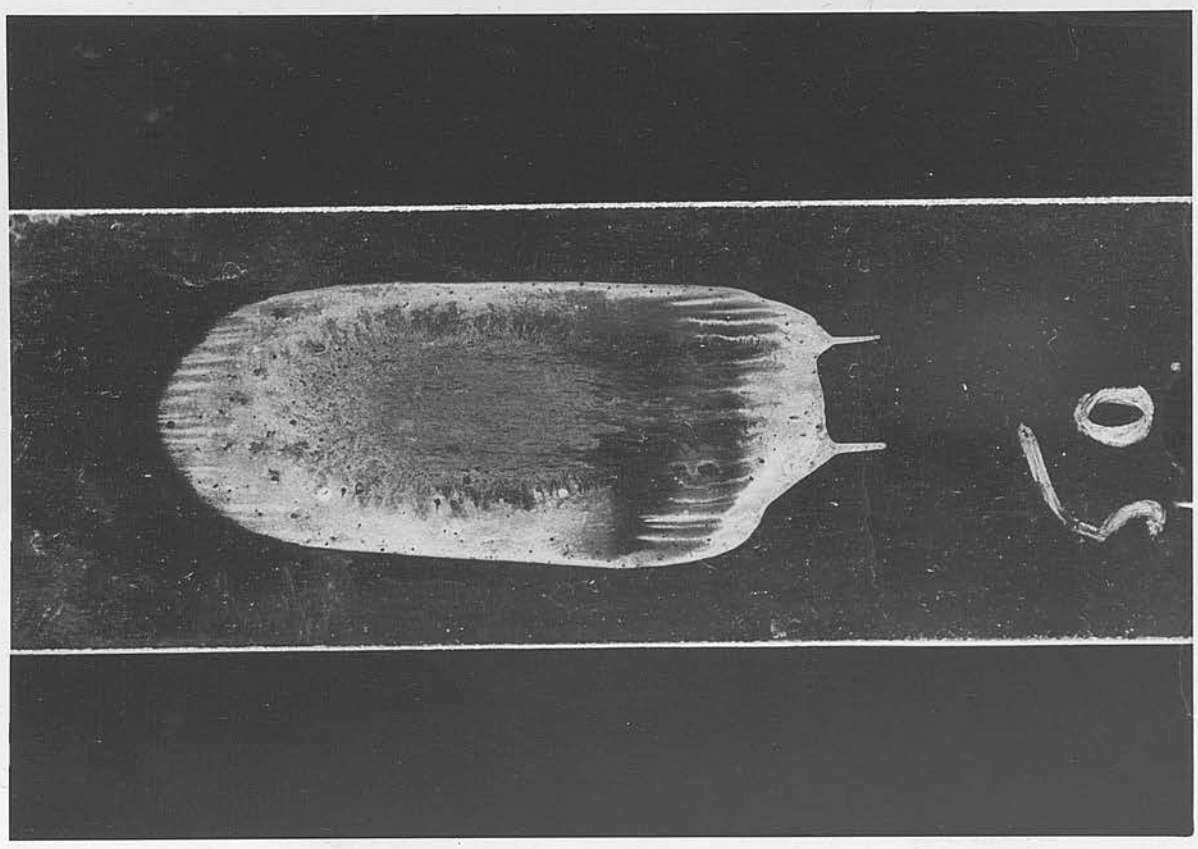


Fig. 11. Showing general appearance of marrow spread.

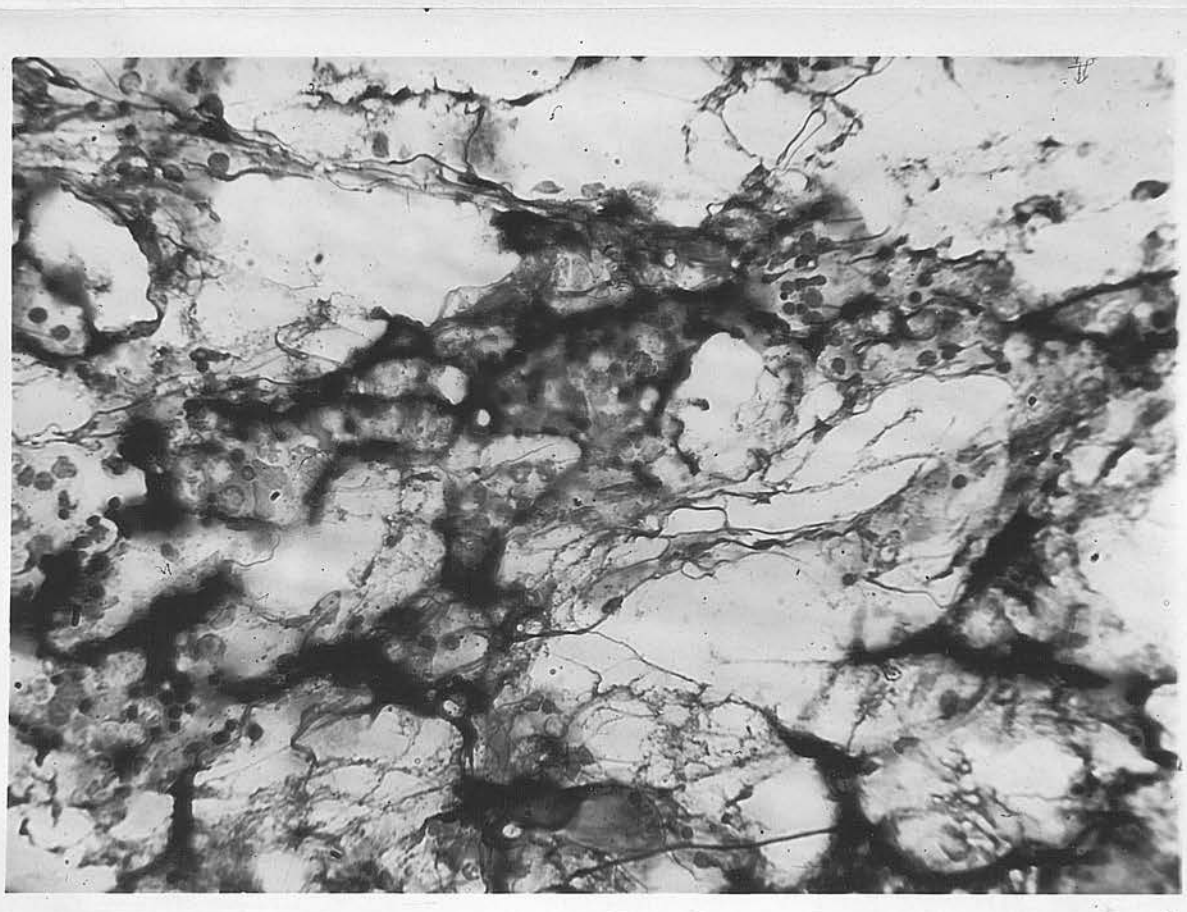


Fig. 11a. Showing reticulum in centre of marrow spread. X 350.

it in spread preparations.

General Appearance of the Marrow Spread. (Fig. 11)

Examination of the stained marrow spread under the x 2/3 objective showed that the centre of the spread was occupied by a loose syncytium enclosing clear spaces; some groups of cells were observed to be lying in columns in the walls of the syncytium, but the majority of the cells were collected round the borders of the central loose mesh work. In all spread preparations an area of the spread was composed of non-nucleated erythrocytes, among which were scattered marrow cells, and along the borders or edges of the spread marrow cells were closely grouped. There was a tendency for the erythroblasts to occur in wedges of up to fifty cells.

When the central syncytium was examined under 1/12 oil immersion objective, it was found to consist of connective tissue framework in which could be seen at irregular intervals, small primitive nuclei with a finely reticular chromatin; both fibrils and nuclei stained dark red. Fig. 11a. This description is in agreement with that made by Orsos (1927) as the result of a study of the fibrillar structure of normal and pathological marrow.

The Methods employed in the Examination of Marrow Samples obtained by Sternal Puncture Biopsy.

Spreads.

The method of spreading the marrow flecks has already been described. The differential cell count was based on the examination of at least 1,000 cells. In almost all spreads there were areas in which the preparation of the spread had disturbed cell morphology, but it was always found that a sufficient number of cells was recognisable/

recognisable to make a differential count possible. Every attempt was made to select groups of cells near the centre of the spread for examination, and good fields were frequently found bordering the marrow reticulum. These areas were chosen because it was considered that the minimum disturbance of marrow architecture was likely to have occurred here, and consequently the cells would be found in the nearest proportions to those pertaining in life. During the differential cell count a record of the cells seen in mitosis was made. The stages differentiated were prophase, metaphase, anaphase, and telophase. (Maximow & Bloom, 1938). From the results of the differential cell counts, haemomyelograms (Pontoni, 1936) were constructed showing the incidence of the various cells expressed as a percentage.

The ratio of the granuloblast to the erythroblast elements was calculated on the basis of erythroblast elements as unity, and is presented as the Myeloid/Erythroid ratio, hereafter known as the M/E ratio. In the construction of the haemomyelogram, and the calculation of M/E ratio mature granulocytes were included. Dacie and White (1949) state that a better expression of the relative proportions of leucopoietic to erythropoietic tissue is obbained by exeluding the mature leucocytes, because they believe that their presence is due to admixture with blood. Leitner (1949) disputes this view and was able to demonstrate mature granulocytes in appreciable numbers in the marrow in cases of agranulocytosis, where they were completely absent from the peripheral blood. Justification for their inclusion as true marrow cells in the sheep is possible from the fact that in the peripheral blood they are at least/

least equalled and generally exceeded by the lymphocytes, whereas in the marrow, lymphocytes are of comparatively rare occurrence compared with the mature granulocytes.

In the case of the neutrophil and eosinophil granuloblasts, and of erythroblasts, the distribution of the cells at different stages of maturity was expressed as a maturation curve for each of the three series (Pontoni, 1936).

Cellularity estimation of Marrow Spreads.

The information to be obtained from haemomyelograms, M/E ratios, and maturation curves is limited by the absence of any quantitative estimate of marrow picture. In the presentation of the results of marrow examination in human medicine, a statement of degree of cellularity is included, the terms normal, increased, and decreased being used to denote grades of cellularity. The limitations of such a classification are obvious and Propp and Schwind (1944) devised a method of grading the cellularity of marrow films by comparing their cellular content with that of blood films. Thus, Grade 1 had approximately the number of cells found in peripheral blood films; Grade 2, 4-5 times and Grade 3, 10-12 times the number. In Grade 4 marrows there were more nucleated cells than erythrocytes. The fact that my preparations were crushed marrow flecks rather than films of marrow blood made the classification devised by Propp and Schwind inapplicable. It was therefore considered desirable to evolve a method of grading cellularity as seen in marrow spreads.

It was noted that the crushing of marrow flecks caused a characteristic disposition of the cells, and reference has already been made to this under the heading of "general appearance of the marrow Spread".

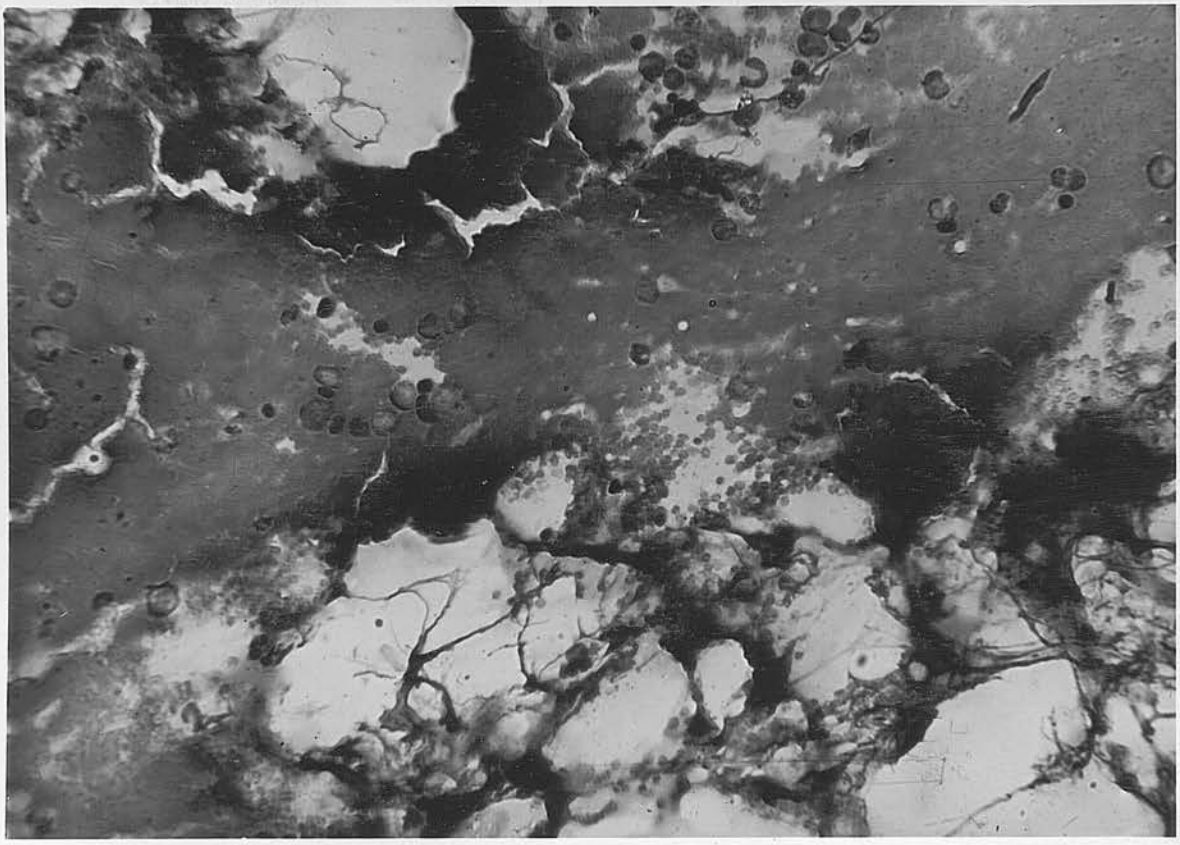


Fig. 12. Cellularity Grade O. X 350.

Fig. 13. Cellularity Grade I. X 350.

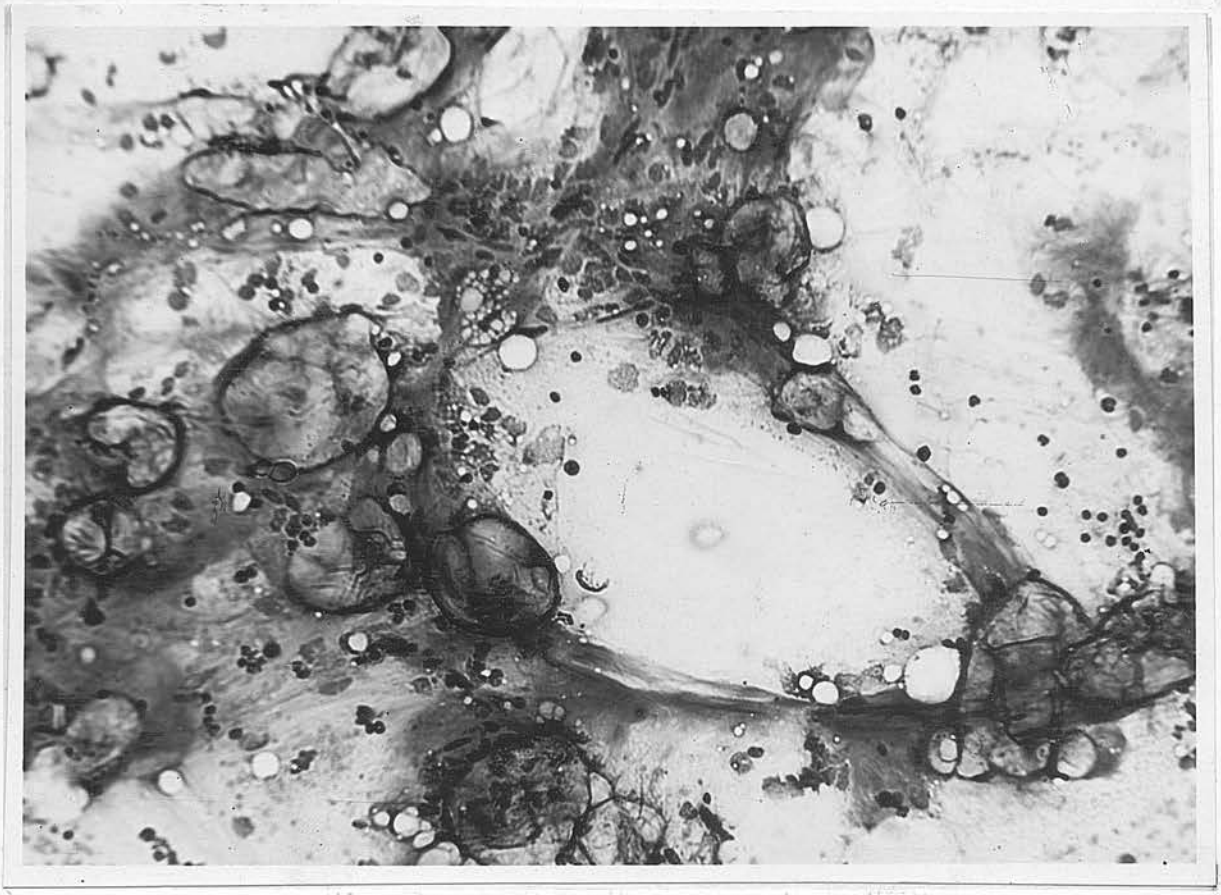


Fig. 14. Cellularity Grade II. X300.

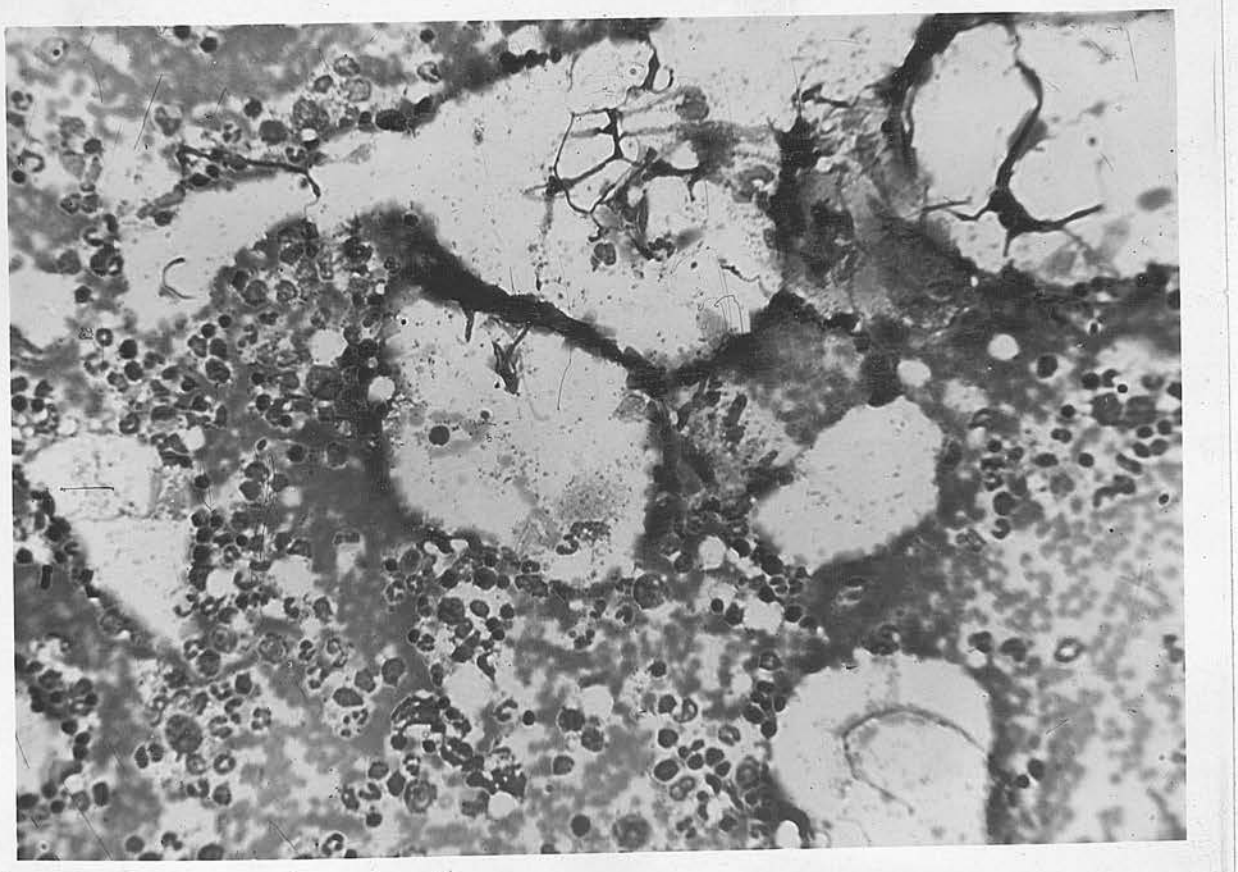


Fig. 15. Cellularity Grade III. X400.

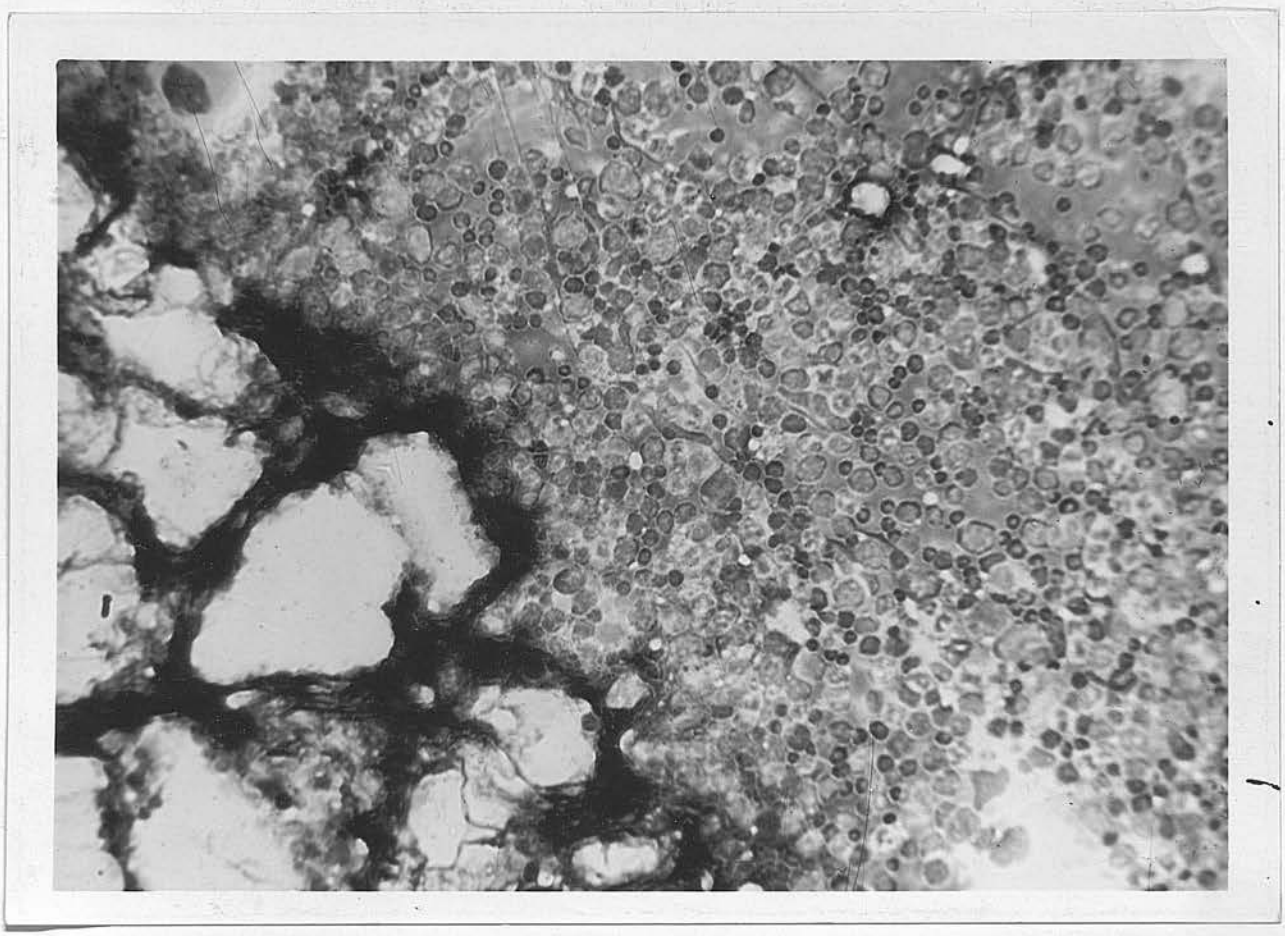


Fig. 16. Cellularity Grade IV. X 400.

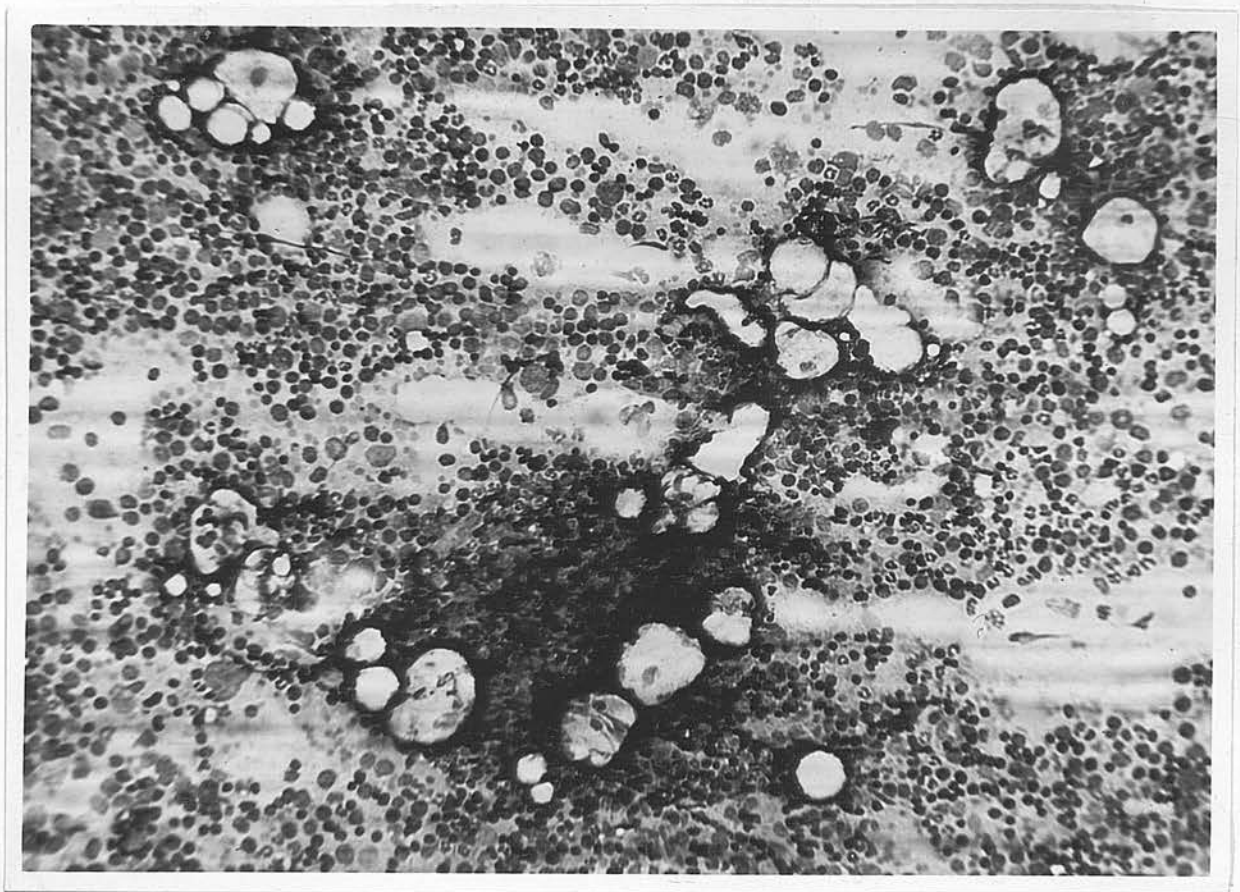


Fig. 17. Cellularity Grade V. X 300.

The estimation of cellularity was carried out by inspection under the 2/3 objective, of the zone of cells which surround the central loose stroma of the syncytium. In spreads in which the cells were very scanty in this zone, the edge of the spread was also examined for density of cellular concentration.

The cellularity was graded from 0 to V. the criteria used being as follows:-

Grade 0. Cells very sparse or absent, both round the reticulum and along the edges of the spread.

Grade I. Cells sparse round reticulum but fairly plentiful along the edges of the spread.

Grade II. Cells round the reticulum in a density comparable with that of leucocytes along the edges of a normal blood film.

Grade III. Cells fairly dense around the reticulum but not touching each other and with no tendency to be in the interstices of the stoma.

Grade IV. Cells in dense masses round the reticulum and actually touching each other, and appearing in columns in the interstices of the stroma.

Grade V. Whole spread showing great cellularity as noted for Grade IV. with the addition of dense masses of cells appearing in the stroma of the reticulum instead of narrow columns as found in Grade IV.

Grades 0 and V. were only found in hypo- and hyperplastic marrows respectively.

The six grades of cellularity are illustrated in Figs. 12-17.

Nucleated cell counts in marrow blood.

Quantitive cell counts were not made on all samples of marrow collected, but when carried out the technique used was as follows:-

a. As has been described, the 0.3 ml. of marrow aspirate which was withdrawn through the Salah needle was expressed on to a watch glass.

b. After a delay of about ten seconds to allow the marrow flecks to sediment on to the watch glass, approximately 0.25 ml. of marrow blood was transferred, by means of a pipette, from the watch glass to glass thimbles having a diameter of 5 mm. and a capacity of 0.45 ml. These thimbles contained ammonium and potassium oxalate in the proportions recommended by Wintrobe (1946) as an anticoagulant for peripheral blood. The marrow blood was mixed with the oxalate by repeated inversion of the thimble.

c. Using a Sahli pipette, 0.02 ml. of marrow blood was transferred to another glass thimble for dilution with 0.06 ml. of 3% acetic acid.

After thorough mixing, the diluted marrow blood was further diluted 1/20 with acetic acid in a 'white' Thoma pipette and loaded into the counting chamber of an improved Neubauer slide.

d. The count was made according to the method used for leucocyte counts in the peripheral blood.

e. The resulting figure, after the adjustment made necessary by the dilutions, was recorded as the total nucleated cell count per cu. mm. of marrow blood.

f. All cell counts on marrow blood were made within four hours of collection.

Preparation of Histological Sections from Marrow Flecks.

In the case of a number of the marrow biopsies to be described later in this thesis, histological sections were cut from the flecks contained in the aspirate. The method employed was based on a procedure described by Campbell (1948) for the histological preparation of bone marrow particles.

i. The marrow flecks required for a spread preparation were first removed from the watch glass. By means of a pipette as much blood as possible was withdrawn, so as to leave the remaining marrow particles adhering to the watch glass.

ii. Fixation, which was carried out within five minutes of the collection of the sample, was by corrosive formol (10% formolin in saturated aqueous mercuric chloride), and methyl alcohol in equal parts. Half an hour was allowed for fixation, at the end of which time the fixative was decanted and the tissue gently washed with distilled water.

iii. The fixed marrow particles were now removed by fine pointed forceps to a glass slide, where they were aggregated into a small conical mass.

iv. As much water as possible was then withdrawn from the tissue by means of filter paper.

v. A glass ring of 5 mm. inside diameter and 15 mm. deep, and having a flat ground lower rim, was now placed round the aggregated particles. The end of the ring in contact with the slide had been previously coated with a thin layer of vaseline.

vi. Into the ring, 2-2½ % agar at approximately 50°C. was pipetted to a depth of about 8 mm.

vii/

vii. The agar set within five minutes with the tissue embedded at the end of the glass ring nearest the slide. The agar block could now be pushed out of the glass ring, trimmed and, following dehydration, mounted in solid paraffin.

viii. Sections were cut at 4 μ.

Staining was by haematoxylin and eosin, the staining times being: haematoxylin,5 minutes and eosin, 2 minutes.

It was not possible to study the details of morphology of individual cells in the sections prepared in this way, but from them an overall estimate of marrow activity could be obtained, and their use in furnishing confirmatory evidence in suspected hypoplasia is described in Section VII.

Discussion of the techniques of examination of marrow obtained by bioptic methods.

In a discussion of the technique of sternal puncture as a method of marrow biopsy Leitner (1949) has outlined the sources of error. The first is a general criticism applicable to all sampling, namely that the material obtained is not necessarily truly representative of the tissue as a whole in the body. The second source of error applies to the aspirate itself, and is due to the fact that the technique of collection alters the material in such a way as to reduce its value as a true sample of the tissue from which it comes.

The first source of error depends on the lack of homogeneity of the bone marrow. Helpap (1937), Reiter (1938) and Domanus (1937) have demonstrated considerable difference in the cellular composition of the marrow in different sites in the human. On the other hand/

hand, Stasney and Higgins (1939) found the fluctuation in the marrow composition in different parts of the body to be within narrow limits, and considered the source of error due to lack of uniformity was not great. Leitner is in agreement with this latter contention, which is supported by the results of his examination of marrow from sternum, ribs, and vertebrae in human autopsies immediately after death. It may well happen however that the sample obtained is not truly representative of the tissue as a whole, but where results are at variance with peripheral blood findings and with clinical signs, further sampling is possible, as Stodmeister and Buchman (1939) have shown that repeated punctures do not influence haemopoiesis.

The sources of error inherent in the aspirate itself are due to dilution with blood and the fact that the more primitive cells have been shown to adhere to the stroma (Damashek, Henstell and Valentine, 1937). This criticism will apply particularly to preparations made by smearing drops of marrow blood, and less to the methods which make use of marrow fragments. Davidson (1941), in a description of his technique (details of which have been given in Section II. as being the technique used throughout my investigations) maintains that an accurate assessment of prevailing cell types may be made by this technique, with little contamination by marrow blood. It has already been shown in this thesis (page 98) that these flecks are indeed units of marrow tissue composed of a stroma of loose fibrous syncytium, in which appear columns of marrow cells.

The unavoidable statistical errors involved in making differential marrow counts are reviewed by Osgood and Seaman (1944). These authors state that no differential cell count can be considered accurate/

accurate unless twenty examples of the specific cell type under consideration are counted. As far as the more primitive cells are concerned this would entail a considerable addition to the length of time required to carry out a differential marrow count, and it is doubtful if the added accuracy so obtained would be justifiable in a technique designed as a routine procedure to aid diagnosis, and in which only gross departures from the normal can be considered significant.

Dacie and White (1949) drew attention to the difficulty experienced in comparing the figures of different authors, due to the impossibility of accurately dividing into arbitrary classes cells in which every gradation of development is seen. This difficulty is most noticeable in the classification of erythroblasts, but I consider that the adoption of a uniform technique in the preparation of material for examination and a clear statement of the criteria upon which cells are separated, coupled with the adoption of a standard nomenclature, would considerably reduce the variation shown in the figures for cell incidence among different workers.

Note. The photomicrographs shown in the following pages as Plates 1 to 7 are black and white exposures of colour transparencies which appear in the original thesis and therefore some of the features mentioned in the legend adjoining the plates are not discernable. It was not possible to make a black and white copy of the reticulum cell (Plate 5.A)

Plate 1.

The field illustrates four stages in the maturation of the granuloblastic series - myeloblast, neutrophil myelocyte, neutrophil metamyelocyte, and neutrophil polymorphonuclear. The colourless neutrophil granules are well shown in the myelocyte and the prescence of a cytocentrum may be observed opposite the 'hilus' of the nucleus. The field also includes a pro-erythroblast and it is thus possible to compare the morphology of the youngest differentiated precursors of the granuloblastic and erythroblastic series. Both cells show nucleoli. (X 2,000)

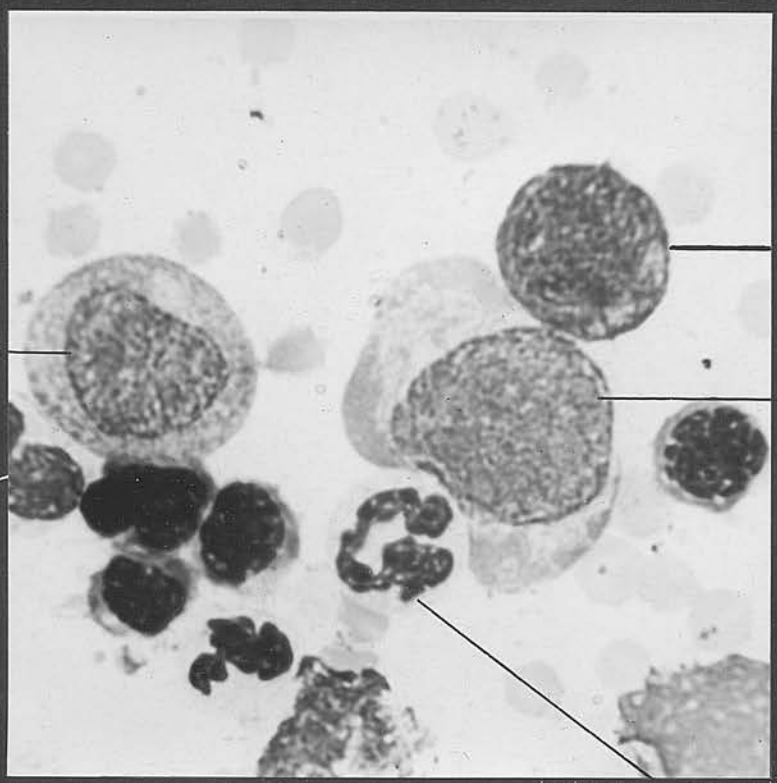

Pro-erythroblast.
Neutrophil Myelocyte
Myeloblast.
Neutrophil metamyelocyte
Neutrophil polymorphonuclear.

PLATE 1.

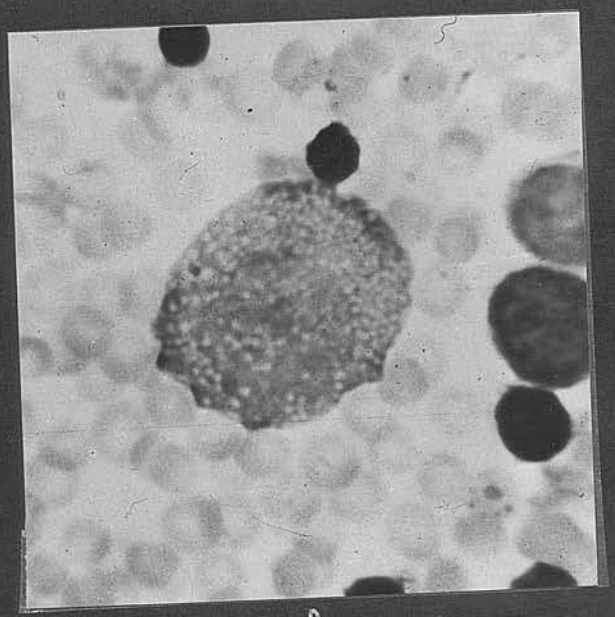
Plate 2.

A. The centre of the field is occupied by an eosinophil myelocyte. The granules are seen to be overlying the nucleus. Four types of granule may be differentiated - the golden eosinophil granule, the pale blue granule, the azure granule and the basophil granule. The blue cytoplasm is in evidence behind the granules. (X 1,800)

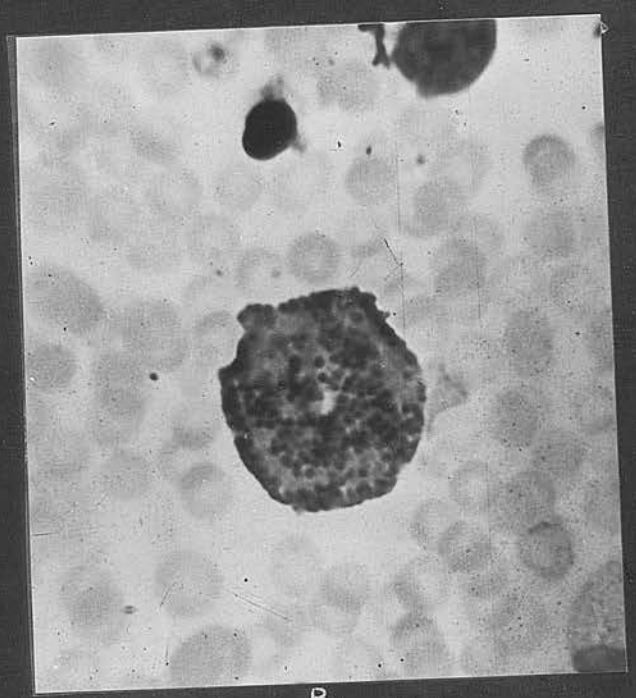
B. This 'transparency' illustrates the basophil metamyelocyte. The dark granules are the predominant structures in the cell obscuring detailed morphology. The field also includes an eosinophil metamyelocyte and an intermediate normoblast in the prophase stage of mitosis. (X 2,000)

C. The basophil myelocyte shown in this field is atypical in that it is possible to appreciate more cell detail than is usually evident in these cells as seen in marrow preparations. On the other hand the basophil meta--myelocyte represents more accurately the form in which this series is usually encountered. (X 2,500)

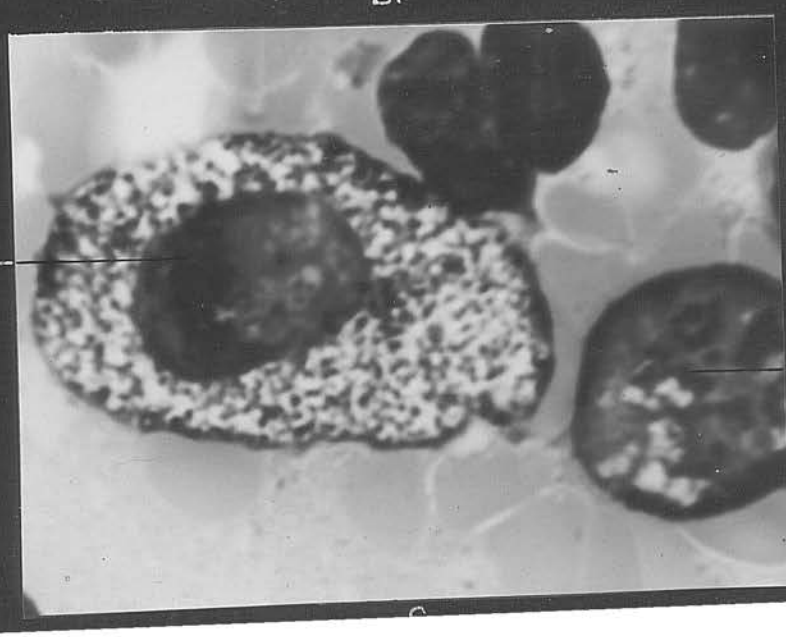
PLATE 2



A.



B.



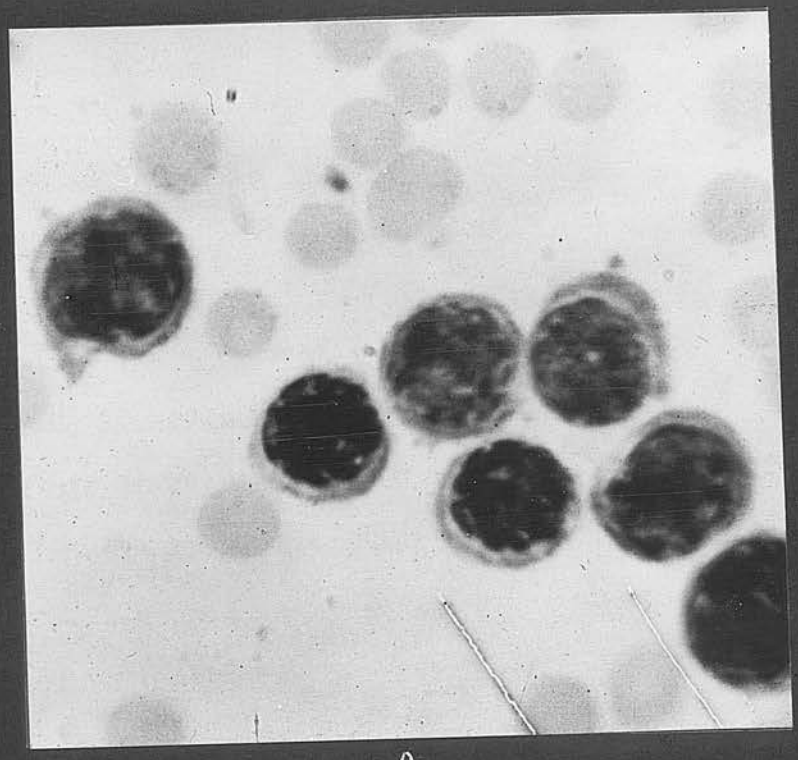
C

Plate 3.

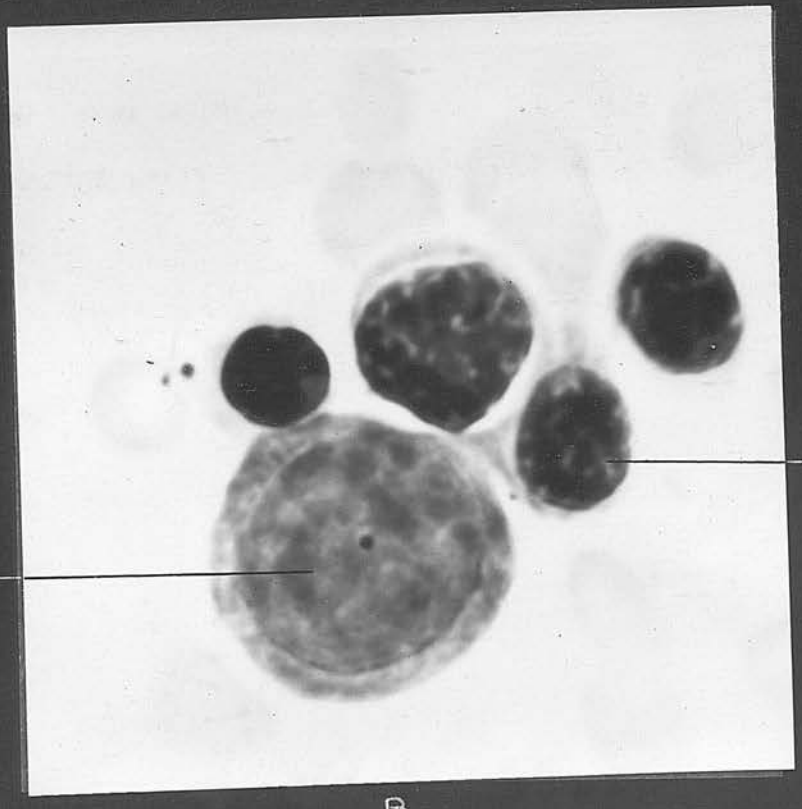
A. The group of intermediate normoblasts shown in this plate illustrates the varying degrees of clumping of nuclear chromatin found in these cells. It also shows the basophilic affinity of the cytoplasm, and in a number of the cells the perinuclear halo is demonstrated. The field also includes a late normoblast and an erythrocyte showing punctate basophilia. (X 2,200)

B. The large cell in the centre of the field is an early normoblast. There is some clumping of the nuclear chromatin, and no nucleoli are present. The cytoplasm is seen to be more basophilic than that of the adjacent intermediate normoblasts, but much lighter than that found in the pro-erythroblast illustrated in Plate 1. The smallest intermediate normoblast shows marked pyknosis forcasting in this respect the nuclear structure of the late normoblast. (X 2,500)

PLATE 3.



A.



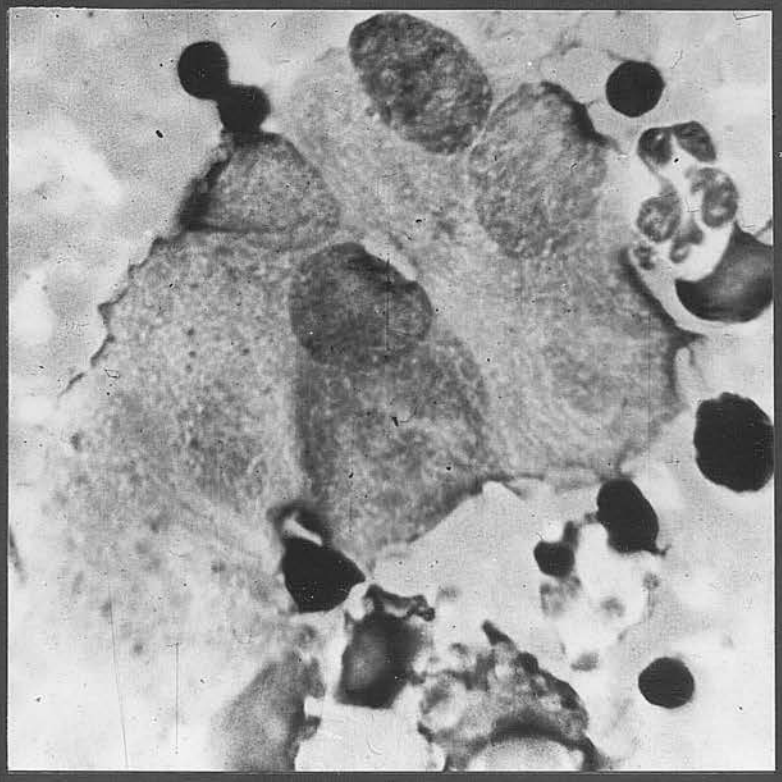
B.

Plate 4.

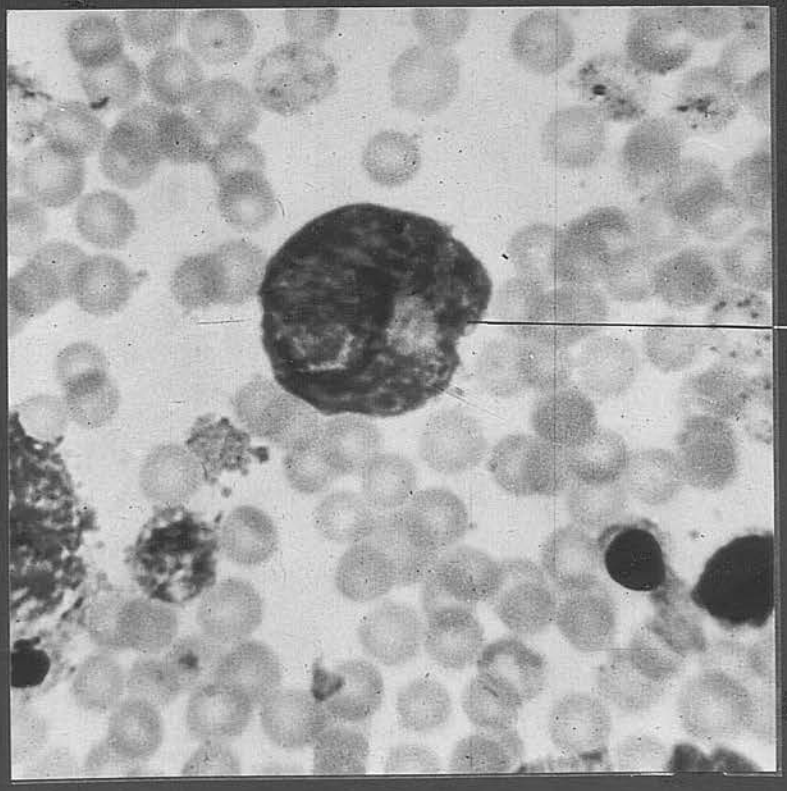
A. This field illustrates a group of immature plasma cells in varying stages of maturity. The more primitive cells have more finely reticulated nuclei which are pink in colour and have a pale blue cytoplasm which is foamy in consistency and contains azure particles. Groups of plasma cells such as this were only rarely seen. (X 1,800)

B. This cell is representative of the plasma cell as more commonly seen in the marrow of the sheep. In contrast to the cells illustrated in Plate 4 A. the cytoplasm is deeply basophilic and contains the characteristic pale area almost invariably present in the mature plasma cell. (X 2,000)

PLATE 4.



A.



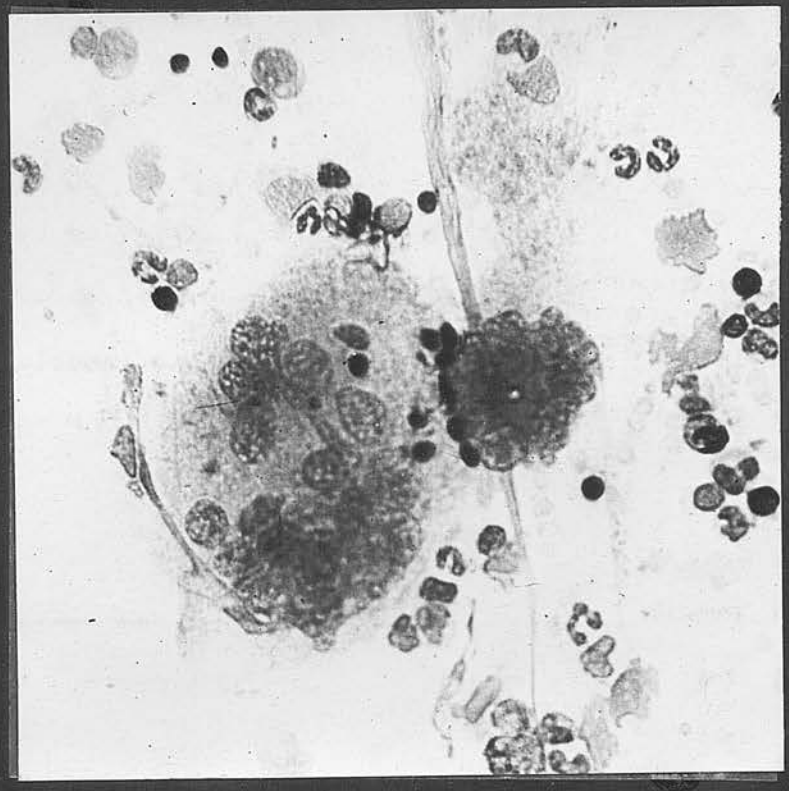
B.

Plate 5.

A. The cell in this field is a reticulum cell. The primitive structure of the nucleus is well shown. The cytoplasm is foamy in consistency due to vacuolation and contains green and purple inclusions. (X 2,000)

B. Two megakaryocytes are shown in this plate. In the larger cell lobulation of the nucleus is more evident indicating that it is more mature than the smaller adjacent cell. Other marrow cells may be seen adhering to the cytoplasm of the larger megakaryocyte. A fibril of marrow reticulum is seen crossing the field from top to bottom. (X 800)

PLATE 5.



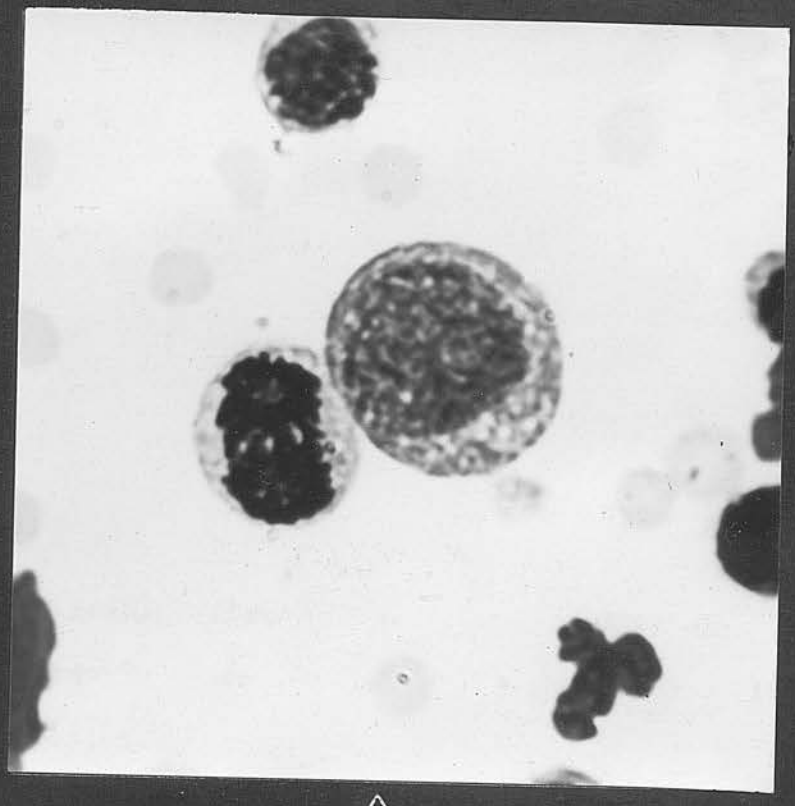
B.

Plate 6.

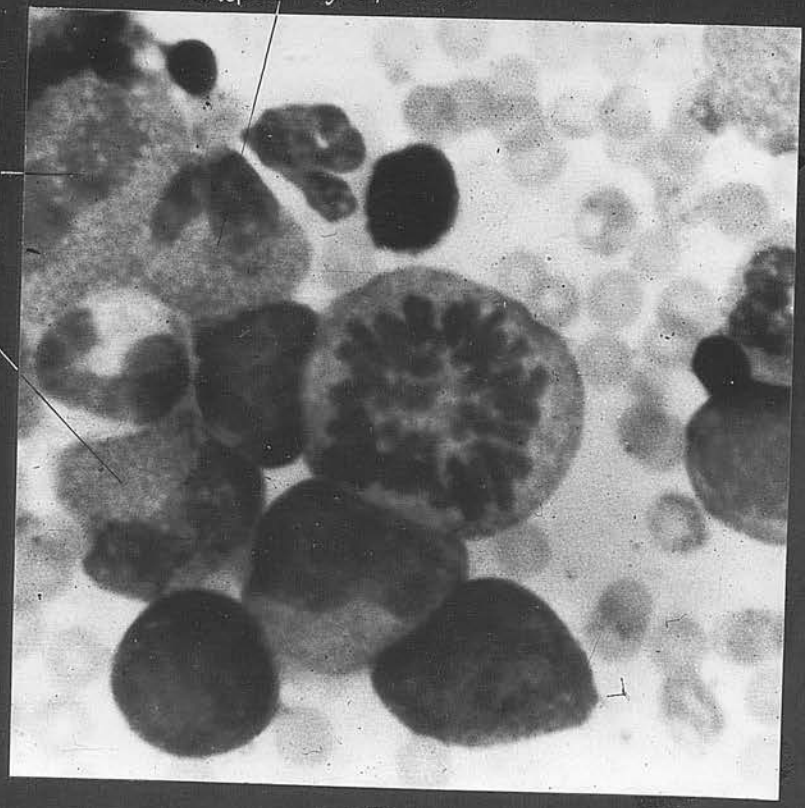
A. The smaller of the two centrally placed cells is an early normoblast in the anaphase stage of mitosis. The larger adjacent cell is seen to contain granules and is classified as a 'young' neutrophil myelocyte in which granulation is just becoming evident. (X 2,000)

B. The cell occupying the centre of the field is classified as a neutrophil myelocyte in the metaphase stage of mitosis. Fragmentation of the nucleus is well shown. The field also contains the three stages of eosinophil granulocyte viz. myelocyte, metamyelocyte, and polymorphonuclear. (X 1,700)

PLATE 6.



A.

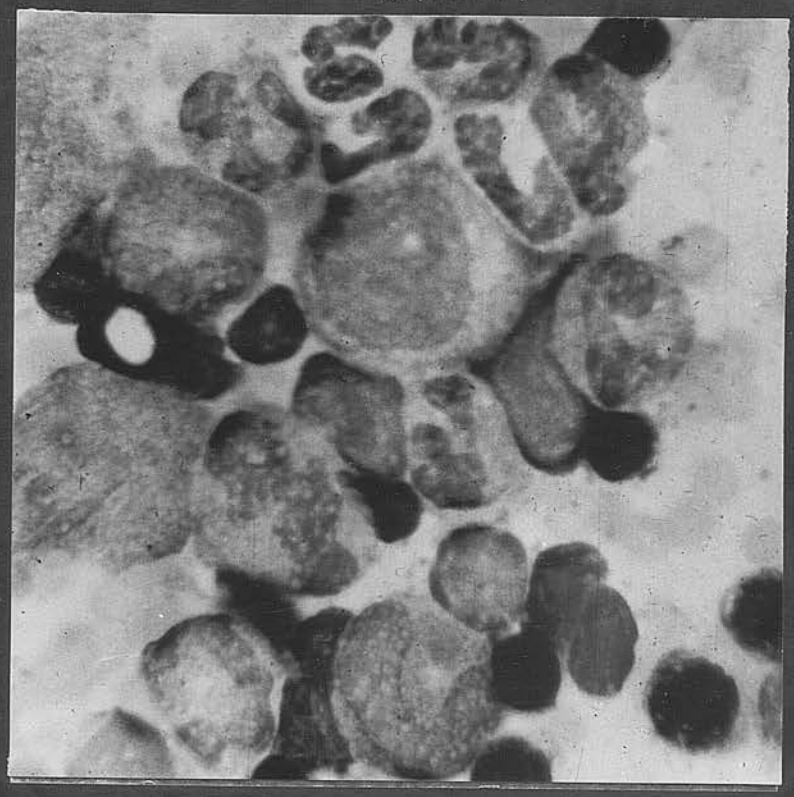

Eosinophil Polymorphonuclear
Eosinophil Myelocyte
Eosinophil Metamyelocyte

B.

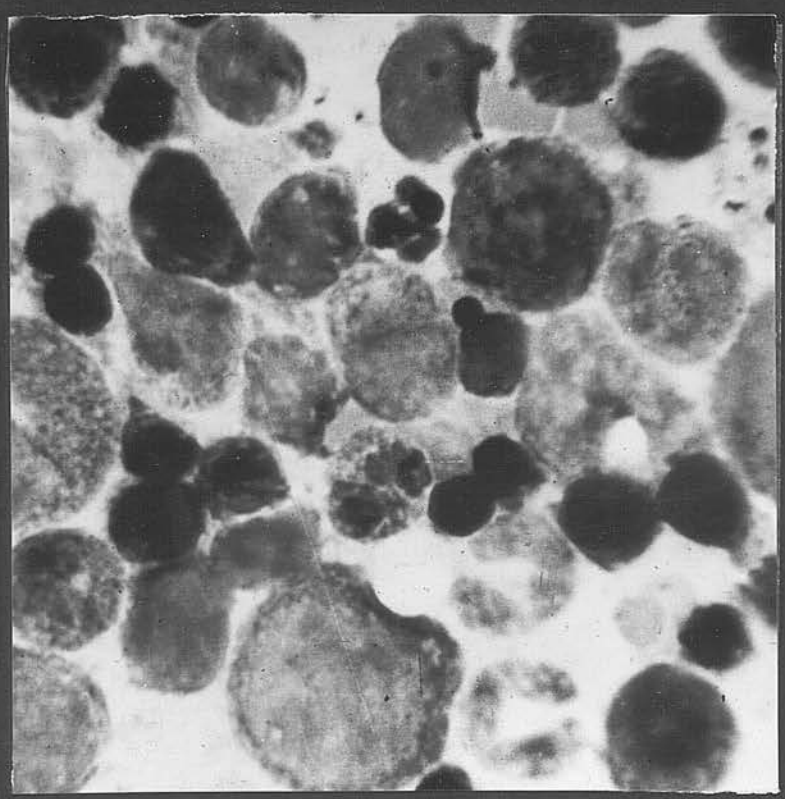
Plate 7.

A. & B. These fields are included to illustrate typical fields of marrow cells and contain many different cells. Nucleoli are plainly visible in the more primitive cells . In Plate 7 B. there is a tendency for the nuclei of the intermediate normoblasts to be 'clogged' with stain. (X 2,000)

PLATE 7.



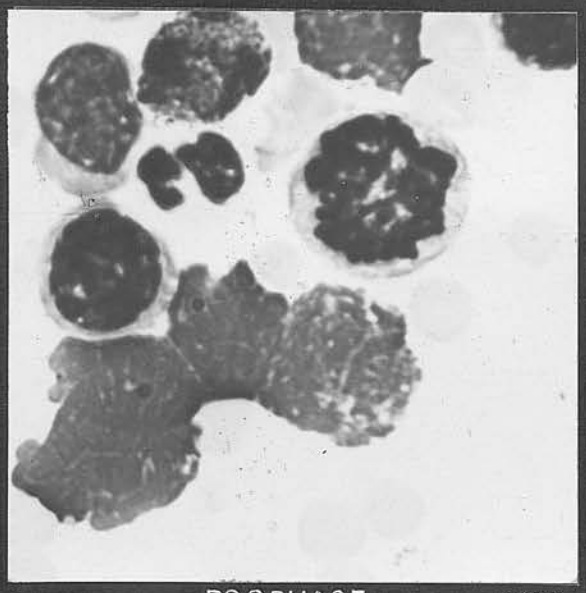
A.



B.

PLATE 8.

MITOSIS.



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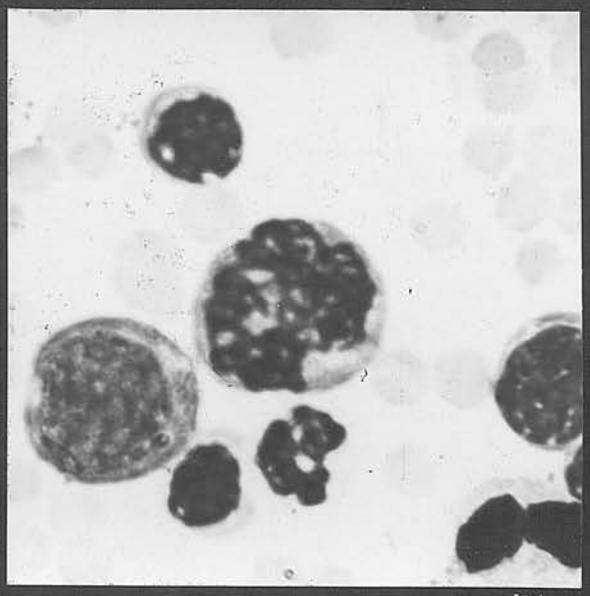

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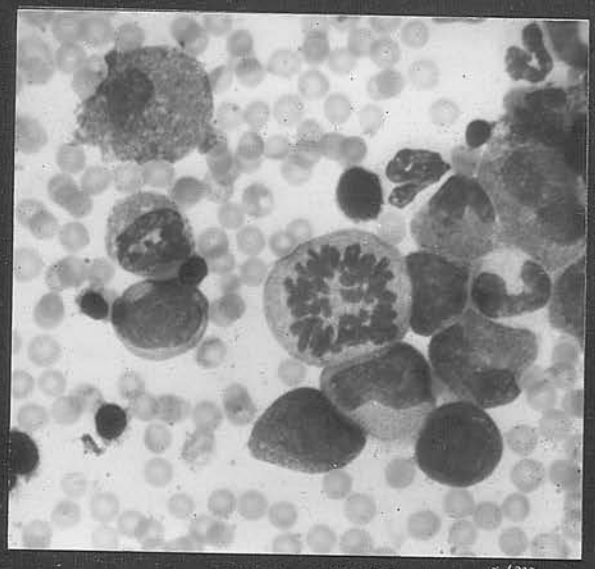

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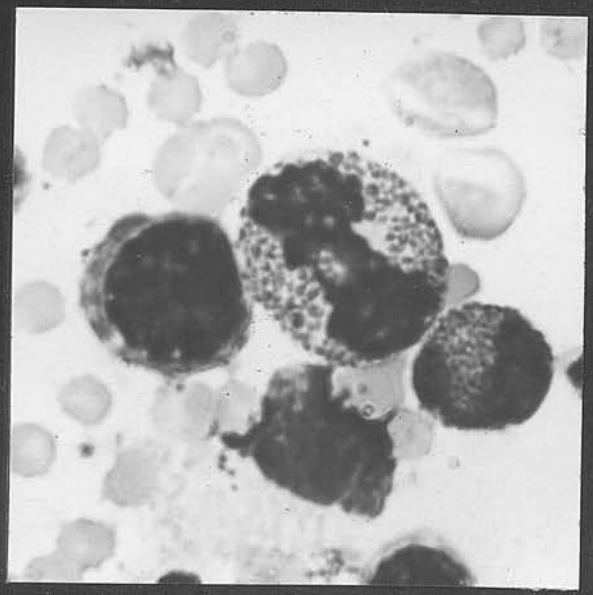

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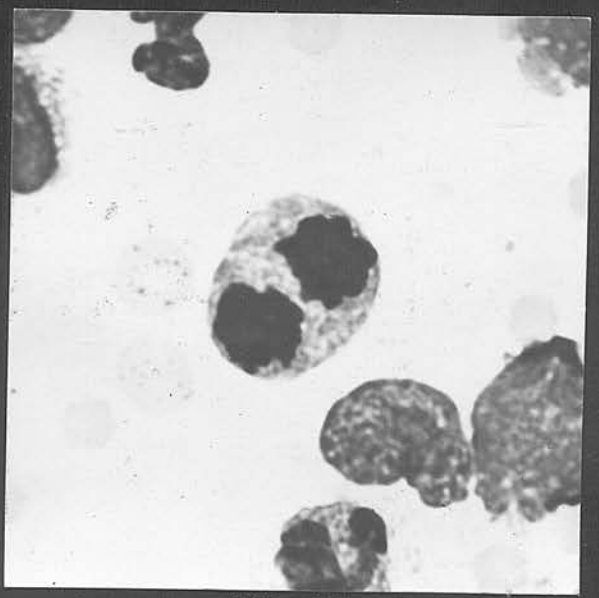

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SECTION III.

Bioptic Sampling by Sternal Puncture as a means of illustrating the response of the Erythropoietic Tissue to the stimulus of Blood Loss.

The series of experiments was undertaken with two objects in mind. The first was to test the possibility of detecting, by the sternal puncture method of marrow biopsy in the sheep, regenerative changes in the erythroid elements. The second object was to correlate these marrow changes with the evidence of regeneration in the peripheral blood.

The material consisted of three crossbred sheep of approximately fifteen months of age. Throughout the experiment they were housed and fed on hay ad lib. and 1½ lb. crushed oats daily. Identification of the sheep was by ear ring, the numbers allocated being 50, E63, and V. 35. Their weights at the beginning of the experiment were 90 lb. 64 lb. and 60 lb. respectively. By adopting Holman's figure of 1.052 for the specific gravity of sheep's blood, and calculating on the basis of 8% of the body weight representing the total blood volume (Dukes, 1942), it was estimated that the total blood volume in respect of the three sheep was as follows:- 50, 3117 ml; E.63, 2216 ml; V. 35, 2078 ml.

Bleeding.

Blood was removed by means of a needle of wide bore from the jugular vein and collected for measurement in a graduated cylinder. In the case of V.35 the stimulus consisted of the removal by a single bleeding of 600 ml. This represented a loss of 28.8% of total blood volume. From sheep 50 and E. 63, measured amounts of blood/

Table XVIII.

Intervals at which samples were collected and details of the bleeding.

Sheep No. 50			Sheep No.E.63			Sheep No. V.35		
On Day	Blood withdrawn ml.	Details of sampling	On day	Blood withdrawn ml.	Details of sampling	On day	Blood withdrawn ml.	Details of sampl
0	780	B & M	0	400	B & M	0	600	B & M
1	780	B	1	325	B	1	-	B
2	-	B	2	500	B	2	-	B
3	250	B & M	3	580	B	3	-	B
4	250	B	4	420	B	4	-	B
5	250	B	5	500	B & M	5	-	B & M
6	-	B	6	-	B	43	-	B & M
7	250	B & M	7	-	B			
8	250	B	8	150	B			
9	250	B	9	-	B			
10	-	B	12	-	B			
11	250	B	13	-	B			
12	-	B	18	-	B			
14	-	B	20	-	B & M			
31	-	B & M	69	-	B & M			
41	-	B & M						

B = Peripheral Blood. M = Marrow.

blood were withdrawn over a period (Table XVIII) until the P.C.V. was reduced to under half the value observed at the start of the experiment. This degree of reduction was chosen as according to Castle and Minot (1936), 'anaemia to produce outstanding symptoms in a moderately active individual requires that the values for the concentration of red cells or haemoglobin be reduced to half'. From sheep 50 this entailed the removal of 3,310 ml. over a period of 12 days, and from E. 63, 2,875 ml. in 8 days. In the case of 50 this represented a loss of 106.7% and in E. 63 a loss of 129.5% of total blood volume.

Sampling.

Samples of blood and sternal marrow were collected using the techniques described in the previous sections. The intervals at which samples were collected and details of the bleeding are shown in Table XVIII.

Examination of Samples.

1. Peripheral Blood. Using the techniques already described, the following examinations were carried out on all samples of blood collected:- P.C.V., Hb., R.B.C., W.B.C., and D.L.C. Estimation of regeneration as shown by reticulocyte counts in films supravitally stained was carried out according to a method devised by Boyce (1949). The technique is as follows:- One part oxalated blood is mixed with 3 parts of a 1% solution of Brilliant Cresyl Blue in normal saline (0.85%) and allowed to stand for 3-5 minutes, when films are made in the usual way. Estimation of regeneration as shown by counts of cells showing punctate basophilia, polychromasia, and the presence of nucleated red cells was carried out on films/

films stained by Leishman Giemsa, the times being those used for marrow spreads. M.C.V. and M.C.H.C. were calculated in respect of all samples collected.

The specific gravity of blood and plasma was estimated by means of the copper sulphate method (Hawk, Oser & Summerson, 1947). The object of making these measurements was to examine the variation encountered following bleedings, and to determine how closely the changes noted in the S.G. of whole blood agreed with the alterations in erythrocyte level as measured by P.C.V.

2. Marrow Samples. Differential marrow counts were made on spreads prepared by the method already described in Section II. The method of differential marrow cell counting was as follows:-

A survey of the cell distribution on the slide, made under the low power, gave a general indication of areas likely to be suitable for study; however, the actual selection of fields for differential counting was carried out under the 1/12 oil immersion objective. The only factors considered in the selection of these fields were that the cells should be recognisable and that the fields should be as near the centre of the squashed fleck as possible. The object of the latter precaution was to reduce the error due to the alteration in the relative distribution of the cells by the act of spreading, the supposition being that this would be less towards the centre of the spread.

In the case of each sheep approximately 340 cells on three slides were differentiated, making a total count of over 1,000 cells.

Details of the nomenclature used have already been described in Section II.

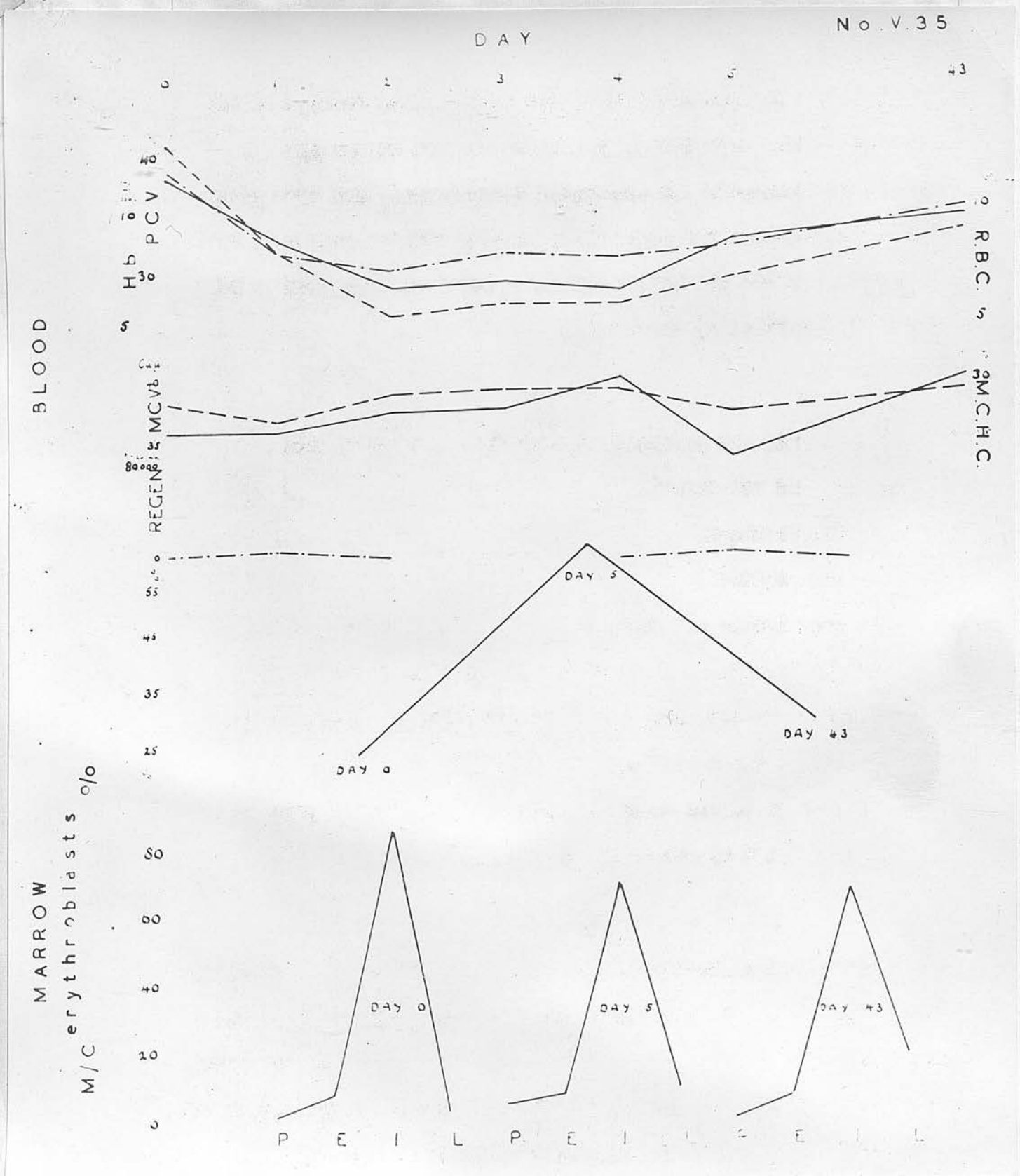


Fig. 18. Sheep V. 35. Changes in the erythrocyte picture in peripheral blood and erythroblastic tissue in marrow

-·-·-·-·- Punctate basophilia. Hb.
- - - - - - Polychromasia. P.C.V. M.C.H.C.
——— Reticulocytes. R.B.C. M.C.V.

From the figures obtained by the differential count, myeloid erythroid ratios were calculated, and maturation curves drawn in respect of Neutrophil and Eosinophil Granulocytes, and Erythroblasts. During the differential count cells seen in mitosis were recorded. An estimate of the cellularity of the spreads was made, using the standards described in Section II.

Results.

The results are presented in respect of the three sheep separately, and are considered from the effect of the bleeding on

a. The erythron,

b. The leukon.

c. Cellularity of Spreads.

Holman's (1944b) standards for Maximum Allowable Difference were used to interpret the variations occurring in M.C.V. and M.C.H.C. in the three sheep.

The results of the S.G. estimations appear under a separate heading at the end of the Section.

V. 35.

The results of the experiment in respect of V. 35 are shown in Figs. 18 and 19 , the data from which they were compiled appearing in the Appendix pages A18-19. Fig. 18 illustrates the variations encountered in the erythron. The changes in the erythrocytic properties of the peripheral blood and in the incidence of regenerative forms are shown above in the diagram, and the variations in the percentage of erythroblasts found in the marrow, together with the maturation curves are drawn below.

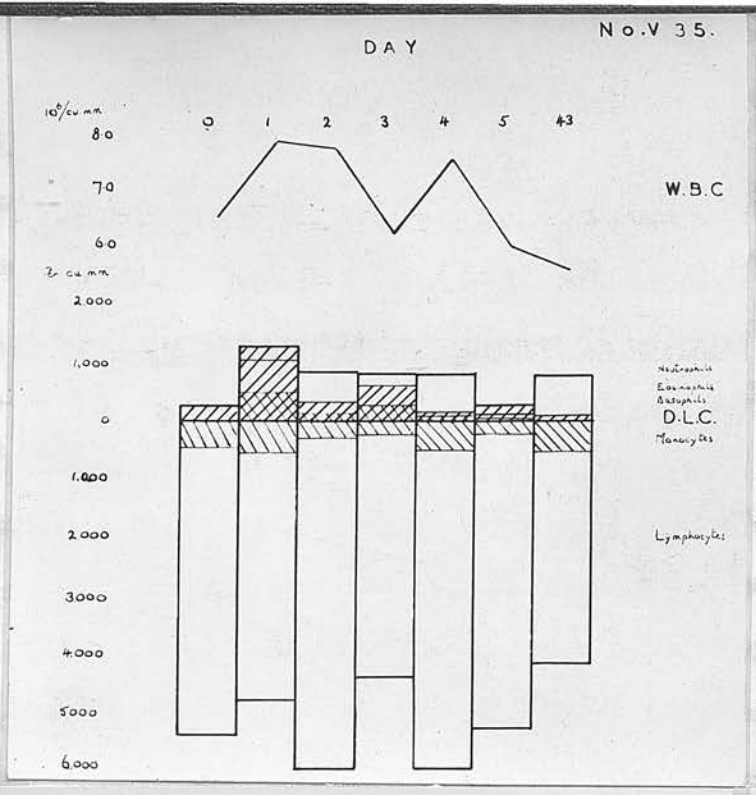
Fig. 19 shows the total and differential leucocyte counts in the peripheral blood and maturation curves for neutrophil and eosinophil granuloblasts in the marrow.

a. Erythron (Fig. 18). The loss of 28.8% of total blood volume by a single bleeding caused a reduction to 42.4% of the initial R.B.C. four days after the bleeding. Thereafter the count rose, but had not reached the pre-bleeding level by the 43rd day. The only regenerative changes observed were seen 24 hours after bleeding, and again on the 5th day, and took the form of under 1,000 erythrocytes per cu. mm. showing punctate basophilia. No reticulocytes were seen. From the M.C.V. and M.C.H.C. the anaemia was classified as normochromic normocytic.

The marrow sample collected five days after the bleeding showed a marked increase in erythroid elements in the marrow (from 25.7% pre-bleeding, to 61.9%). This was shown by the maturation curve to be due to an increase in the pro-erythroblasts and late normoblasts. A final marrow sample collected 43 days after the bleeding showed that the percentage of erythroid cells in the marrow was 31.6%, but there was still a slightly higher incidence of late normoblasts than at the start of the experiment.

Mitotic figures were observed in 0.6% of the erythroblasts before bleeding. On the 5th day this percentage rose to 1.3%, returning in the final sample to 0.2%.

b. Leukon. (Fig. 19). The highest count of both eosinophils and neutrophils was recorded 24 hours after withdrawing the 600 ml. of blood. The only other point of interest in the variation shown in the leukon was an increase in the incidence of myeloblasts in the/



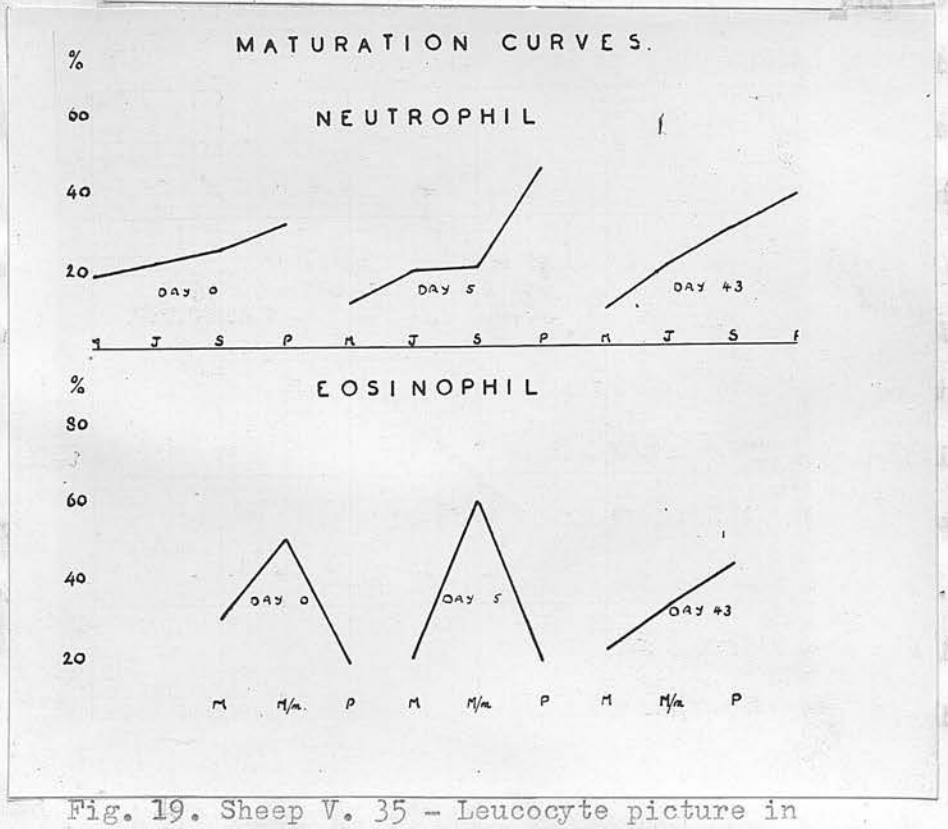


Fig. 19. Sheep V. 35 - Leucocyte picture in peripheral blood and maturation curves of granuloblastic tissue in marrow.

the the marrow sample collected on the 5th day. The percentage of granuloblastic cells showing mitosis remained constant at 0.1%.

c. Cellularity of Spreads. Cellularity was graded III. in the first marrow sample, rose to V. on the 5th day and was back to IV. on the 43rd day after bleeding.

E. 63.

The results of the experiment in respect of E. 63 are shown in Figs.20 and 21 , the data from which they are compiled appearing in the Appendix, pp. Fig. 20 shows the erythron picture. Fig.21 the leucocyte levels in peripheral blood and the maturation curves for neutrophil and eosinophil granuloblasts.

a. Erythron.(Fig. 20) Over a period of eight days this sheep lost 129.5% of its total blood volume, causing a reduction of R.B.C. to 55.9% of the original count. A rise in erythrocyte properties towards initial levels was seen to have started five days after the last bleeding and was complete 62 days after the last occasion on which blood was removed.

The low M.C.V. value on the 5th day was considered to have no significance in view of the equally low figure recorded on the 69th day, when the R.B.C. had returned to normal. Thus, with the absence of any significant change in M.C.H.C., the anaemia was classified as normochromic normocytic.

The signs of regeneration included the presence of reticulocytes, and erythrocytes showing punctate basophilia, and polychromasia. Punctate basophilia was the first regenerative sign to appear; it reached a higher level than the other two forms, and persisted/

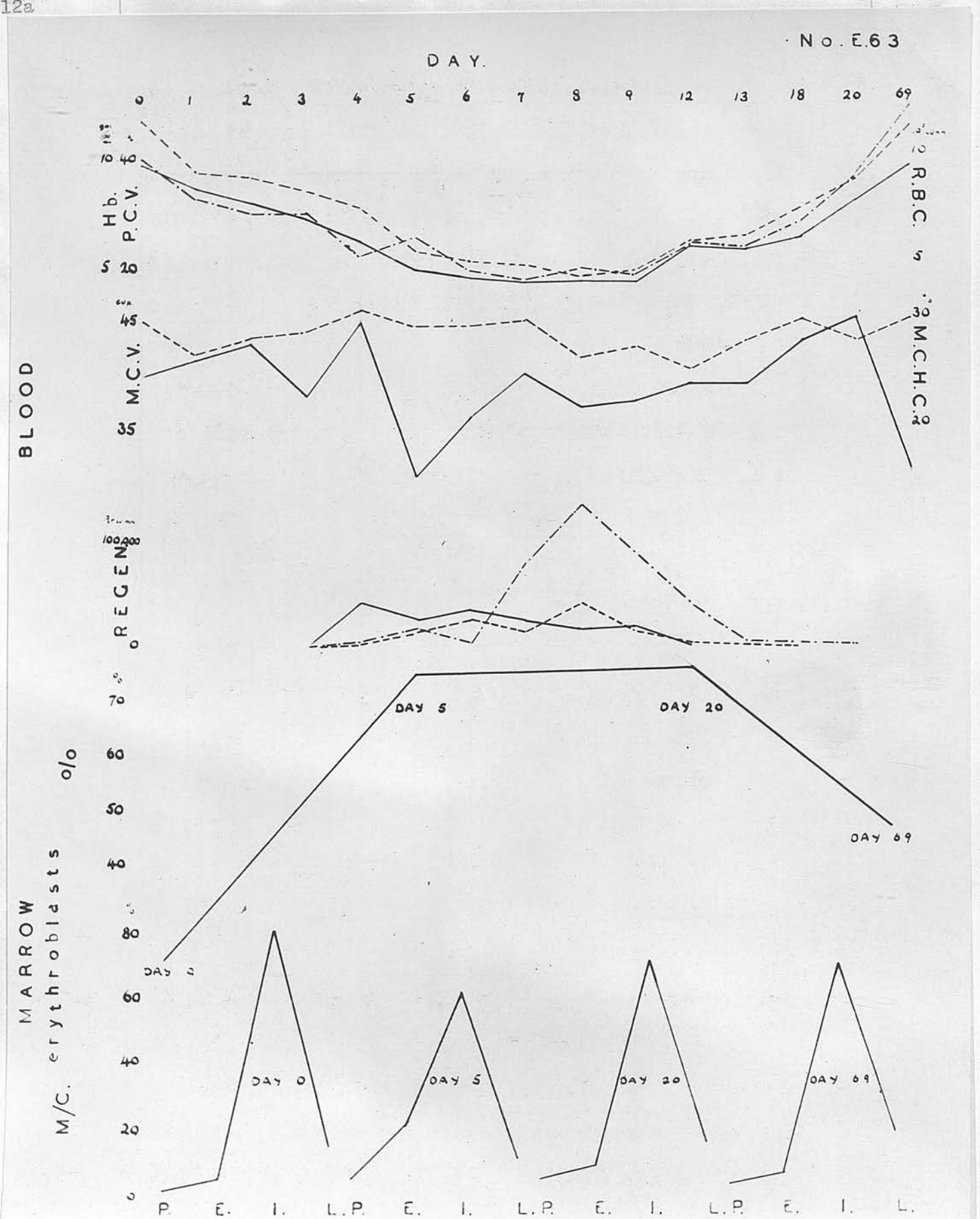


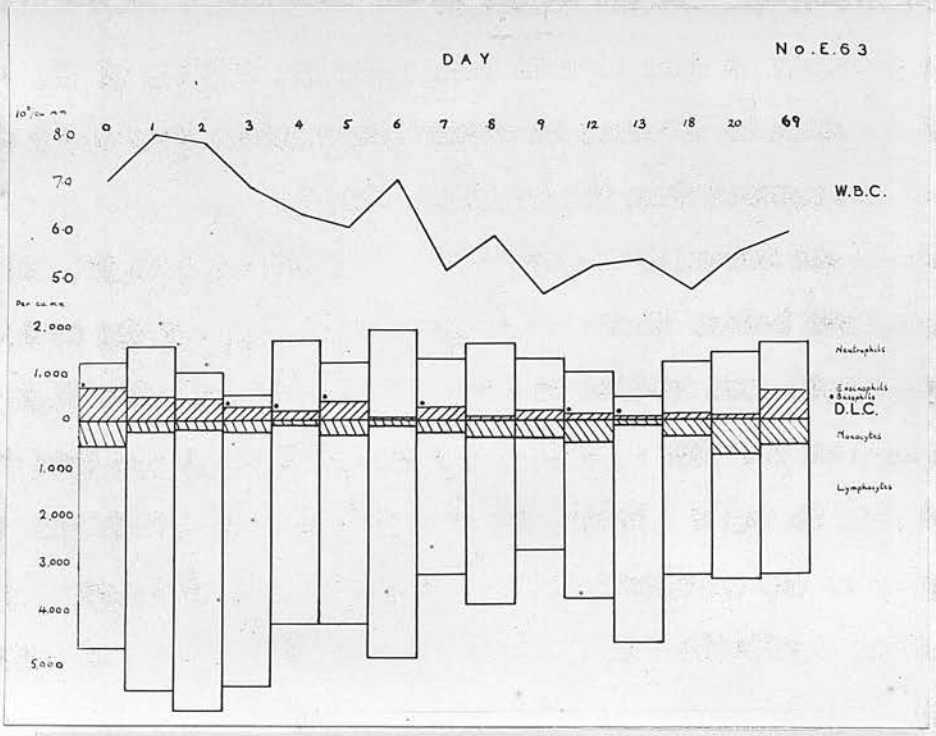
Fig. 20. Sheep E. 63. Erythrocyte picture in peripheral blood and erythroblastic tissue in marrow.

—·—·—·— Punctate basophilia. Hb.
— — — — Polychromasia. P.C.V. M.C.H.C.
———— Reticulocytes. R.B.C. M.C.V.

persisted in the peripheral blood after they had disappeared. No cells showing punctate basophilia could be found twelve days after the final bleeding. In the course of the examination of the blood films, late normoblasts, and cells showing Howell Jolly bodies were noted on the 4th, 6th, 7th and 8th days, being most numerous on the 8th day, which was when regeneration was at its peak. Thereafter they were absent.

Marrow samples taken on the 5th day, viz., after the removal of 2,225 ml. of blood, showed the percentage of the erythroid elements had increased from 23.4% to 75.4%. The maturation curve for erythroblasts in this preparation showed a slight increase in the pro-erythroblasts and a marked increase in early normoblasts over the pre-bleeding levels. A marrow sample taken 13 days after the last bleeding showed the erythroid elements in the marrow now comprised 76.6% of cells present in the marrow, but that the early normoblasts had decreased, and late normoblasts increased as compared with the previous sample. Polychromasia and reticulocyte counts showed a close agreement. The picture in the marrow sample taken on the 20th day is of interest, as it shows erythroid hyperplasia to be still present, with the shift to the left seen in the previous sample now having been converted to a shift to the right. It was accompanied by no signs of regeneration in the peripheral blood and with the erythrocyte count only 7,000 lower than the initial level. In the final marrow sample examined, the percentage of erythroblasts, although more nearly approaching the pre-bleeding level, stood at 47.2%, which was above the initial figure of 23.4% and the maturation curve showed the late normoblasts to be higher

in/



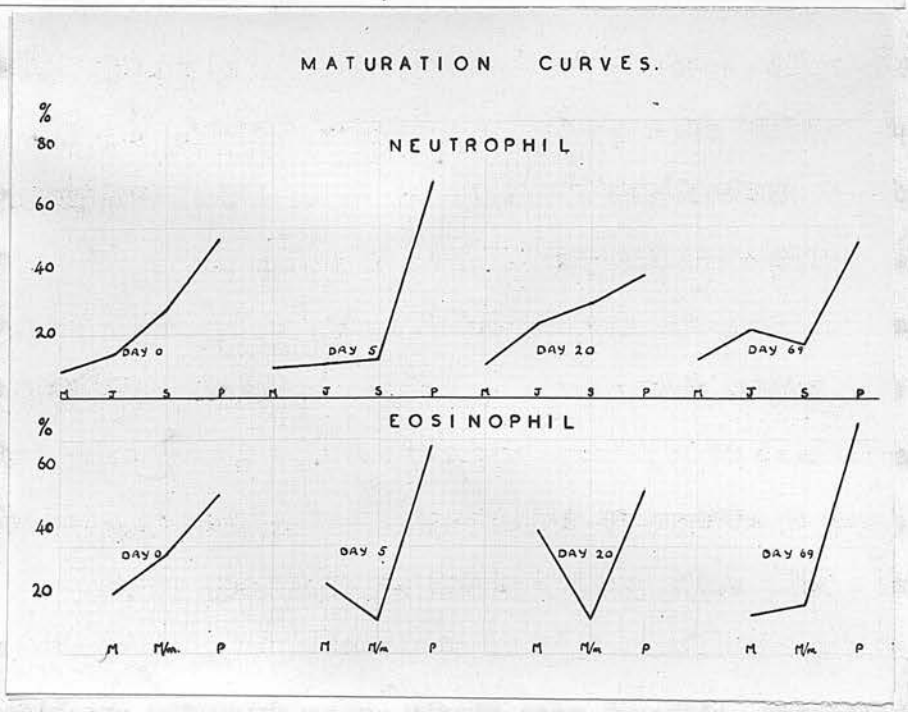


Fig. 21. Sheep E.63 - Leucocyte picture in peripheral blood and maturation curves in granuloblastic tissue in marrow.

in incidence than in the three previous samples, although pro-erythroblasts and early normoblasts were both represented in proportions similar to the initial findings.

Thus, it may be seen that in this sheep the effects of the haemorrhage could be detected in marrow preparations long after all signs had disappeared from the peripheral blood.

Mitosis was recorded in 0.1% of the erythroblasts in the marrow spread prepared before bleeding began. By the 5th day the number of erythroblasts seen in mitosis had risen to 0.8% and this figure rose to 0.9% on the 20th day. In the sample taken on the 69th day it had fallen to 0.3%. These findings are consistent with the hyperplasia of the erythroid tissue seen, but it is of interest that the increase in mitotic activity did not persist after normal erythrocyte levels had been restored in the blood.

b. Leukon. (Fig. 21). There was a tendency for the W.B.C. to fall throughout the experiment, but it was not possible to associate the fluctuations in the incidence of either the granulocytic or mononuclear leucocytes with this trend.

The maturation curves for neutrophil and eosinophil granulocytes in the marrow showed great variability, but no consistent shift to the left or right was observed. The percentage of myeloid elements seen in mitosis in the four marrow samples collected was 0.3%; 0.6%; 0.2%; 0.2%, the highest value occurring on the 5th day and coinciding with the highest level for mitosis in the erythroblasts.

c/

c. Cellularity of Spreads. The cellularity in the spreads made before bleeding began was graded at IV; by the 5th day it had risen to V. and on the 20th and 69th days was IV. and III. respectively.

Sheep 50.

The results of the experiment in respect of sheep 50 are shown in Figs.22 and 23 . The data from which they are constructed appears in the Appendix, pages A22 and A23.

a. Erythron. Over a period of 12 days this sheep lost 106.7% of its total blood volume, causing a reduction of R.B.C. to 30.1% of the initial R.B.C. by the 10th day. Thereafter the count rose and 30 days after the final bleeding was just above the initial level. All three forms of regeneration were noted during this experiment, viz., reticulocytes, punctate basophilia and polychromasia. Cells showing punctate basophilia were most numerous throughout and erythrocytes showing polychromasia least numerous. The fluctuations in the incidence of reticulocytes and polychromatophilic cells showed a marked degree of agreement. Regenerative forms of all three types were first seen on the 4th day, and all three rose to their highest levels on the 6th day, when punctate basophilia was evident in 2.02% of erythrocytes, 1.14% reticulocytes were present, and cells showing polychromasia amounted to 0.58%. By the 14th day reticulocytes and polychromasia had disappeared, but punctate basophilia was still present.

As the R.B.C. fell to its lowest level the M.C.V. gradually rose and M.C.H.C. fell, and although the deviations did not exceed Holman's M.A.D. for these indices, the consistent nature of the change/

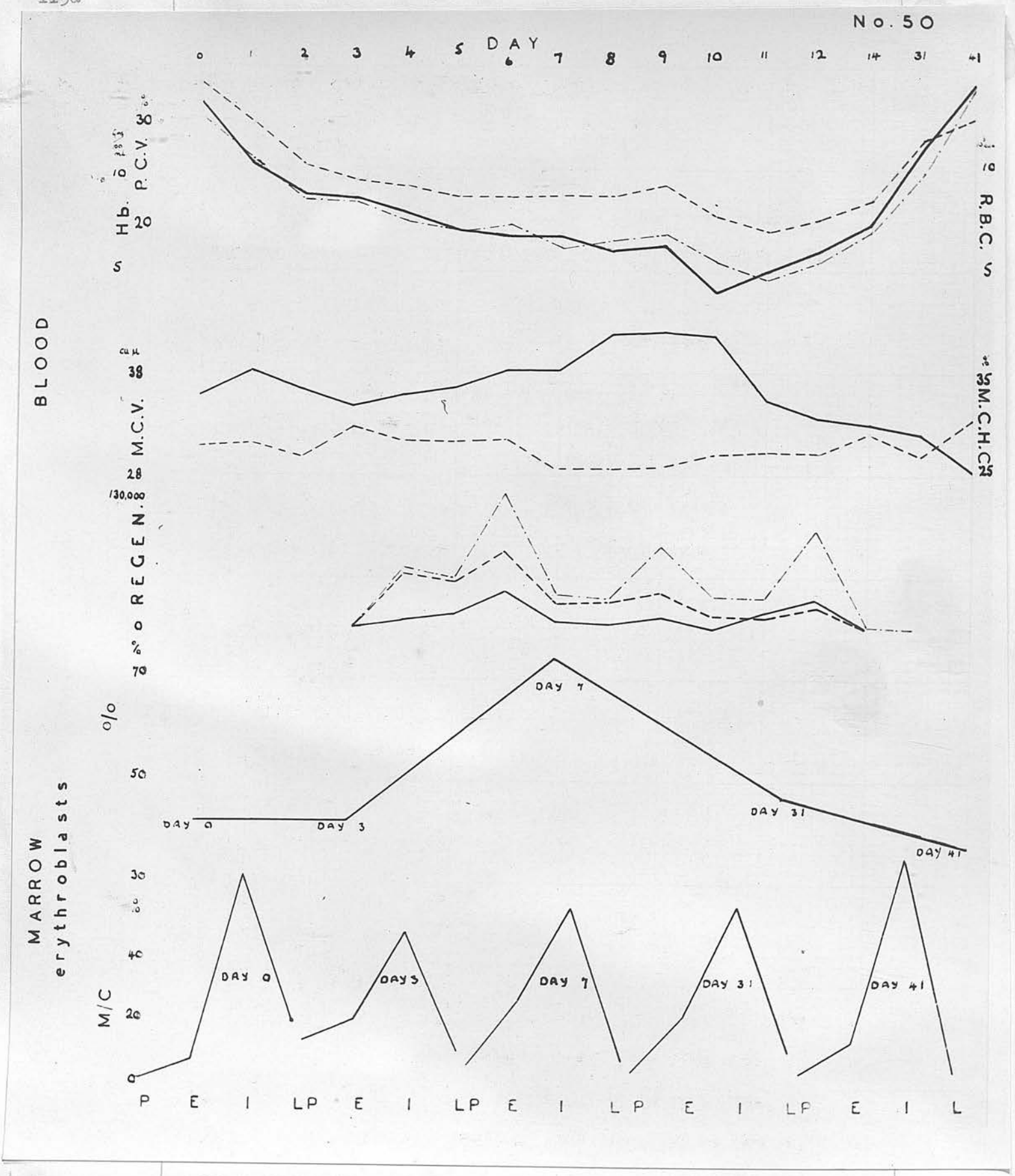


Fig. 22. Sheep 50. Erythrocyte picture in peripheral blood and erythroblastic tissue in marrow.

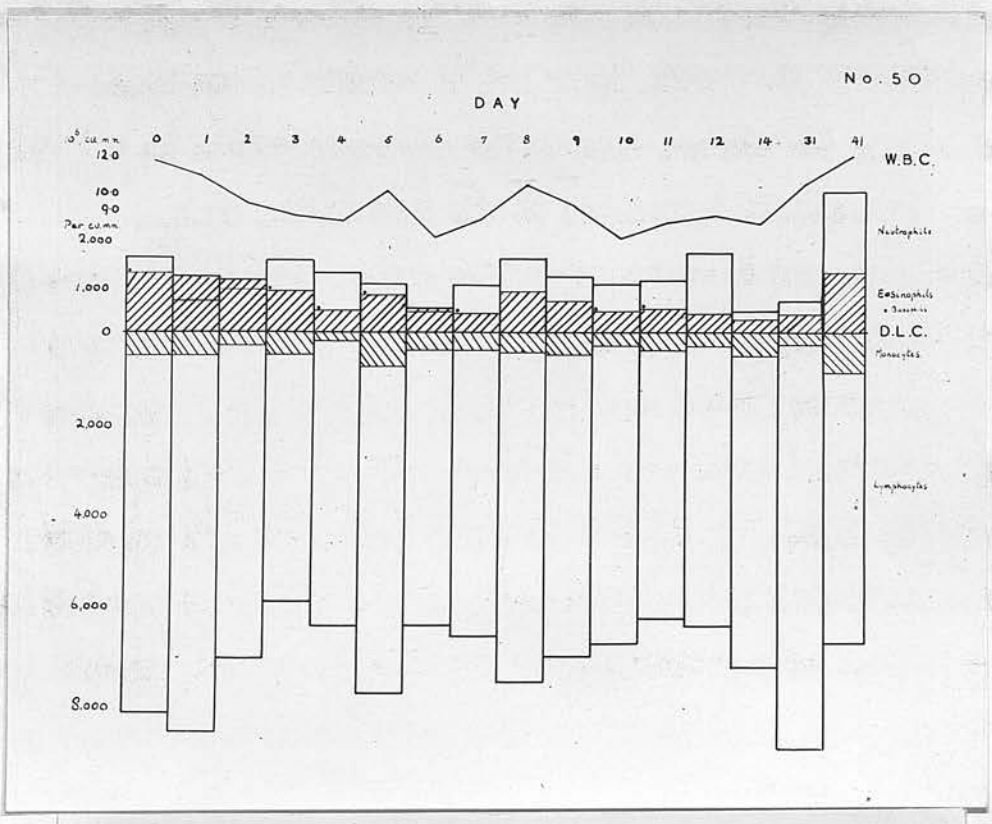
-·-·-·- Punctate basophilia. Hb.
– – – – – Polychromasia. P.C.V. M.C.H.C.
——— Reticulocytes. R.B.C. M.C.V.

change suggests the changes were significant, and there was in fact a tendency for the anaemia in this sheep to be macrocytic and hypochromic.

No regenerative forms were noted on the 31st and 41st days of the experiment. The only late normoblasts observed, were seen on the 7th day.

A marrow sample taken on the third day disclosed no increase in the percentage of erythroid elements present as compared with the pre-bleeding sample; however, the maturation curve for the erythroblasts on the 3rd day showed a decrease in late normoblasts and a marked increase in pro-erythroblasts and early normoblasts. This change had occurred before evidence of regeneration in the peripheral blood appeared. By the 7th day the percentage of erythroblasts in the marrow had increased to 74% from 42.6% at the previous sampling. The maturation curve on the 7th day showed the pro-erythroblasts to be lower in incidence than on the 3rd day, but there had been a further increase in early normoblasts, and a slight decrease in late normoblasts. By the 31st day the marrow reaction had altered in character, and the percentage of erythroblasts had fallen to 47.4%, but there was still evidence of stimulation of the erythroid tissue in the fact that the early normoblasts were still more numerous than at the start of the experiment. The higher incidence of early normoblasts was still evident on the 41st day, viz., 30 days after the last bleeding.

The percentage of erythroblasts seen in mitosis at the various samplings was as follows:- Pre-bleeding, 0.4%; 3rd day 0.2%; 7th day, 1.4%; 31st day, 1.2%, and 41st day, 0.1%. Thus mitosis was most evident at the time of greatest marrow reaction.



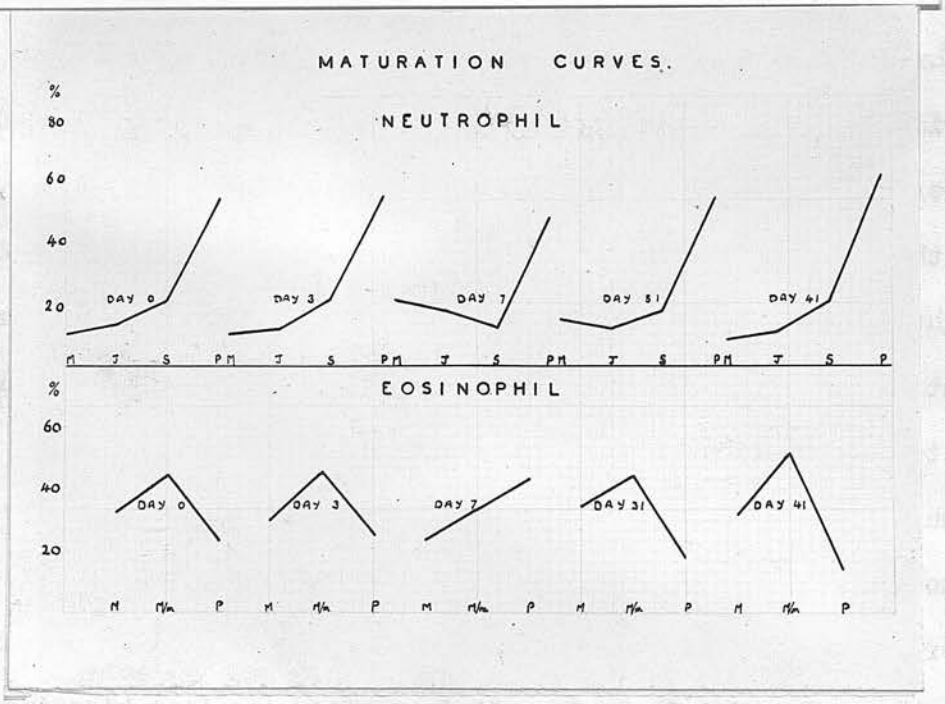


Fig. 23. Sheep 50. Leucocyte picture in peripheral blood and maturation curves for granuloblastic tissue in marrow.

b. Leukon. Fig. 23). As with the previous sheep, the fluctuations in the W.B.C. could not be related to the blood loss. Nevertheless, the changes seen in the erythroid tissue on the 7th day were accompanied by a shift to the left in the neutrophil granuloblasts, and from the maturation curves it was seen that the neutrophil myelocytes comprised 20.9% of the neutrophil granuloblasts, as against 10.2% and 10.7% at the two previous samplings. Their incidence fell to 14.6% on the 31st day, and by the 41st day was back to 8.7%. Marked variation in the maturation curves for the eosinophil series was observed. Little change was noted in the numbers of granuloblasts seen in mitosis at the various smplings.

c. Cellularity of Spreads. The cellularity of the spreads prepared before bleeding and on the 41st day were graded at IV. but on the 3rd, 7th and 31st days the cellularity was graded at V.

Mitosis in Erythroblasts.

In Table XIX an analysis of the mitosis seen in the erythroblasts is given for the three sheep. The erythroblasts are classified according to age, and it will be seen that cell division was most common in the intermediate normoblasts and that it increased in all three sheep at the time of the maximum marrow response to the bleeding. There was also a slight increase in the mitotic activity in the pro-erythroblasts and early normoblasts.

Table XIX.

An Analysis showing stage at which Erythroblasts were most commonly seen in division.

Sheep No.	Day	Pro-erythroblasts %	Early Norms. %	Interm. Norms. %	Late Norms. %	Total %
V.35	0			0.6		0.6
	5	0.2	0.1	1.0		1.3
	43			0.1		0.1
E.63	0			0.1		0.1
	5		0.2	0.6	0.2	1.0
	20	0.1	0.2	0.7	0.1	1.1
	69	0.1		0.2		0.3
50.	0	0.1	0.1	0.3		0.5
	3	0.1	0.1	0.1		0.3
	7		0.2	1.4	0.1	1.7
	31		0.1	1.1		1.2
	41			0.1		0.1

Eosinophil Leucocytes.

From Figs. 19, 21 and 23 it will be seen that in sheep V. 35 and 50 the eosinophil counts were generally higher than those found in Sheep E. 63. Thus, on one occasion in the case of V. 35 the eosinophil count was 1,326 per cu. mm; and in 50 the count exceeded 1,100 per cu. mm. on four occasions, whereas the highest count recorded in E. 63 was 665 per cu. mm. From the maturation curves for the eosinophil granuloblasts in the 3 sheep it will be seen that in sheep V. 35 and 50 there was a marked tendency for the eosinophil metamyelocyte to be in highest incidence, whereas in E. 63 the lobulated eosinophil was the most predominant cell in the series. Thus the higher counts in the peripheral blood appear to be reflected by a shift to the left in their precursors in the marrow.

Discussion.

The results of these experiments showed that by bioptic marrow sampling it was possible to observe the changing response of the marrow to a stimulus such as blood loss. Two forms of change were shown to occur in the marrow. One consisted of an erythroblastic hyperplasia, and marrow samples taken at the point when the erythrocyte count was around its lowest level showed this state to be well marked in all three sheep, and an increase in the cellularity of the spread was recorded in the case of each sheep. Further samples collected when the erythrocytes had returned to their pre-bleeding level showed the M/E ratio to have returned approximately to the initial figure. The other change was in the form of an alteration of the erythroblastic maturation curve. It consisted of an increase in the relative incidence of either pro-erythroblasts or of early normoblasts, or of both, the increase coinciding with the state of erythroid hyperplasia in all three sheep. In sheep 50 this change also appeared before the erythroid hyperplasia. Some disturbance of the maturation curve was seen in the marrow in all three sheep even after the erythrocytes had returned to their initial level. In the case of sheep 50, the early normoblasts were found to be higher than at the beginning of the experiment, although 30 days had elapsed since the last bleeding.

Samples taken from sheep V. 35 and E. 63 , 6 weeks and 9 weeks respectively after the last withdrawal of blood showed that late normoblasts were still higher than had been found in samples taken before bleeding started. These findings suggest that the maturation curve may be a sensitive guide to marrow response to blood loss and/

and should always be included in the examination of the marrow of the sheep.

These marrow changes were accompanied during the stage of maximum marrow response by signs of regeneration in the peripheral blood, but persisted much longer than the blood changes. It was noteworthy that only at the peak of marrow stimulation did nucleated red cells appear in the blood, and then only in small numbers, and they quickly disappeared once recovery set in. This indicates that the demand for erythrocyte replacements was met by a speeding up of maturation in the marrow, so that relatively few very immature cells escaped into the peripheral blood. This power of the sheep's marrow to accelerate the ripening of its erythroblasts was also demonstrated by the fact that once the removal of blood was stopped there was a rapid reduction in the incidence of reticulocytes and cells showing punctate basophilia and polychromasia. My results in this respect confirm the findings described by Wirth (1938) in a comparative study of the powers of regeneration in the domestic animals. He showed that although the sheep can recover normal levels following blood loss as quickly as any other species, the signs of regeneration in the peripheral blood disappear much more quickly than is the case in the dog, cat, or pig. He also recorded the infrequence of nucleated red cells in the blood during regeneration. Coffin (1944), as the result of subjecting sheep to repeated weekly bleedings, noted that most animals withstood a 'rather high' level of bleeding for an appreciable period before anaemia became apparent. This adds further confirmation to the conclusion that the marrow of the sheep is capable of/

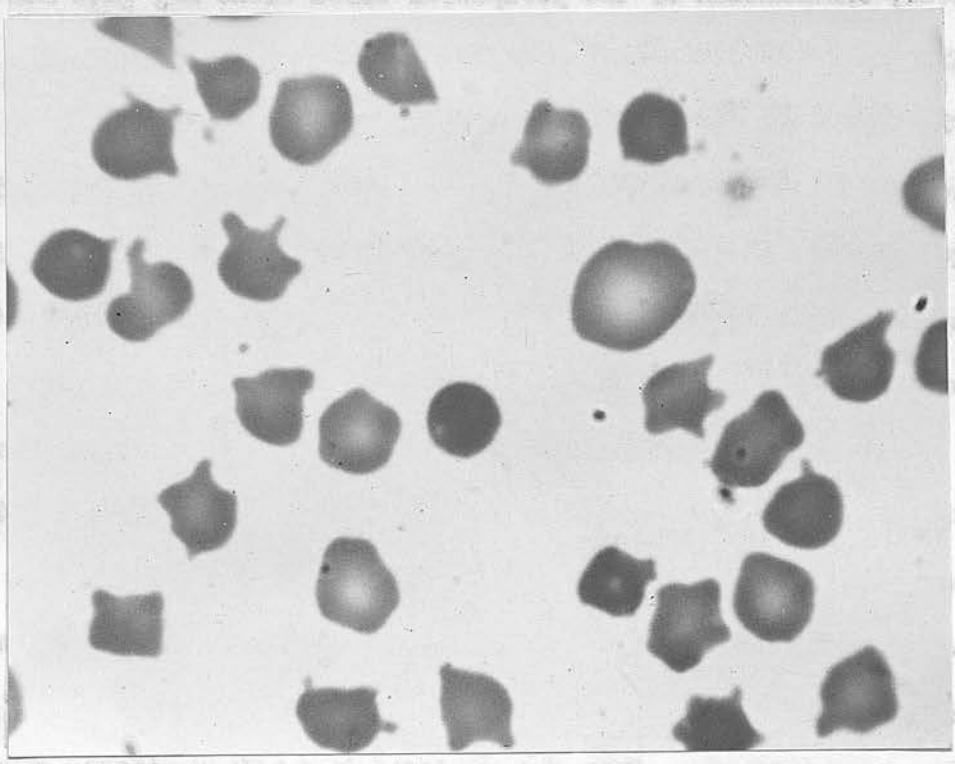


Fig. 23b. Erythrocytes showing anisocytosis and poikilocytosis.
(× 2.500)

for reticulocyte examination in the sheep.

The anisocytosis found in the blood films during the anaemic phase in my sheep (Fig. 23b) has been reported by a number of previous workers (Fourie, 1931; Holman, 1945a; Bennetts & Beck, 1942; and others) as a constant feature in anaemia in sheep. Holman has pointed out that the normocytic anaemia usually found following blood loss is due to macrocytes and microcytes balancing each other. This was found to be the case in sheep E. 63 and V. 35, but there was a tendency in sheep 50 for the anaemia to be macrocytic, and this, it is suggested, was due to the preponderance of immature cells which appeared as macrocytes.

Conclusions.

From the results of bioptic marrow examination of sheep made anaemic by bleeding it was concluded as follows:-

1. It was possible from an examination of material obtained by sternal puncture to detect the marrow response to blood loss.

2. The marrow response consisted of an erythroid hyperplasia accompanied by an increase in the more primitive erythroblastic elements.

3. The marrow changes bore a close relation to those seen in the peripheral blood.

4. There was some evidence to show that in the sheep, the response of the marrow to blood loss included a speeding up of maturation in the bone marrow.

5. It was possible to detect a residual disturbance of the marrow picture after the blood picture had returned to normal.

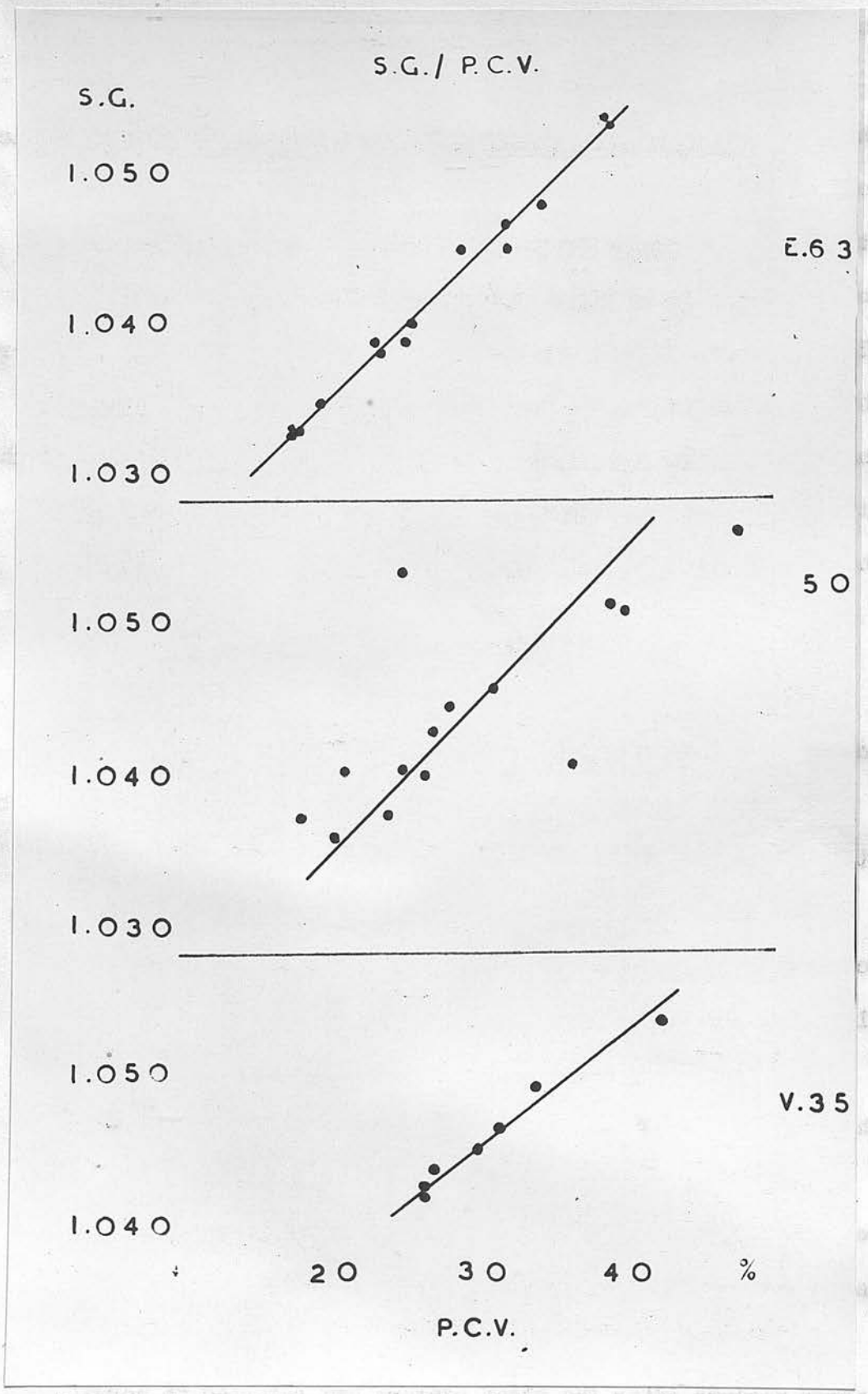


Fig. 24. Showing correlation of Specific Gravity and packed cell volume.

Specific Gravity of Blood and Plasma.

a. Correlation between Specific Gravity of Blood and Packed Cell Volume.

Table XX. shows the values obtained for correlations between specific gravity of blood and packed cell volume in respect of the three sheep E. 63, 50, and V. 35.

The dot diagrams in Fig. 24 shows the correlation between specific gravity and packed cell volume in the three sheep. The regression lines are drawn from the values obtained using the equation $y = a + b(x - \bar{x})$ where P.C.V. = y and S.G. = x.

Table XX.

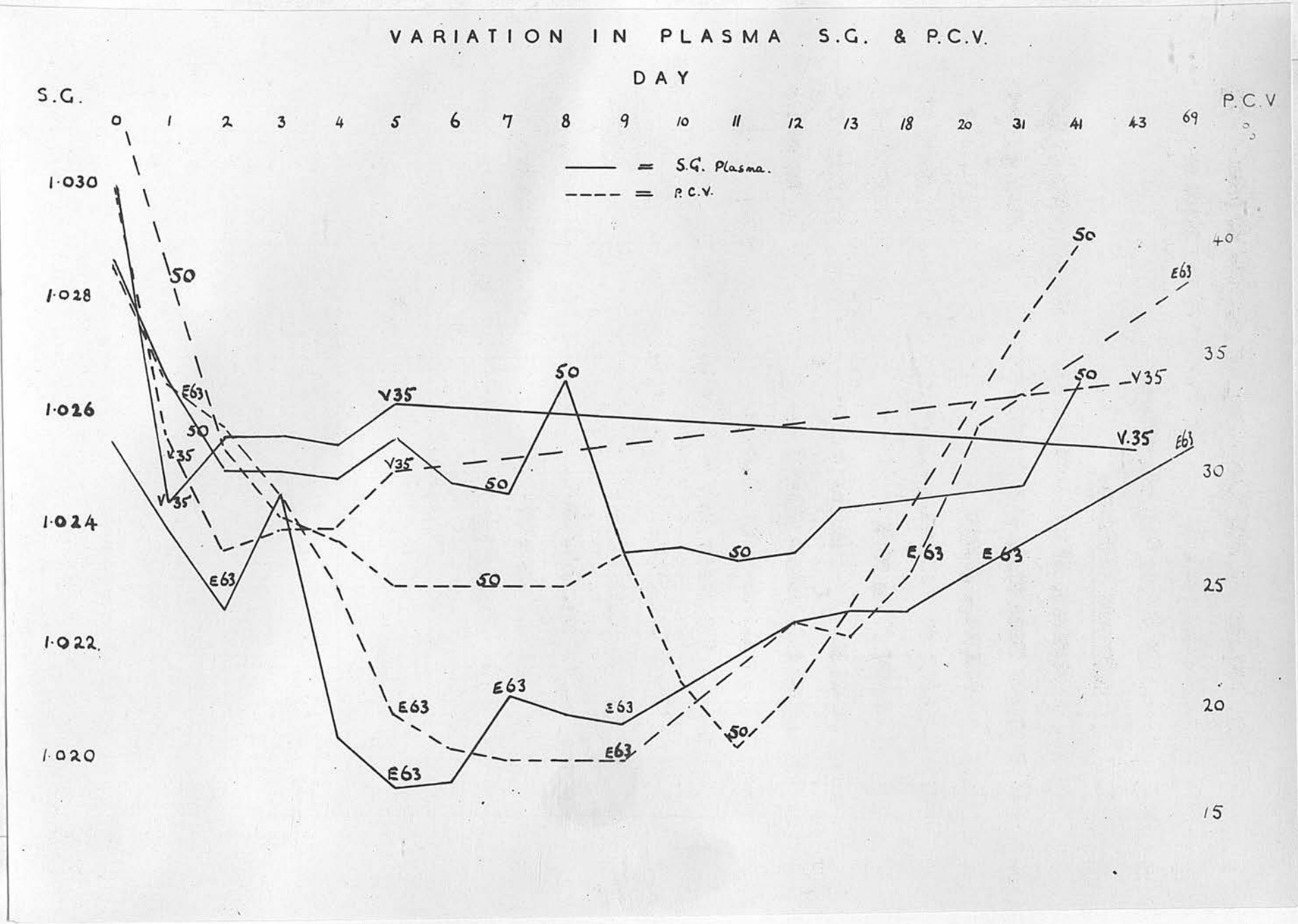
Values obtained for correlations between Specific Gravity of Blood and Packed Cell Volume.

Sheep's Number	E. 63	50	V.35
Number of samples examined	13	16	7
Coefficient of Correlation between S.G. of Blood & Packed Cell Volume	0.98 **	0.76 **	0.99 **
Average Specific Gravity ($\bar{x}$)	1.04172	1.04339	1.04609
Average packed cell Volume (a)	27.31	28.68	31.36
Regression (b) of packed cell Volume on Specific Gravity	0.105	0.098	0.134

Algebraic symbols in brackets refer to the regression equation $y = a + b(x - \bar{x})$ where x = S.G. and y = P.C.V.

** Significant @ 1%.

Fig. 25.


VARIATION IN PLASMA S.G. & P.C.V.
DAY
S.G.
P.C.V
0 1 2 3 4 5 6 7 8 9 10 11 12 13 18 20 31 41 43 69
1·030
1·028
1·026
1·024
1·022
1·020
40
35
30
25
20
15
= S.G. Plasma.
= P.C.V.
50
E63
V35
V.35

These results indicate that it may be possible to predict the Packed Cell Volume from the Specific Gravity of the blood as estimated by the Copper Sulphate method.

b. Specific Gravity of Plasma.

The fluctuations in the level of the specific gravity of the plasma in the three sheep is shown in Fig. 25, in which are also shown the successive changes in packed cell volume. It will be seen that there is some measure of agreement between the shape of the two curves; thus, as the packed cell volume falls there is a corresponding reduction in the specific gravity of the plasma, and as the packed cell volume rises there is an increase in the specific gravity of the plasma. The explanation for the reduction in the S.G. values in the plasma following the bleeding lies in the dilution of the constituents of the plasma by the fluid which passes from the tissues to restore total blood volume. With the restoration of corpuscular volume which occurred once bleeding was discontinued, there was a concurrent return of the plasma constituents to more normal levels, and a consequent rise in the values for plasma specific gravity.

Section IV.

A Study of the Weekly Variation in the Erythron and Leukon in Sheep

a. On a diet providing for maintenance and production.

b. On a diet providing half maintenance.

The object of this experiment was twofold. First to investigate the weekly variation likely to be encountered in normal sheep on a full diet, and secondly to test the influence on this variation of a marked reduction in nutrition. It was considered that before the significance of variation between individual sheep could be assessed it was necessary to examine the variation likely to occur in a series of samples taken from the same sheep. The reason for investigating the variation of the marrow picture in sheep on a low plane of nutrition was that the significant changes reported in Section I as occurring in the peripheral blood had taken place at a time of the year when the nutritional level was low. Under natural conditions low nutrition is often associated with increased parasitism. It is at least theoretically possible for both these factors to influence erythropoiesis. It was therefore considered desirable to observe the changes in blood and bone marrow occurring in sheep on a low plane of nutrition under conditions in which parasitism was kept at the lowest possible level.

Material. Six ten month old castrated grey face male sheep were selected at random from a flock of 40 lambs. These six lambs were divided at random into two groups of three. They were numbered by ear ring, E. 98, E. 99 and E. 100, to form Group E; and B. 198, B. 199, and B. 200, forming Group B. The groups were confined in separate indoor pens having concrete floors.

Diet. A week was allowed to elapse between bringing the lambs in from pasture and the start of the experiment, in order to accustom them to the artificial food and environment. During this week the diet consisted of meadow hay ad lib. Water was available in unlimited amounts to all sheep throughout the experiment. Measured diets were started after the sheep had been in the pens one week and the details are as follows:-

1. Group E. was given 4½ lb. of chopped hay and 4 lb. of bruised oats daily. This diet was calculated to provide for full maintenance and production in the form of growth, according to Woodman's (1948) suggested requirements for a sheep weighing 100 lb.

2. Group E. received 4 lb. of chopped meadow hay only per day. This diet was calculated to provide half maintenance only.

No attempt at individual feeding was made, the food being put in a feeding box in the pen. These diets were adhered to throughout the experiment.

Worms. It was recognised that sheep in an enclosed space are particularly liable to build up a heavy worm burden. To control the effects of parasitism certain measures were adopted. During the first week in the pens, and before sampling commenced, worm egg counts were carried out on faeces samples from all six sheep, using the Gordon Whitlock technique (Gordon & Whitlock, 1939). The results of these counts showed the lambs to be carrying a light worm burden, and each lamb was dosed with 20 gm. of Phenothiazine. Two subsequent worm egg counts were carried out, one half-way through the experiment, and one at the end. To prevent faecal contamination the food was fed from boxes and the pens were swept out every three days.

Sampling. Samples were taken on the day before controlled feeding started and thereafter at weekly intervals for six weeks. All six sheep were sampled on the same day; Group B. in the morning and Group E. in the afternoon. At each sampling blood and bone marrow were collected. Using the techniques already described in Section I. blood was collected from the jugular vein and subjected to the following examination:- P.C.V., Hb., R.B.C., W.B.C., and D.L.C.

Marrow samples were obtained by sternal puncture as previously described in Section II. The second to the sixth sternebrae were used for aspiration of marrow, and each sternebra was given at least three weeks in which to recover from the effects of the previous puncture before being used again. The examination of the material so collected consisted of total nucleated cell counts on marrow blood, and differential marrow cell counts on spread preparations. An estimate of cellularity of the marrow spreads was also made. Sections were cut from aggregated marrow flecks on three occasions during the experiment. The procedures used for preparation and examination were those already described in Section II. From the results of the differential marrow counts haemomyelograms and maturation curves were constructed, and the incidence of mitosis seen during differential counts was recorded.

Results. *

a. Weekly Variation on a diet providing full maintenance and production. Group E.

Weight. The weekly weights for the three sheep in Group E. are shown in Table XXII.

* The full data are given in the Appendix pages A.24 to A.29.

Table XXI.

Weekly weights of sheep in Group E.

Sheep's No.	Initial weight in lbs.	Weight in lbs at the end of;-					
		1st week	2nd week	3rd week	4th week	5th week	6th week
E.98	108½	107	108¾	104¾	107	107½	108¾
E. 99	91¾	92¼	91	90	92	91	92½
E.100	109¾	110¼	109½	109¼	103½	105½	106

There was marked fluctuation in the weekly weights in all three sheep. Both E. 98 and E. 99 showed some loss in weight, which was most marked at the end of the third week. The weights of both sheep at the last sampling however were above the initial weights. In the case of E. 100 there was a fall in weight at the end of the fourth week and at the end of the sixth week the weight was found to be 3¾ lb. below the weight at the start of the experiment. From the weights for these sheep it may be said that the diet roughly maintained body weight, but allowed no gain, although the diet was calculated to provide for both maintenance and production in the form of weight gain. This was accounted for by the fact that almost invariably the sheep refused to consume completely the hay component of the ration.

Worms. The results of the worm egg counts made before dosing with Phenothiazine, during the 4th week of the experiment, and at the/

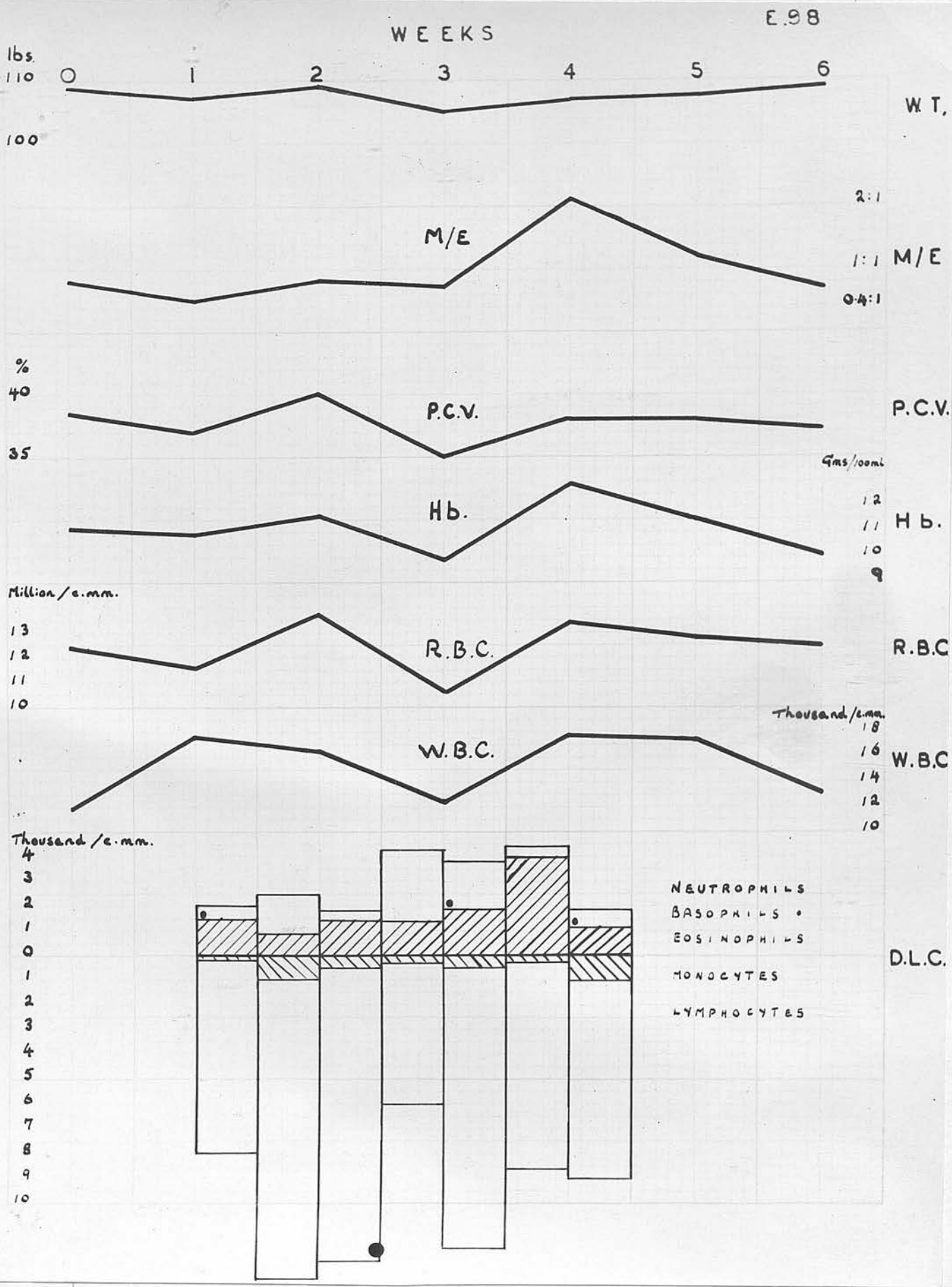

E.98
WEEKS
lbs.
110
100
0
1
2
3
4
5
6
W.T.
M/E
2:1
1:1
0.4:1
M/E
%
40
35
P.C.V.
P.C.V.
Gms/100ml.
12
11
10
9
Hb.
Hb.
Million/c.mm.
13
12
11
10
R.B.C.
R.B.C
Thousand/c.mm.
18
16
14
12
10
W.B.C.
W.B.C
Thousand/c.mm.
4
3
2
1
0
1
2
3
4
5
6
7
8
9
10
NEUTROPHILS
BASOPHILS
EOSINOPHILS
MONOCYTES
LYMPHOCYTES
D.L.C.

Fig. 26.

the time of final sampling are shown in Table XXII.

Table XXII.

Worm Egg Counts

before dosing, during 4th week of experiment, and at final sampling.

Sheep's No.	Before dosing	During 4th week	At final sampling
	Eggs per gm.		
E. 98	2,000	200	0
E. 99	1,400	400	0
E. 100	7,100	900	0

The results show that the methods adopted for the control of worms in these sheep were successful and that parasitism was negligible throughout the experiment.

Peripheral Blood.

The results of the examination of the peripheral blood of Group E. are shown in the form of graphs. Figure 26. shows the results of E. 98; Fig. 27. those of E. 99, and Fig. 28. those for E. 100. These figures also show the weight curves and the fluctuations in M/E ratio.

Holman (1944b) has calculated for the various haematological constituents a value which he has called the Maximum Admissable Difference (M.A.D.) as a guide to the daily and monthly variation of these constituents in individual sheep. He has defined the M.A.D./

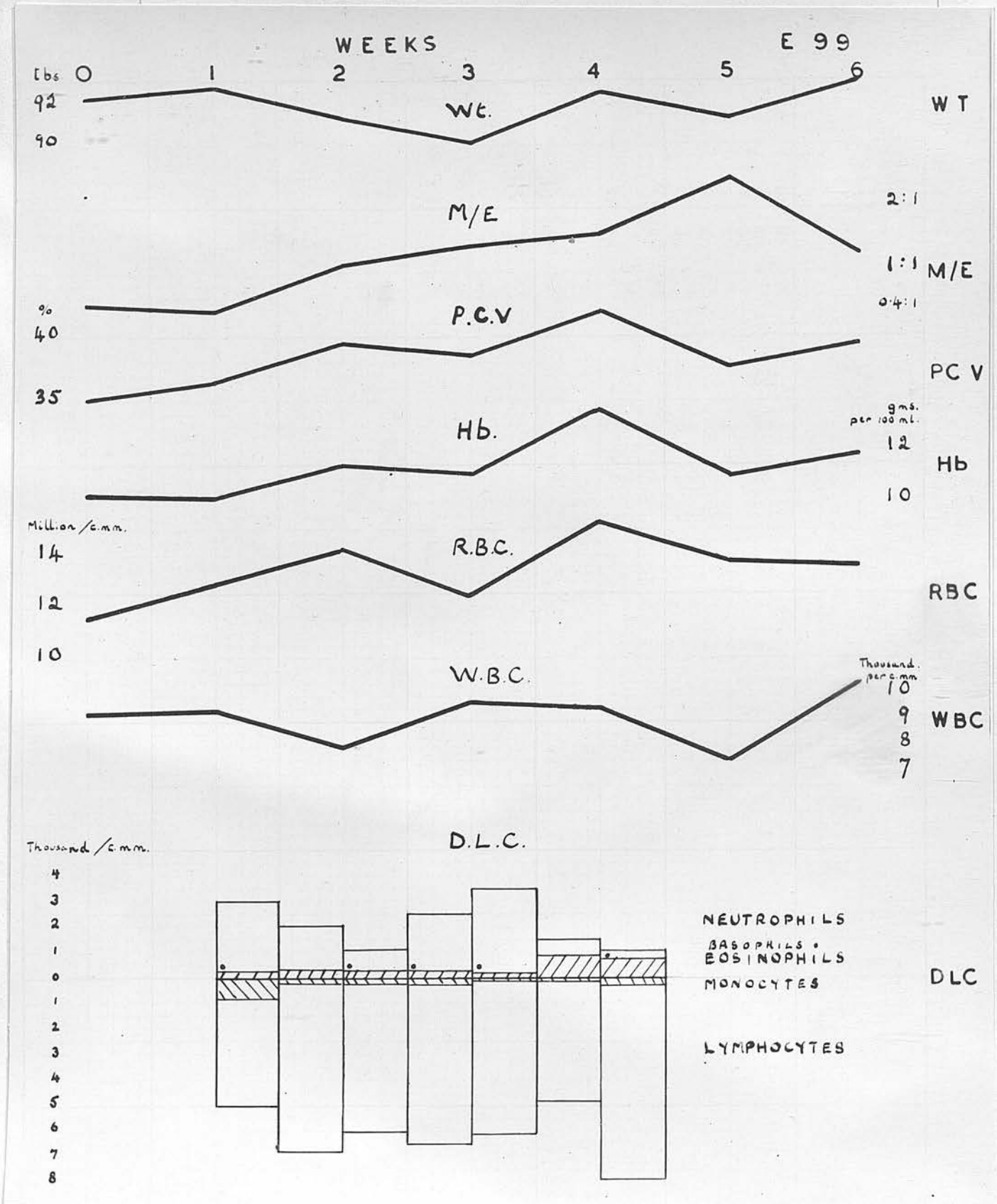

WEEKS
E 99
lbs
0
1
2
3
4
5
6
92
90
Wt.
WT
M/E
2:1
1:1
0·4:1
M/E
%
40
35
P.C.V
PC V
gms. per 100 ml.
Hb.
12
10
Hb
Million/c.mm.
14
12
10
R.B.C.
RBC
Thousand per c.mm
W.B.C.
10
9
8
7
WBC
Thousand/c.mm.
D.L.C.
4
3
2
1
0
1
2
3
4
5
6
7
8
NEUTROPHILS
BASOPHILS
EOSINOPHILS
MONOCYTES
LYMPHOCYTES
DLC

Fig. 27.

(95%)

M.A.D./as being such that the difference of two readings made on the same sheep at an interval of 24 hours or one month should not exceed these respective values in more than 5% of the cases in the absence of some interfering factor.

To test the significance of the variations observed in the blood picture of the three sheep, in the case of each sheep pairs of readings taken at four-weekly intervals were compared. Thus, the figures for the samples taken on the first day were compared with those for the fifth sampling, and so on. The significance of the difference between such pairs of readings was assessed by comparison with Holman's M.A.D. (monthly) for the constituent under consideration. As there were only seven samples taken, there were no values with which to compare those taken at the end of the 3rd week. These observations were therefore compared with those for the first and last samplings, although they were only separated from the observations made at the end of the 3rd week by three instead of four weeks.

By this method of analysis it was found that the M.A.D. was exceeded in respect of some components in one or more samples, in all three sheep.

In E. 98 the variation in P.C.V., Hb., and R.B.C. was within the M.A.D. for these properties, but in the sample collected at the end of the 3rd week a significant rise in the neutrophil count was found; this neutrophilia however was not accompanied by any shift to the left and was well within Holman's range for normal sheep. It was not considered permissible to analyse the variations occurring in the Eosinophil count of this sheep on the basis of Holman's/

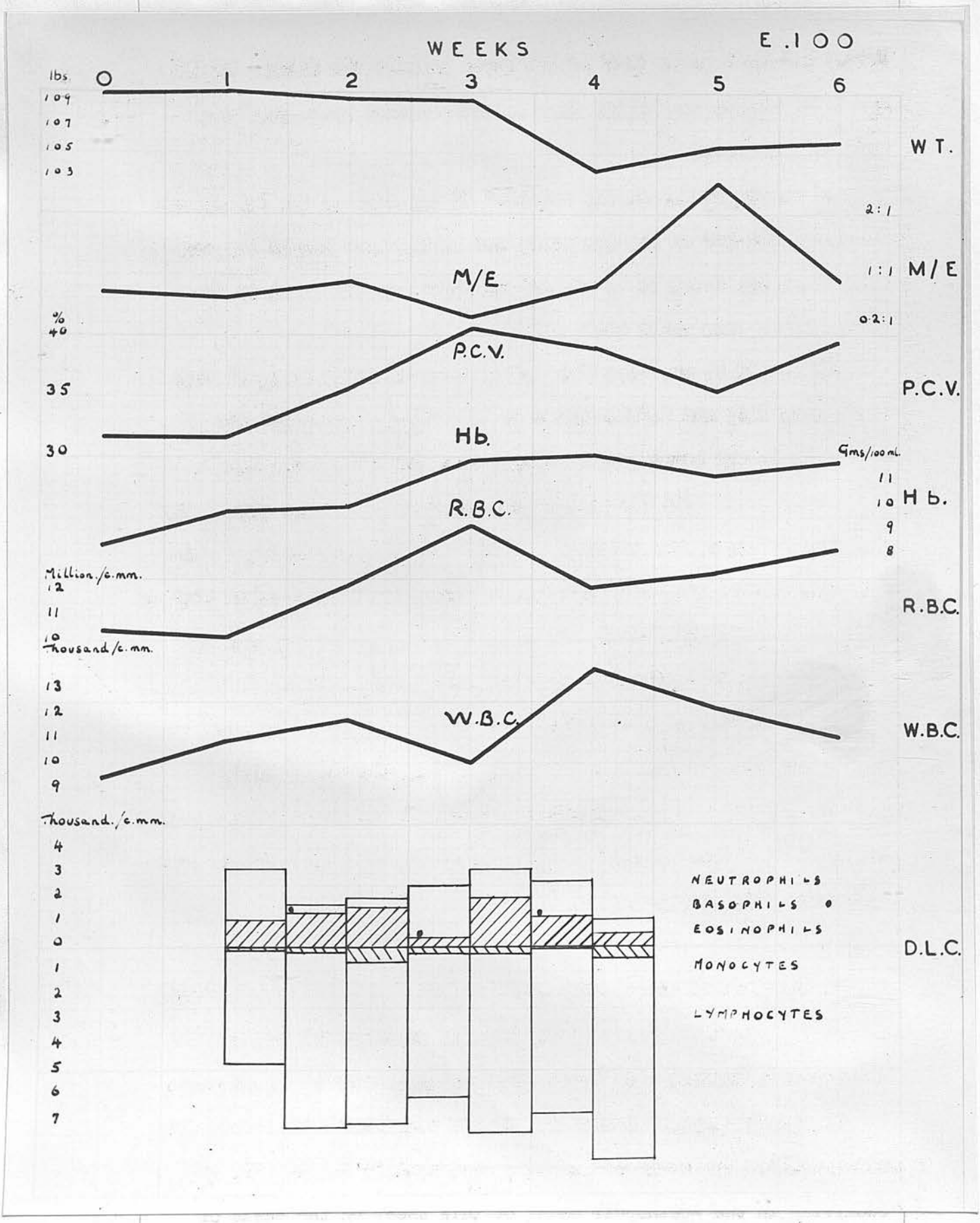

E.100
WEEKS
0 1 2 3 4 5 6
lbs.
109
107
105
103
WT.
2:1
1:1
0.2:1
M/E
%
40
35
30
P.C.V.
Hb.
Gms/100 ml.
11
10
9
8
Hb.
Million/c.mm.
12
11
10
R.B.C.
Thousand/c.mm.
13
12
11
10
9
W.B.C.
Thousand./c.mm.
4
3
2
1
0
1
2
3
4
5
6
7
NEUTROPHILS
BASOPHILS
EOSINOPHILS
MONOCYTES
LYMPHOCYTES
D.L.C.

Fig. 28.

Holman's M.A.D. as in four of the seven samples the figure for the eosinophil count was higher than Holman's normal range for this cell in the sheep.

In E. 99 the M.A.D. was exceeded in the case of the P.C.V. and R.B.C. at the end of the 4th week, and in the same sample the eosinophil count was found to be significantly higher than that in the sample taken four weeks previously.

In E. 100 it was also found that there was a significant rise in P.C.V., Hb., and R.B.C. at the end of the 4th week, but the variation in the leucocytes in this sheep fell within the M.A.D.

It was concluded from the foregoing analysis of the results of the examination of the blood of the three sheep that the variations encountered were generally those to be expected in successive samples from the same sheep, but that in E, 99 and E. 100 there was a tendency for a polycythaemia to develop at the end of the 4th week, and this is illustrated in the curves for the erythrocytic properties in Figs. 27 and 28. This polycythaemia was probably associated with a slight anhydraemia caused by a reduction in fluid intake in these two sheep. No cause could be found for the eosinophilia which was discovered in E. 98.

Marrow.

The results of the examination of the marrow will be considered under the following headings:- 1. Cellularity. 2. M/E ratio, 3. Maturation Curves. 4. Total nucleated cell count. 5. Mitosis.

1. Cellularity. Evaluation of the cellularity of the marrow spreads, using the standards already described in Section II, was carried/

carried out for all marrow samples collected, and the results are given in Table XXIII.

Table XXIII.

Grading of Cellularity of Marrow Spreads.

Sheep's No.	Initial grading	Grading of Marrow Cellularity at the end of week					
		1	2	3	4	5	6
E. 98	IV.	III.	IV.	IV.	I.	II.	IV.
E. 99	IV.	IV.	IV.	IV.	II.	III.	II.
E. 100	IV.	IV.	III.	IV.	III.	II.	I.

It was considered that the method used to assess cellularity gave great scope for subjective bias, and to avoid this, examination of the spreads was not carried out until the end of the observations so that all the slides could be re-numbered by an independent person prior to examination. Thus, the grading of the cellularity was made without the pre-knowledge of the sheep from which the slide came or of the week in which the marrow was collected. Table XXIII. shows that in the first four samples the cellularity was dense, but thereafter the spreads became less cellular, with only one preparation graded as 'IV'..

2. Myeloid/Erythroid Ratio. For each marrow examined the M/E ratio was calculated from the results of the differential cell count, details of which appear in the Appendix, pp. A.24. A.26 - A.28. The weekly variations for the three sheep are shown in Table XXIV. and Figs. 26, 27, and 28.

Table XXIV.
Myeloid/Erythroid Ratio.

Sheep's No.	Myeloid/Erythroid Ratio in marrow at:-						
	Initial Sampling	The end of 1st week	2nd week	3rd week	4th week	5th week	6th week
E.98	0.8 :1	0.5 :1	0.8 :1	0.7 :1	2.1 :1	1.2 :1	0.7 :1
E. 99	0.5 :1	0.4 :1	1.1 :1	1.4 :1	1.6 :1	2.5 :1	1.3 :1
E. 100	0.7 :1	0.6 :1	0.9 :1	0.3 :1	0.8 :1	2.5 :1	0.9 :1

From Table XXIV. it will be seen that the ranges for the M/E ratio for E. 98, E. 99, and E. 100 were 0.5 - 2.1, 0.4 - 2.5, and 0.3 - 2.5 respectively. Examination of the weekly variation for the three sheep shows that the range is considerably narrowed by the exclusion of the maximum value when the ranges become:- E. 98, 0.5 - 1.2; E. 99, 0.5 - 1.6; and E. 100, 0.3 - 1.1. In sheep E. 98 this 'peak' value was obtained at the end of the 4th week, whereas in E. 99 and E. 100 it appeared at the end of the 5th week. That these 'peaks' in the M/E ratio are not due to any proliferation of granulocytic tissue is shown by the fact that there was a decrease in the cellularity of the spreads at this time, furthermore, there was no sign of a shift to the left in the granuloblasts. It

follows/

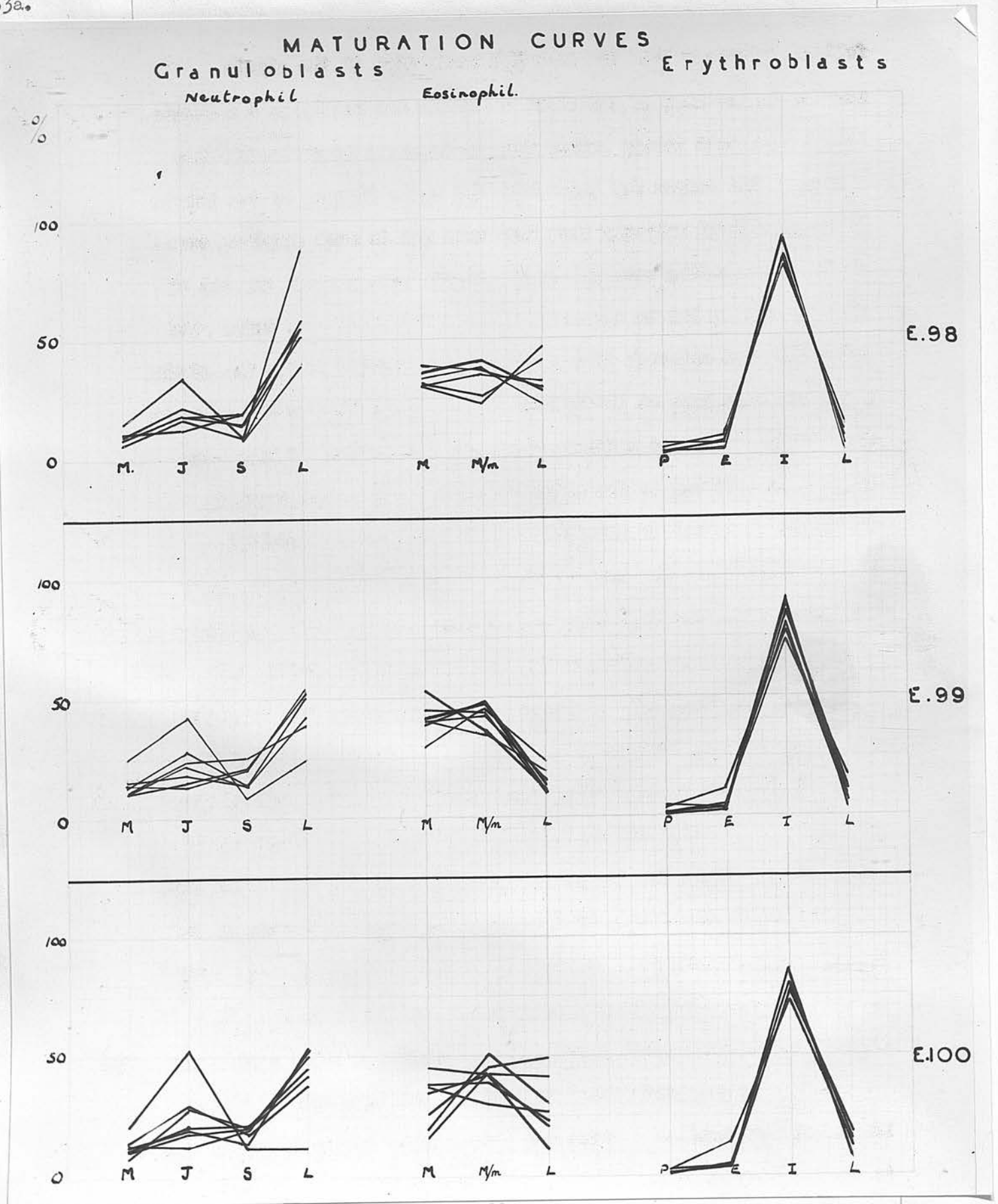

MATURATION CURVES
Granuloblasts
Neutrophil
Eosinophil.
Erythroblasts
%
100
50
0
M. J S L
M M/m L
P E I L
E.98
E.99
E.100

Fig. 29.

follows therefore that the high M/E ratio seen in the single samples in the case of all three sheep are due mainly to a reduction in the erythroid tissue rather than an increase in granuloblastic tissue. The reason for these high M/E ratio figures is not clear. It is considered unlikely that they were due to some sampling error, as the high reading occurred in all three sheep towards the end of the experiment, but in one sheep it was recorded a week before the other two, and although it constituted an abrupt rise for two of the sheep the peak came at the end of a gradual rise in the M/E ratio in the third. It seems probable that this phenomenon may have been related in some way to the artificial feeding and management to which the sheep had to adjust itself, but the factor involved remains obscure. The importance of the finding lies in the fact that when sheep are kept under artificial conditions such as these, this may be a common variation for which allowance must be made.

3. Maturation Curves. Maturation curves were constructed for the erythroblasts, neutrophil granuloblasts, and eosinophil granuloblasts in respect of the seven marrow examinations for each of the three sheep. These are shown in Fig. 29. The variation in the shape of the curves for the granuloblasts is seen to be greater than that for the erythroblasts in all three sheep. In the case of the eosinophil granuloblasts the range of variation was very wide both in the same sheep and between the sheep. On the other hand the curves for the neutrophil granuloblasts showed a greater measure of agreement, the greatest variation being in the incidence of the lobulated neutrophils. There was a remarkable degree of constancy in the maturation curves of the erythroblasts in all three sheep.

In/

In this connection it was noted that in the examination of the spreads there was a tendency for the erythroblasts to be seen in columns in which the cells appeared in roughly the same proportions as shown by the maturation curves, whereas there appeared to be no such orderly arrangement for the granuloblasts. This fact may be a contributory cause for the difference in the variations encountered in the maturation curves for these two groups of cells.

4. Nucleated cell count in Marrow Blood. Table XXV. shows the weekly nucleated cell count in the marrow blood of the three sheep.

Table XXV.

Nucleated Cell Counts in Marrow Blood.

Sheep's No.	Nucleated cell count of marrow blood in thousands per cu.mm. in the:-						
	Initial Sample	Sample taken at the end of 1st week	2nd week	3rd week	4th week	5th week	6th week
E. 98	257	122	144	51	39	129	101
E. 99	266	C	127	117	37	10	48
E.100	210	121	37	154	145	C	C

C = clotted sample.

The highest counts in all three sheep were made at the first sampling, and thereafter, although there was some variation from week to week, the counts were all lower than those of the first sampling. No explanation for this can be found, but it is probably associated with the artificial conditions of husbandry, the effect of which has already/

already been postulated in relation to the variation in the M/E ratio. It was not found possible to correlate the nucleated cell counts with the gradings for cellularity in the spreads. At first sight it might be supposed that the quantitive cell count of the marrow blood would give useful information on marrow activity, but Dacie & White (1949) have drawn attention to the wide variation experienced in the figures for the human marrow cell counts, a fact which is confirmed for the sheep by my results. The variation is attributed by Dacie & White to the uncontrollable factor of dilution with peripheral blood, and to the tendency for marrow cells to adhere together in clumps of varying sizes. This latter phenomenon was observed as occurring in the counting chamber whilst carrying out the counts in my sheep. Reich & Kolb (1942), in a quantitive study of the variations in multiple sternal marrow samples taken simultaneously have also shown that the technique is extremely inaccurate. The lack of correlation between nucleated cell counts in marrow blood and the degree of cellularity for marrow spreads which has been shown in the three sheep sampled in this experiment, supports the view advanced by the writer (19 51) that the application of the results of quantitive estimations made on marrow blood to the results of qualititive examinations of marrow spreads is illogical. It is therefore considered that no useful purpose is served by the estimation of numbers of nucleated cells in marrow blood in the sheep.

Mitosis. Table XXVI shows the variation observed in the incidence of cells seen in mitosis during the differential marrow counts for the three sheep. In some preparations no cell mitosis was seen, while the greatest number observed in division was 0.7%. Mitosis was seen more frequently among the erythroblasts than the granuloblasts in E. 99 and E. 100, but in E. 98 the position was reversed. Among the granuloblasts no cell beyond the stage of myelocyte was observed in mitosis, while all ages of erythroblast, except the late normoblast, were seen in division. This is in agreement with the findings of many previous workers investigating mitosis in marrow cells (Leitner, 1949; Dacie & White, 1949, and others). The erythroblast most commonly seen in mitosis was the intermediate normoblast.

Table XXVI.

Incidence of Mitosis
among Granuloblasts and Erythrocytes

Sheep's No.	Series of cell	Percentage of cells seen in division at:-						
		Initial sampling	The end of each week					
			1st	2nd	3rd	4th	5th	6th
E. 98	Granuloblast	0.3	0.0	0.1	0.4	0.1	0.1	0.3
	Erythroblast	0.0	0.0	0.0	0.1	0.2	0.2	0.0
	Total	0.3	0.0	0.1	0.5	0.3	0.3	0.3
E. 99	Granuloblast	0.0	0.0	0.2	0.0	0.0	0.3	0.2
	Erythroblast	0.2	0.2	0.0	0.1	0.0	0.2	0.3
	Total	0.2	0.2	0.2	0.1	0.0	0.5	0.5
E.100	Granuloblast	0.1	0.0	0.1	0.0	0.0	0.2	0.1
	Erythroblast	0.3	0.4	0.1	0.7	0.1	0.0	0.3
	Total	0.4	0.4	0.2	0.7	0.1	0.2	0.4

The total number of cells seen in division during the examination of the 21 spreads was 58. Of these, 56.8% were in the prophase, 8.6% in the metaphase, 22.6% in the anaphase, and 12% in the telophase.

Results. *

b. Weekly variation on a diet providing half maintenance (Group B.)

Weights. The weekly weights for the sheep in Group B. are shown in Table XXVII.

Table XXVII.

Weekly Weights of Sheep in Group B.

Sheep's No.	Weight in lbs. at						
	Initial sampling	The end of 1st week	2nd week	3rd week	4th week	5th week	6th week
B.198	101	96	97	91¼	91¼	89½	85
B.199	111¾	108	106¾	102¾	102½	101½	101
B.200	104¼	102¾	100¼	95¼	94	91	87

There was a progressive loss of weight in all three sheep. It was greatest in B. 200, in which it ammounted to a loss of 16.7%. In B. 198 the loss was 15.8% and in B. 199, 9.6%.

Worms. The results of the worm egg counts made before dosing with Phenothiazine, during the 4th week and at the time of the final sampling, appearing in Table XXVIII. show that the control measures adopted against the development of a worm burden likely to affect the results of the blood and marrow examination were successful.

* The full data are given in the Appendix pages A.30 to A.33.

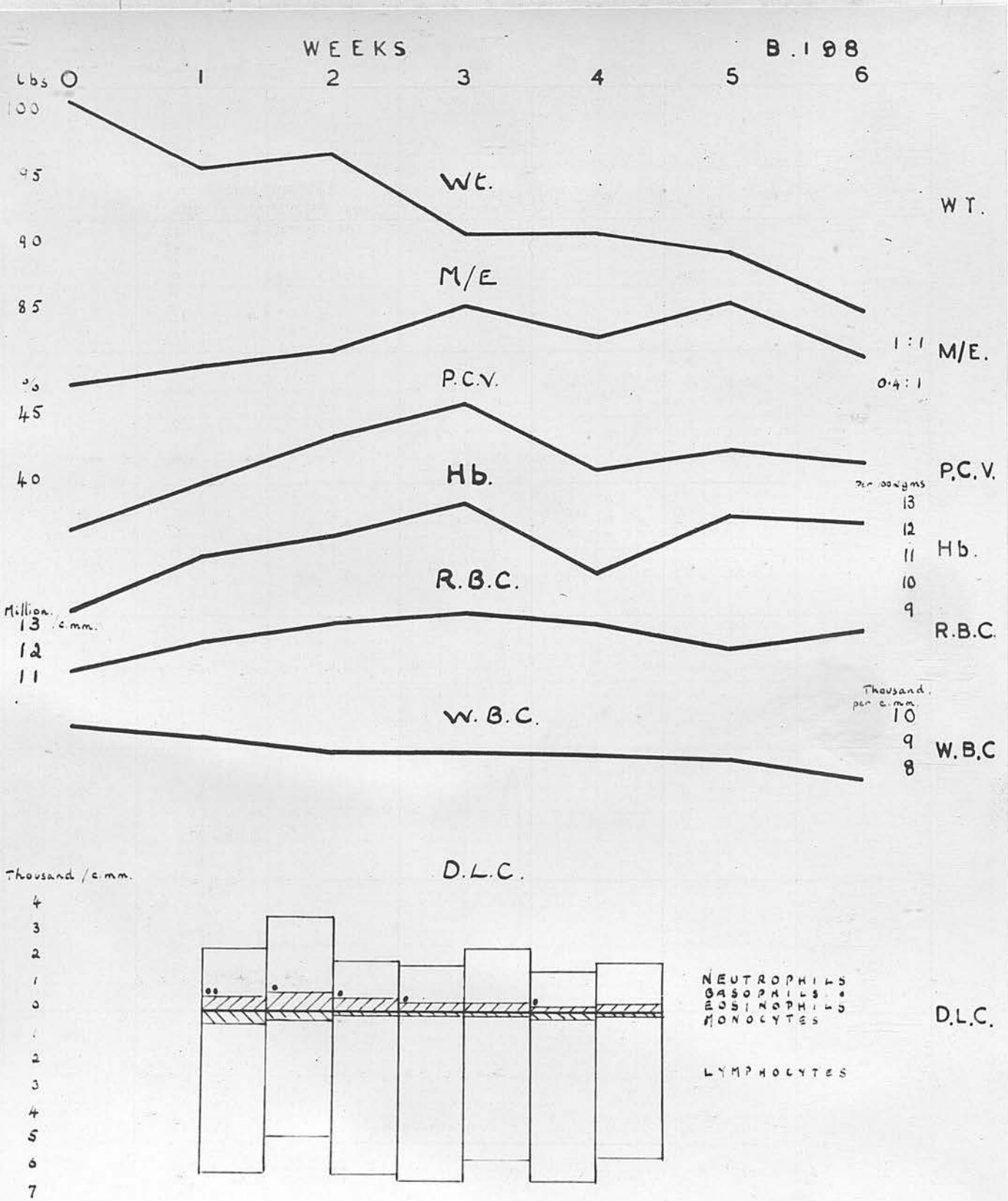

WEEKS
B.188
Lbs
0
1
2
3
4
5
6
100
95
90
85
80
75
45
40
Wt.
M/E
P.C.V.
Hb.
R.B.C.
W.B.C.
WT.
1:1
0.4:1
M/E.
P.C.V.
Hb.
R.B.C.
W.B.C
Million c.mm.
13
12
11
Thousand per c.mm.
10
9
8
per 100 gms
13
12
11
10
9
D.L.C.
Thousand / c.mm.
4
3
2
1
0
2
3
4
5
6
7
NEUTROPHILS
BASOPHILS
EOSINOPHILS
MONOCYTES
LYMPHOCYTES
D.L.C.

Fig. 30.

Table XXVIII.

Worm Egg Counts
before dosing, during 4th week of experiment,
and at final sampling.

Sheep's Number	Worm eggs per gm. of faeces, collected:-		
	Before dosing	During 4th week	At final sampling
B. 198	1,500	900	0
B. 199	5,100	0	0
B. 200	2,000	0	0

B. 200. Three days after the experiment began, sheep B. 200 was found to be suffering from a septic process involving the right fore foot due to 'foot rot'. As this condition persisted throughout the experiment, it was decided that this animal could not be regarded as 'normal'; therefore the results in respect of this sheep are not included with those for the other sheep.

Peripheral Blood. The variations in the blood picture are shown in graph form in Figs. 30 I. and 31, for B. 198 and B. 199 respectively.

In B. 198 there was a successive rise in P.C.V., Hb., and R.B.C., the highest values being recorded at the end of the 3rd week. The significance of this change was tested by reference to Holman's M.A.D. as described for Group E. The readings in respect of P.C.V. and Hb. at the end of the 3rd week were found to be significantly higher than those at the beginning and end of the experiment.

In/

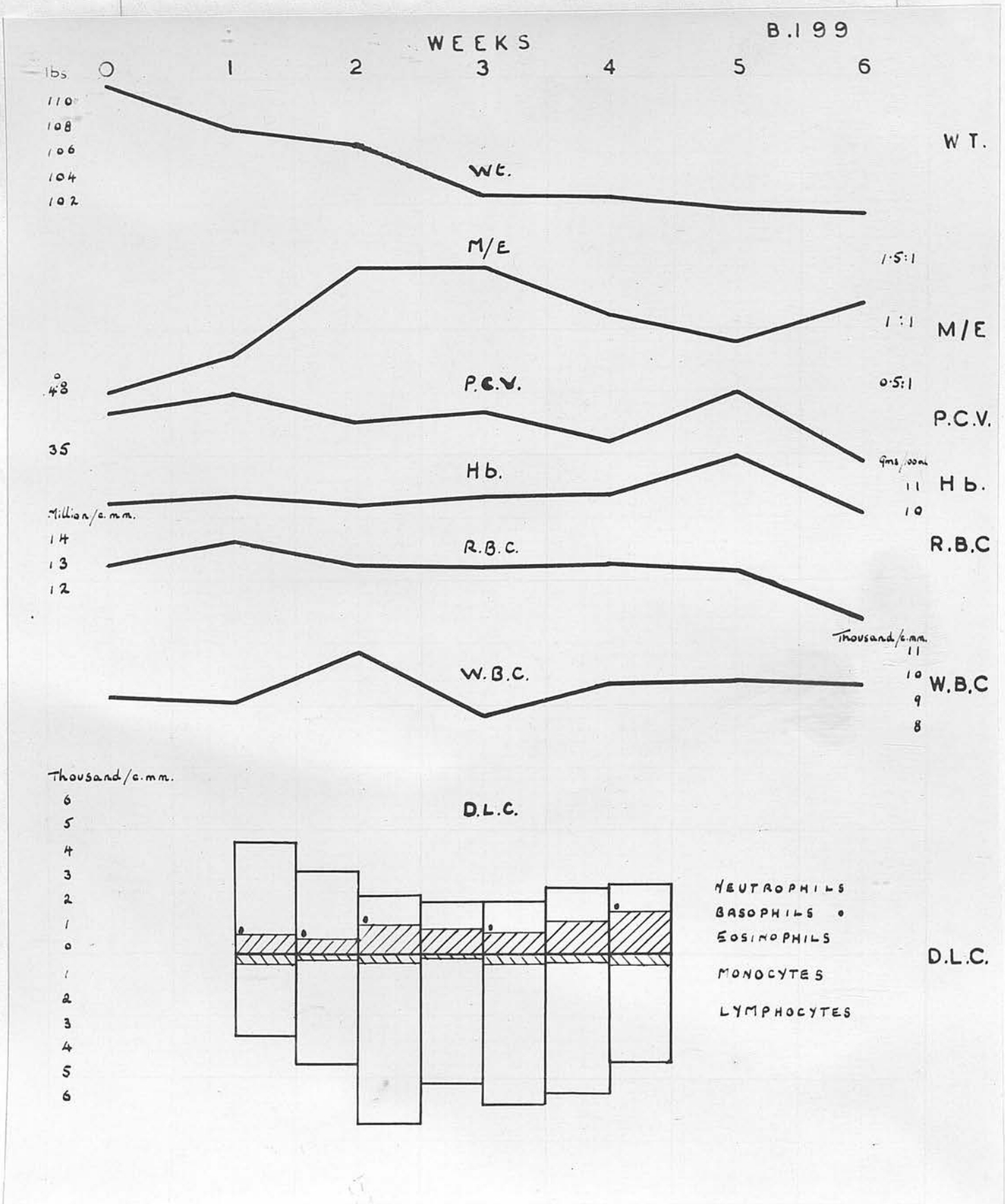

B.199
WEEKS
0
1
2
3
4
5
6
lbs
110
108
106
104
102
Wt.
W T.
M/E
1·5:1
1 : 1
0·5:1
M/E
P.C.V.
48
35
P.C.V.
Hb.
Gms/100c.
11
10
Hb.
Million/c.mm.
14
13
12
R.B.C.
R.B.C
Thousand/c.mm.
11
10
9
8
W.B.C.
W.B.C
Thousand/c.mm.
6
5
4
3
2
1
0
1
2
3
4
5
6
D.L.C.
NEUTROPHILS
BASOPHILS
EOSINOPHILS
MONOCYTES
LYMPHOCYTES
D.L.C.

Fig. 31.

In B. 199 the variations in the erythrocytic properties were found to fall within the range to be expected in successive samples from individual sheep. This also applied to the variation in the leucocytes in both sheep.

It was concluded that in the case of these two sheep the feeding of a diet designed to provide half maintenance, for a period of seven weeks, produced no demonstrable changes in the blood picture, other than a polycythaemia, which was also observed in the controls on a diet providing full maintenance.

Marrow.

Cellularity. In Table XXIX. the results of the evaluation of the cellularity are shown for the 14 spreads from B. 198 and B.199.

Table XXIX.

Grading of Cellularity of Marrow Spreads.

Sheep's Number	Initial grading	Grading of marrow cellularity at the end of the					
		1st week	2nd week	3rd week	4th week	5th week	6th week
B198	IV	II.	II.	III.	III.	I.	I.
B.199	IV.	III.	III.	III.	III.	III.	II.

The grading of the cellularity of these spreads was carried out with the same precautions against bias as were described for the preparations in Group E.

When these results are compared with those for Group E. it is found that although the same high standard of cellularity was shown in/

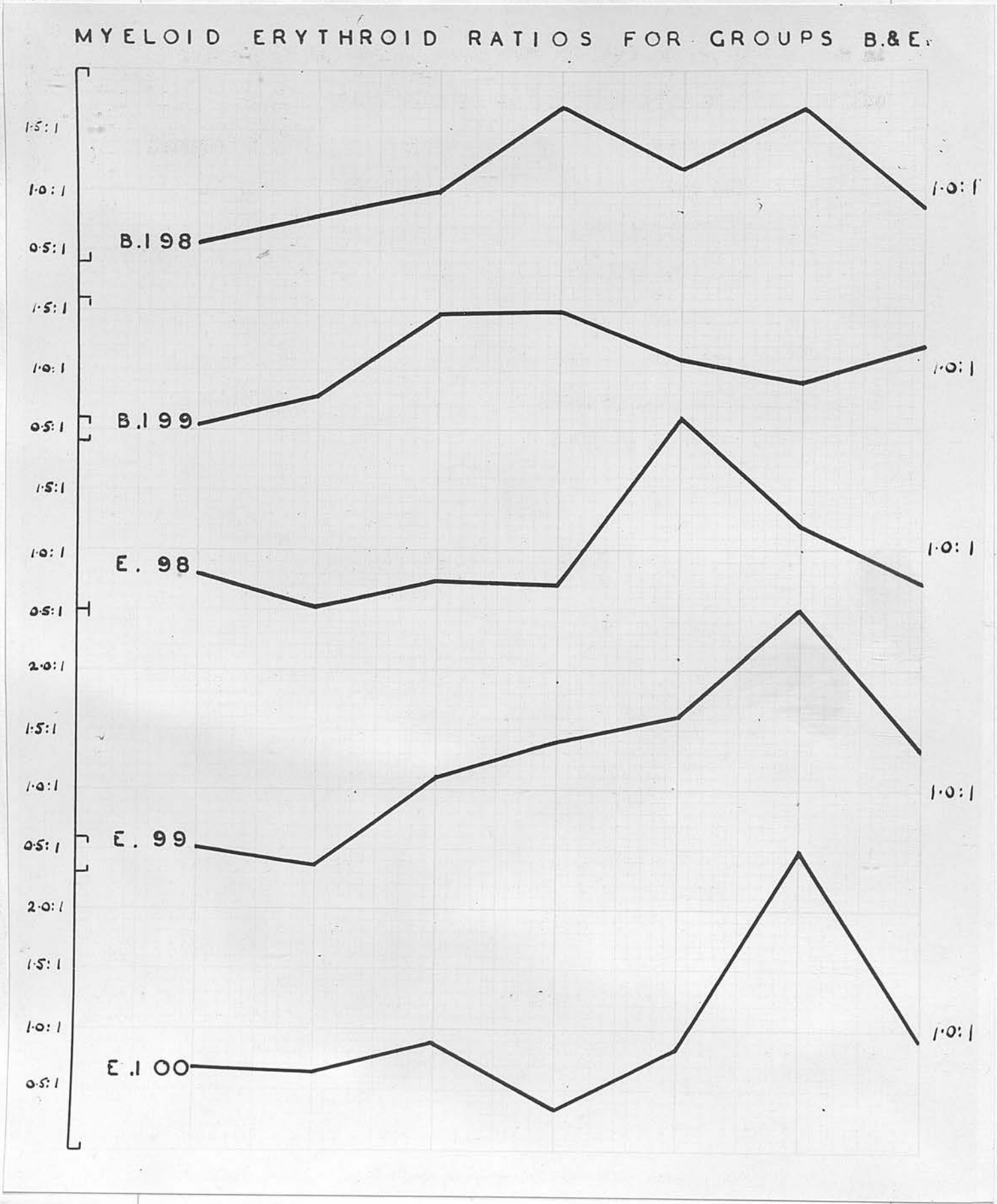

MYELOID ERYTHROID RATIOS FOR GROUPS B.& E.
1·5:1
1·0:1
0·5:1
B.198
1·0:1
1·5:1
1·0:1
0·5:1
B.199
1·0:1
1·5:1
1·0:1
0·5:1
E. 98
1·0:1
2·0:1
1·5:1
1·0:1
0·5:1
E. 99
1·0:1
2·0:1
1·5:1
1·0:1
0·5:1
E.100
1·0:1

Fig. 32.

in the initial samples from all five sheep, B. 198 and B. 199 were subject to a more rapid reduction in the cellularity of their spreads than was shown in the sheep in Group E. and that the overall standard for the spreads, following the initial one was lower in these two sheep than that for the sheep in Group E.

M/E Ratio. The variation in the M/E ratio for the two sheep over the seven samples is shown in Fig.32, which also shows the variation for the sheep in Group E. for comparison. The mean and range of the seven samples in respect of the sheep in both Group B. and Group E. are given in Table XXX.

Table XXX.

Myeloid/Erythroid Ratio
shown as Mean Values & Range for Sheep in Groups B & E.

Sheep's No.	M/E ratio of seven samples examined shown as	
	Mean	Range
B. 198	1.11 : 1	0.5 - 1.7 : 1
B.199	1.07 : 1	0.5 - 1.5 : 1
E. 98	0.97 : 1	0.5 - 2.1 : 1
E. 99	1.25 : 1	0.4 - 2.5 : 1
E. 100	0.64 : 1	0.3 - 2.5 : 1

Fig. 32 shows the same general pattern of variation in both groups, although there is a tendency for the rise in M/E ratio to occur earlier in Group B. than in Group E. From Table XXX. it is seen that the range of variation is less in Group B. than E.

It is considered that the variation in M/E ratio and marrow cellularity/

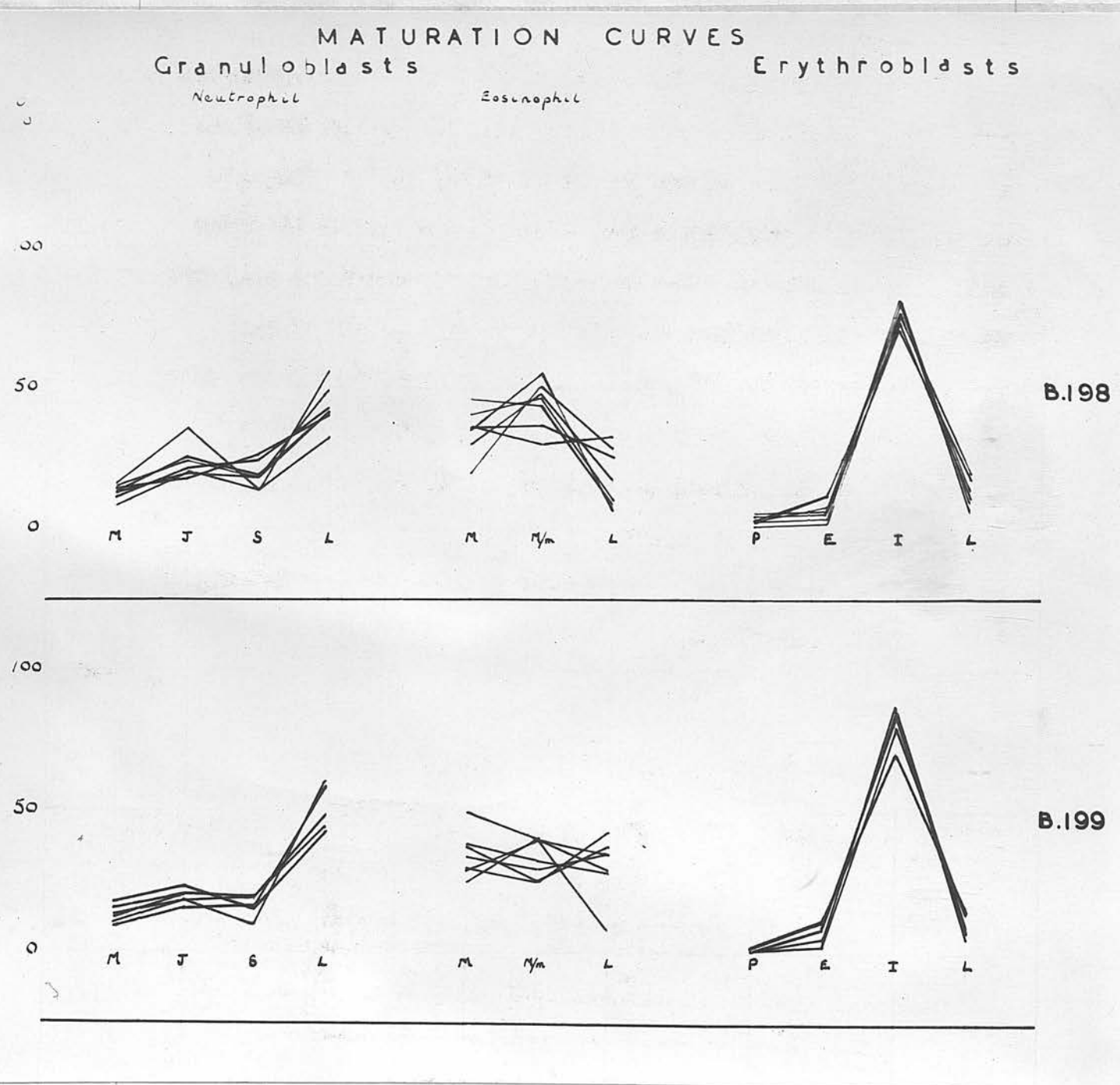

MATURATION CURVES
Granuloblasts
Erythroblasts
Neutrophil
Eosinophil
100
50
0
M J S L
M M/m L
P E I L
B.198
100
50
0
M J S L
M M/m L
P E I L
B.199

Fig.33.

cellularity in the two groups is not significantly different, the same factor operating to cause the variation in both groups. The fact that in Group E. the M/E ratio rose higher than in Group B. suggests that this factor is not related to the quantitive aspect of the diet.

Maturation Curves. The maturation curves for the erythroblasts and neutrophil and eosinophil granuloblasts for the two sheep are shown in Figure 33. As was the case in Group E. the maturation curves for the erythroblasts showed less variation than did those for the granuloblasts. The curves for the erythroblasts were very similar to those for Group E. The relative incidence of the different ages of the neutrophil granuloblasts showed the same distribution in the two groups, while the great variation in the eosinophil curves observed in Group E. was repeated in Group B.

Nucleated cell count in marrow blood.

Table XXXI. shows the weekly nucleated cell counts in the marrow blood for the two sheep.

Table XXXI.

Nucleated Cell Counts in Marrow Blood.

Sheep's No.	Nucleated cell count of marrow blood in thousands per cu. mm. in the						
	Initial Sample	Sample taken at the end of					
		1st week	2nd week	3rd week	4th week	5th week	6th week
B.198	195	52	31	45	55	22	22
B.199	106	25	25	46	59	32	C

C = clotted sample.

As was noted in the sheep in Group E. the first samples gave the highest nucleated cell counts, and the remaining six counts were much lower. Throughout, the counts were lower in Group B. than were found in Group E. Apart from this discrepancy it was not possible to demonstrate any differences in the counts for the two groups.

Mitosis. Table XXXII shows the variation observed in the numbers of cells seen in the marrow preparations from the two sheep.

Table XXXII.

Incidence of Mitosis among Granuloblasts & Erythroblasts.

Sheep's Number	Series of cell	Percentage of cells seen in division at:-						
		Initial sampling	The end of each week					
			1st	2nd	3rd	4th	5th	6th
B.198	Granuloblast	0.1	0.2	0.0	0.1	0.2	0.1	0.0
	Erythroblast	0.2	0.5	0.0	0.1	0.2	0.2	0.7
	Total	0.3	0.7	0.0	0.2	0.4	0.3	0.7
B.199	Granuloblast	0.2	0.1	0.2	0.1	0.1	0.1	0.1
	Erythroblast	0.2	0.1	0.1	0.0	0.0	0.3	0.1
	Total	0.4	0.2	0.3	0.1	0.1	0.4	0.2

From Table XXXII it is seen that in some spreads no cells were observed in mitosis, while the greatest number observed in any one spread during a differential count amounted to 0.7%. This range is in exact agreement with the findings for Group E. Division was seen more frequently among granuloblasts in B. 199 and among erythroblasts in B. 198. Of the 43 cells observed in mitosis during the examination of the 14 spreads, 60.5% were in the prophase, 11.6% in the metaphase/

Table XXXIII.

Haemomyelogram

showing Mean, Maximum & Minimum Values for E. 98, E. 99 & E.100

Sheep's No.	E. 98			E. 99			E. 100		
%	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.
Haemocytoblasts	0.0	-	-	0.0	-	-	0.01	0.0	0.1
Myeloblasts	0.60	0.2	0.8	0.51	0.2	0.9	0.56	0.1	0.9
Promyelocytes	0.04	0.0	0.2	0.04	0.0	0.1	0.03	0.0	0.1
Neutrophil Grans.	27.96	22.6	41.5	36.56	19.6	48.8	30.37	17.7	45.2
Eosinophil Grans.	13.37	11.3	24.2	12.79	5.1	23.6	12.23	5.6	23.6
Basophil Grans.	0.33	0.1	0.7	0.94	0.2	1.1	0.87	0.6	2.1
Erythroblasts	52.79	31.8	58.3	48.36	28.6	74.2	54.63	28.6	75.4
Lymphocytes	0.34	0.0	0.8	0.16	0.0	0.5	0.34	0.0	0.7
Monocytes	0.03	0.0	0.1	0.03	0.0	0.2	0.07	0.0	0.3
Plasma cells	0.13	0.1	0.2	0.11	0.0	0.3	0.23	0.0	0.7
Reticulum cells	0.39	0.2	0.5	0.47	0.1	0.8	0.63	0.0	1.7
Mitosis Grans.	0.17	0.0	0.4	0.10	0.0	0.3	0.07	0.0	0.2
Mitosis Erythros.	0.07	0.0	0.2	0.14	0.0	0.3	0.27	0.0	0.7
M/E Ratio- :1	0.97	0.5	2.1	1.25	0.4	2.5	0.64	0.3	2.5
Total cell count Thousands per cu.mm.	120	39	257	101	10	266	134	37	210

metaphase, 20.9% in the anaphase, and 7.0% in the telophase. The only difference between these results and those for Group E. was that in this group mitosis was more common in the metaphase than the telophase, while in Group E. the reverse was the case. As was the case in Group E. of the four stages of mitosis, cells were most commonly seen at the prophase stage, whilst the next most common stage was that of anaphase. As with Group E. no cell beyond the age of myelocyte was seen in division, and the commonest erythroblast seen in mitosis was the intermediate normoblast. It was concluded that the low plane of nutrition had produced no demonstrable effect on the frequence of mitosis in the sheep of this group.

Conclusions.

A haemomyelogram showing mean values and ranges is presented (Table XXXIII.) in respect of each of the three sheep on a diet providing full maintenance. The erythroblasts and differentiated granuloblasts, viz., neutrophil, eosinophil, and basophil granuloblasts, are not subdivided according to age, as the maturation curves for these cells are shown in Fig. 29.

No alteration in the bone marrow or blood picture could be demonstrated as the result of feeding a diet providing only half maintenance.

It was possible to show certain definite effects on both blood and bone marrow as the result of artificial feeding and management. In the peripheral blood these took the form of an erythrocytic polycythaemia. In the marrow the changes consisted of a reduction in/

in marrow cellularity, which was mainly at the expense of the erythroid tissue. The fact that these changes did not persist right up to the end of the experiment showed a tendency for the sheep to accustom themselves to these artificial conditions.

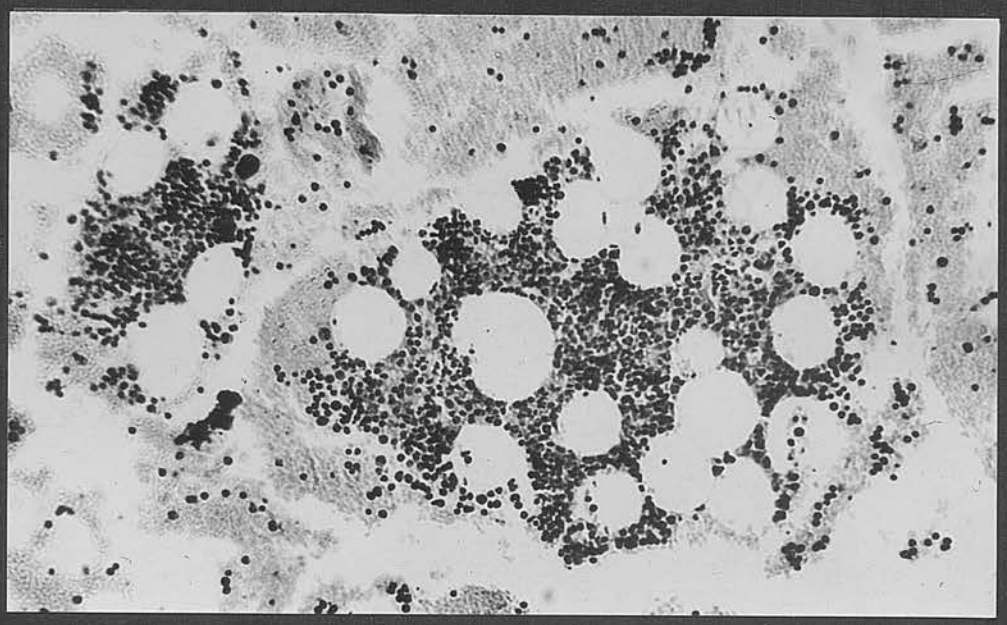


Fig 33a. Photomicrograph. of section of aggregated marrow flecks from sheep B198 showing fat cells appearing as clear areas. The flecks are surrounded by marrow blood. X 250.

SECTION V.

An Investigation of the Changes occurring in the Peripheral Blood and Sternal Marrow in Scottish Hill Sheep from December to June.

The object of this investigation was to study the variation in the bone marrow and its relation to the blood changes over the period during which a significant drop in erythrocyte values had to be recorded in the previous year. (Section I.)

Method. The observations to be described here formed part of a wider experiment conducted under the auspices of the Agricultural Research Council, to study the seasonal variation in the worm burden in Scottish hill sheep. The results of the investigation as far as the worm burden is concerned, and which have been published by Morgan, Parnell & Rayski (1951), were made available to the writer, for correlation with the haematological data here described. The estimation of the worm burden necessitated the slaughter of the sheep, and to this end 28 ewes were slaughtered between December 13th and June 27th; two were slaughtered on the first date mentioned, the next two on January 11th, and thereafter at fortnightly intervals. In the case of all 28 sheep blood samples were taken from the jugular vein, and marrow withdrawn by sternal biopsy, as described in Section II. the day before slaughter. In addition to these samples, it was possible in the case of 14 of the 28 sheep to collect blood samples on September 27th and again on December 13th, and in respect of these sheep an estimate of the change occurring between these dates and the date of slaughter was possible.

The examination of the peripheral blood comprised P.C.V., R.B.C., Hb., W.B.C., and D.L.C. The techniques used in these determinations have been described in Section I., as has the method by which M.C.V. and M.C.H.C. were calculated. The stained blood films were examined for regenerative changes by the inspection of approximately 10,000 erythrocytes. In addition to these examinations, the fragility of the erythrocytes was tested, and the specific gravity of whole blood and plasma estimated.

Fragility was tested by salt solutions varying by 0.02%, from 0.34% to 0.78% sodium chloride, and the tubes showing complete and initial haemolysis noted after standing for 24 hours. This examination was not carried out on all the samples collected but only on the 20 samples collected during the period in which, from the results of the previous year's work, the fall in erythrocyte levels was to be expected. There is some evidence to show that the immature corpuscle is less resistant to haemolysis than the adult (Cruz, Hahn, Bale & Balfour, 1941) and if, as the result of the oligocythaemia which was expected to occur in this period, the peripheral blood contained an abnormal number of immature cells, it was possible that their presence might be indicated by an increase in the fragility of the blood as a whole.

The specific gravity of blood and plasma was carried out on 22 of the samples collected. The copper sulphate method (Hawk, Oser, & Summerson, 1947) was used for these determinations. This examination was included to test the correlation of the results it gave, with the other erythrocyte measurements made, with the object of discovering what degree of accuracy could be expected from S.G. estimations/

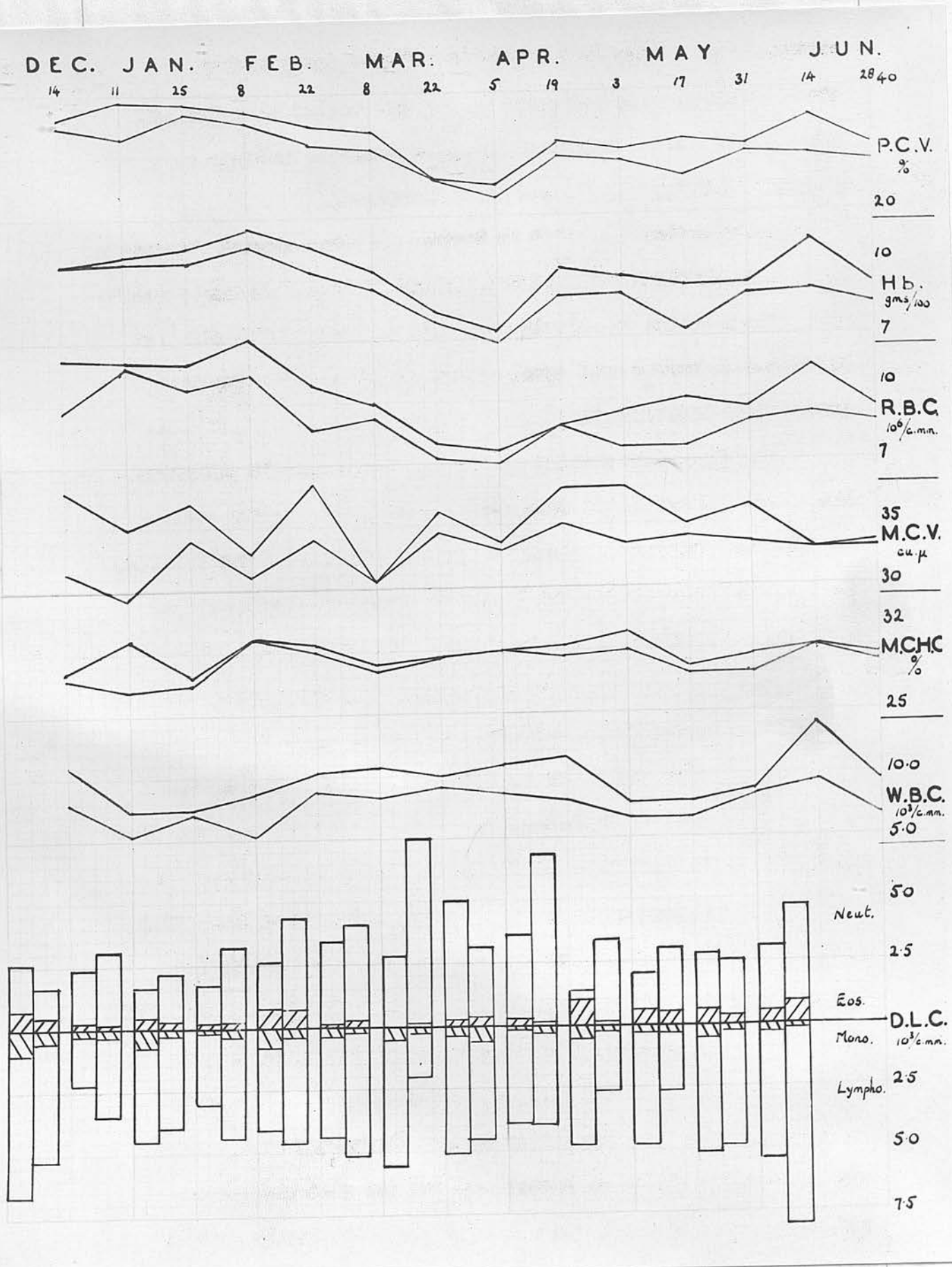

DEC. JAN. FEB. MAR. APR. MAY JUN.
14 11 25 8 22 8 22 5 19 3 17 31 14 28 40
P.C.V. %
20
10
Hb. gms/100
7
10
R.B.C. 10⁶/c.mm.
7
35
M.C.V. cu.μ
30
32
M.C.H.C %
25
10·0
W.B.C. 10³/c.mm.
5·0
50
Neut.
2·5
Eos.
D.L.C.
Mono. 10³/c.mm.
Lympho. 2·5
5·0
7·5

Fig. 34.

estimations if, say, in a survey including a large number of observations, it was necessary to use this method of estimating erythrocyte level, instead of the more laborious and time consuming P.C.V., R.B.C., and Hb. estimations.

By the methods detailed in Section II. the material obtained by sternal puncture was used for the preparation of marrow spreads. An estimate of the cellularity was made on the spreads, and from differential marrow cell counts haemomyelograms and maturation curves were constructed.

Results. The results of the examination of the peripheral blood and sternal marrow comprise 35 separate values for each of the 28 sheep examined. In view of the difficulty of incorporating material of these dimensions in the form of text tables, it was considered preferable wherever possible to present the results in diagrammatic form. The figures from which these diagrams are constructed appear in the Appendix, pages A.34 to A.41.

Peripheral Blood. The results of the examination of the peripheral blood are shown in Fig. 34. The dates of sampling are shown as the abscissa, and it will be noted that on each date, values for two sheep appear. The P.C.V., Hb., and R.B.C., which are shown in that order from above downwards, show a general agreement in trend of variation. The highest levels recorded were in the eight sheep sampled up to and including February 8th. From then until April 5th there was a gradual fall, the lowest readings occurring at the latter date. Thereafter there was an abrupt rise for all three properties, but the rise was not sufficient to constitute a general return to the levels recorded

prior/

prior to Febrary 8th. Table XXIV. illustrates these changes, showing the mean values for P.C.V., Hb., and R.B.C. for the periods referred to above, viz., December 13th to February 8th; from February 22nd to April 5th; and from April 19th to June 28th (all dates inclusive).

Sampling period	Number of Sheep	Mean values for		
		P.C.V. %	Hb. gms/100 ml.	R.B.C. 10^6/cu.mm.
Dec. 13th - Feb. 8th	8	36.43	10.55	10.91
Feb. 22nd - Apr. 5th	8	29.0	8.68	8.31
Apr. 19th - Jun. 28th	12	30.25	8.98	8.80

The number of observations was small in this experiment, but as the trends shown agree with the findings reported in Section I. it is considered that greater significance may be attached to these results than would have been justifiable without the confirmatory evidence supplied by the results in Section I.

Holman (1950) gives the mean and S.D. for erythrocyte count of sheep as 11.5 $\pm$ 1.8 and an examination of the R.B.C. of the 28 sheep on this basis shows that five of the sheep sampled (50, 69, 60, 62, and 66) had erythrocyte counts which could be regarded as somewhat abnormally low and possibly pathological - the counts being less than the mean, minus twice the S.D. However, it has already been shown in Section I. that the normal erythrocyte count in the sheep on this farm was lower than the normal standard laid down by Holman. It is therefore suggested that this fall is more in the nature of an oligocythaemia of the type described by Holman (1950) as/

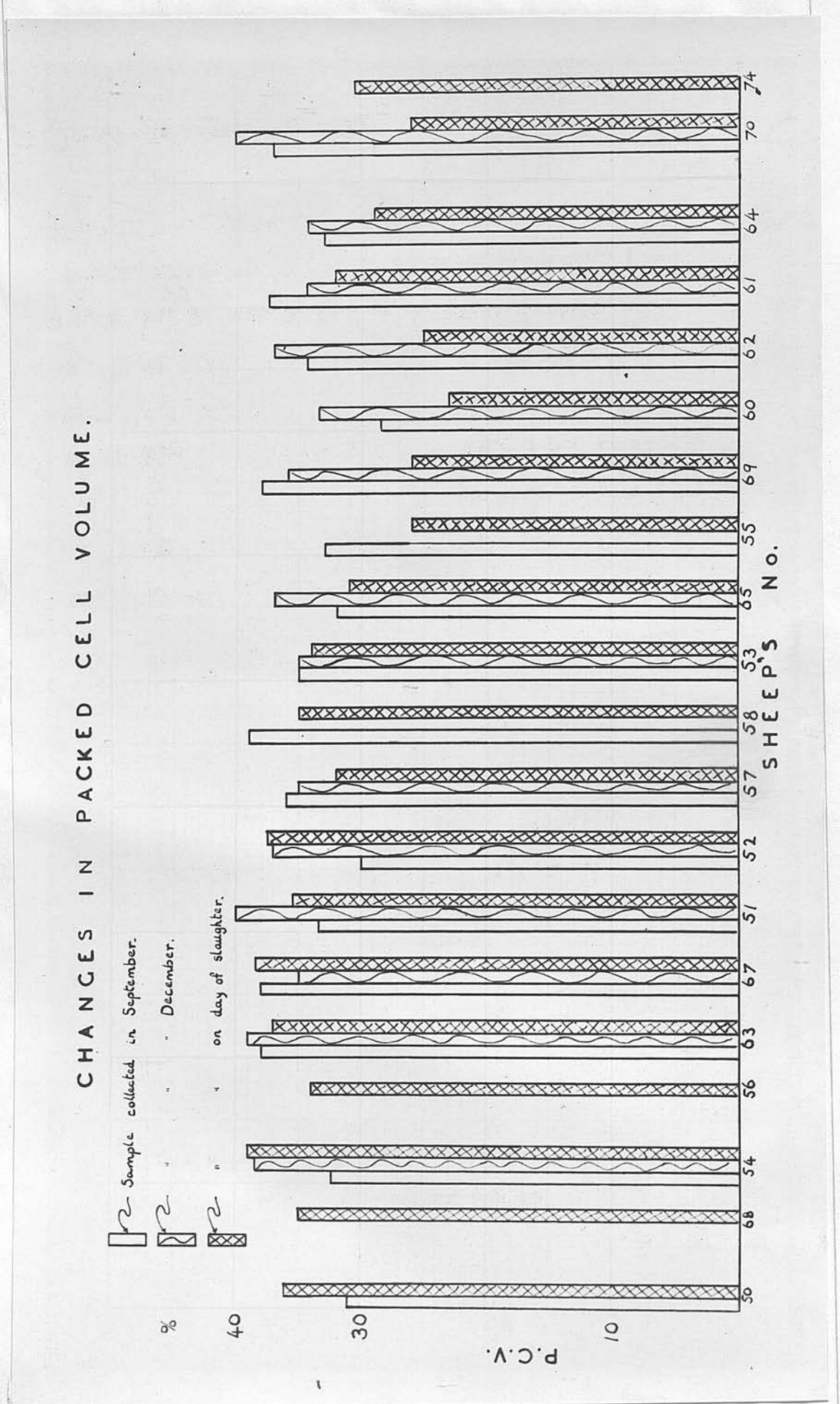

CHANGES IN PACKED CELL VOLUME.
Sample collected in September.
" " December.
" " on day of slaughter.
%
40
30
10
P.C.V.
50
68
54
56
63
67
51
52
57
58
53
65
55
69
60
62
61
64
70
74
SHEEP'S No.

Fig. 35

as occurring in herbivorous animals during the winter and spring months.

There is evidence to show that the changes in the erythrocyte values were not entirely related to individual variation between sheep. Blood samples were collected from fifteen of the sheep in September and from thirteen of them again in December, before the start of the experiment proper. Fig. 35 shows the P.C.V. levels for these sheep on the day before slaughter, together with those for the September and December sampling.

M.C.V. and M.C.H.C. The marked variation in the M.C.V. could not be correlated with any other measurement. The M.C.H.C. was remarkably constant.

Leucocytes. The variations in W.B.C. and D.L.C. were no greater than those to be expected between different sheep sampled at the same time. There was, however, a tendency for the neutrophil leucocytes to be more numerous in sheep sampled from March 22nd to April 19th, a variation which was largely responsible for the higher total leucocytes counts seen at this time.

Evidence of Regeneration in films. No erythrocytes showing either punctate basophilia or polychromasia were observed in the stained blood films from the sheep.

Fragility of Erythrocytes. The results of the fragility tests are shown in Table XXXV. it shows values for complete and initial haemolysis (C. & I.H.) for the twenty samples examined for this property.

Table XXXV.

Specific Gravity of Blood and Plasma and Fragility of Red Corpuscles.

Sheep's No.	Specific Gravity		Fragility	
	Blood	Plasma	I.H.	C.H.
63	1.0526	1.0292	.70	.50
67	1.0540	1.0286	.68	.48
51	1.0521	1.0272	.70	.52
57	1.0497	1.0290	.68	.48
58	1.0518	1.0301	.70	.54
53	1.0488	1.0272	.64	.52
65	1.0486	1.0280	.64	.48
55	1.0471	1.0285	.70	.52
69	1.0456	1.0261	.66	.50
60	1.0415	1.0265	.68	.50
62	1.0441	1.0275	.66	.48
61	1.0490	1.0281	.66	.48
64	1.0466	1.0276	.52	.48
70	1.0491	1.0300	.70	.34
74	1.0487	1.0276	.70	.34
66	1.0464	1.0281	-	-
75	1.0506	1.0301	-	-
59	1.0550	1.0336	.76	.54
73	1.0518	1.0353	.76	.56
76	1.0486	1.0281	-	-
77	1.0471	1.0274	-	-
52	1.0536	1.0275	.68	.46
54	-	-	.68	.52

I.H. = Initial Haemolysis. C.H. = Complete Haemolysis.

Holman (1944a) states that reading after 24 hours and recording only the tube in which complete haemolysis occurs, the normal range for the sheep is 0.45% - 0.65%, and an inspection of Table XXXV shows that the values obtained in my sheep fell within this range, with the exception of sheep 70 and 74. No explanation for the increase in the fragility of the erythrocytes in these sheep can be given.

Mean values, but no ranges, for initial haemolysis are given for sheep by Hamburger, quoted by Kato (1941) and Jolly, quoted by Isaacs (1923). Jolly's figure is 0.74% and Hamburger's, 0.60% . From Table XXXV. it will be seen that with the exception of sheep 64 my results approximated to these standards.

There was no tendency for the variation in corpuscular fragility to be related to time of sampling or any other measurement.

Specific Gravity. The results of the specific gravity estimations for blood and plasma appear in Table XXXV. The S.G. of these sheep was seen to vary from 1.0415 to 1.0550 with a mean of 1.0492. Holman (1944a) found that the S.G. of sheep sampled between June and December was expressed by a mean of 1.052 with a S.D. of ± 0.005, and a range of 1.042 to 1.065. An examination of Table XXXV shows that the S.G. of all the sheep examined fell within the range given by Holman, with the exception of sheep 60, but that the values were generally much lower than Holman's; this was no doubt related to the fact, to which reference has already been made, that my sheep showed lower erythrocyte levels than Holman's. On the other hand, my sheep showed higher figures for S.G. than those of Bonard (Wirth, 1950), who gave the normal range as 1.040 to 1.046.

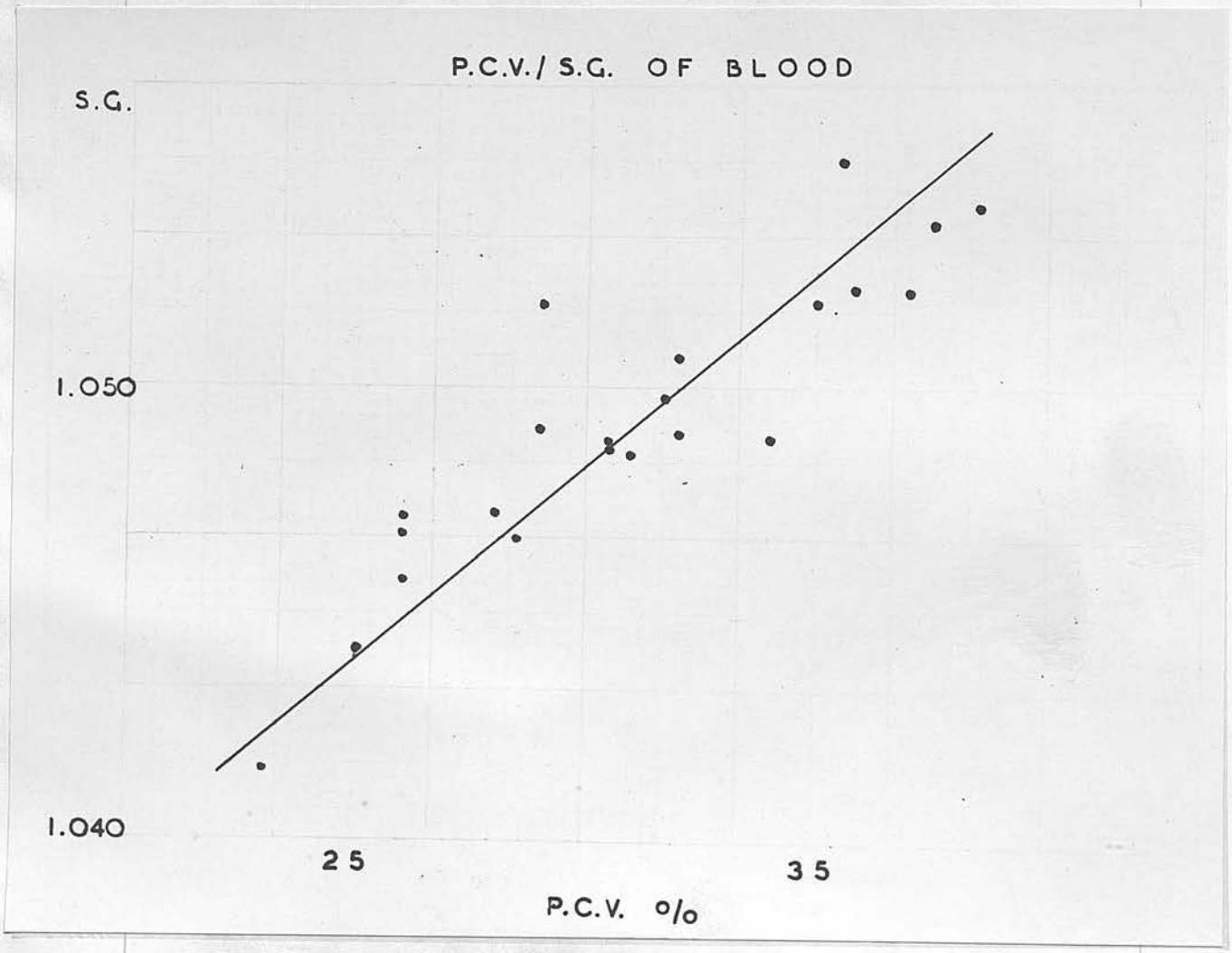


Fig. 36. Showing correlation of Specific Gravity of blood and packed cell volume.

Correlation of S.G. with P.C.V. The scatter diagram appearing as Fig.36 shows the correlation between S.G. of whole blood and P.C.V. It was found that where S.G. is represented by x. and P.C.V. by y, the regression line is given by y = 1173.9x - 1200.636 so that when P.C.V. = 25.0% , the S.G. of whole blood = 1.04407. The correlation coefficient of 0.9019 was shown to be highly significant, i.e. with n = 20, p = less than .001 (Fisher & Yates, 1949. Table VI.). Thus, in these results, which represent those from single samples from a number of individuals, a correlation exists between P.C.V. and S.G. of whole blood. This suggests that when S.G. of whole blood is estimated by the copper sulphate method, it may be possible to forecast the P.C. V. with some degree of accuracy. These results do not confirm the findings of Holman (1944a), who,while showing a significant correlation between P.C.V. and S.G. when different samples were taken from one individual sheep (coefficient of correlation 0.55, n = 18),found that when single samples, taken from a number of individuals, were used the correlation was obscured.

The S.G. of the plasma varied from 1.0251 to 1.0353 with a mean value of 1.02870. There was no trend visible in the variation observed. These values are for the most part within the range given by Kruger and quoted by Wirth (1950), of 1.0255 - 1.0271, but a number of my observations were higher.

Marrow. In the presentation of the results of the examination of the marrow the following divisions have been made for separate consideration:-

1. Cellularity of spreads. 2. Haemomyelograms.
3. Myeloid/Erythroid ratio. 4. Maturation Curves.
5. Mitosis.

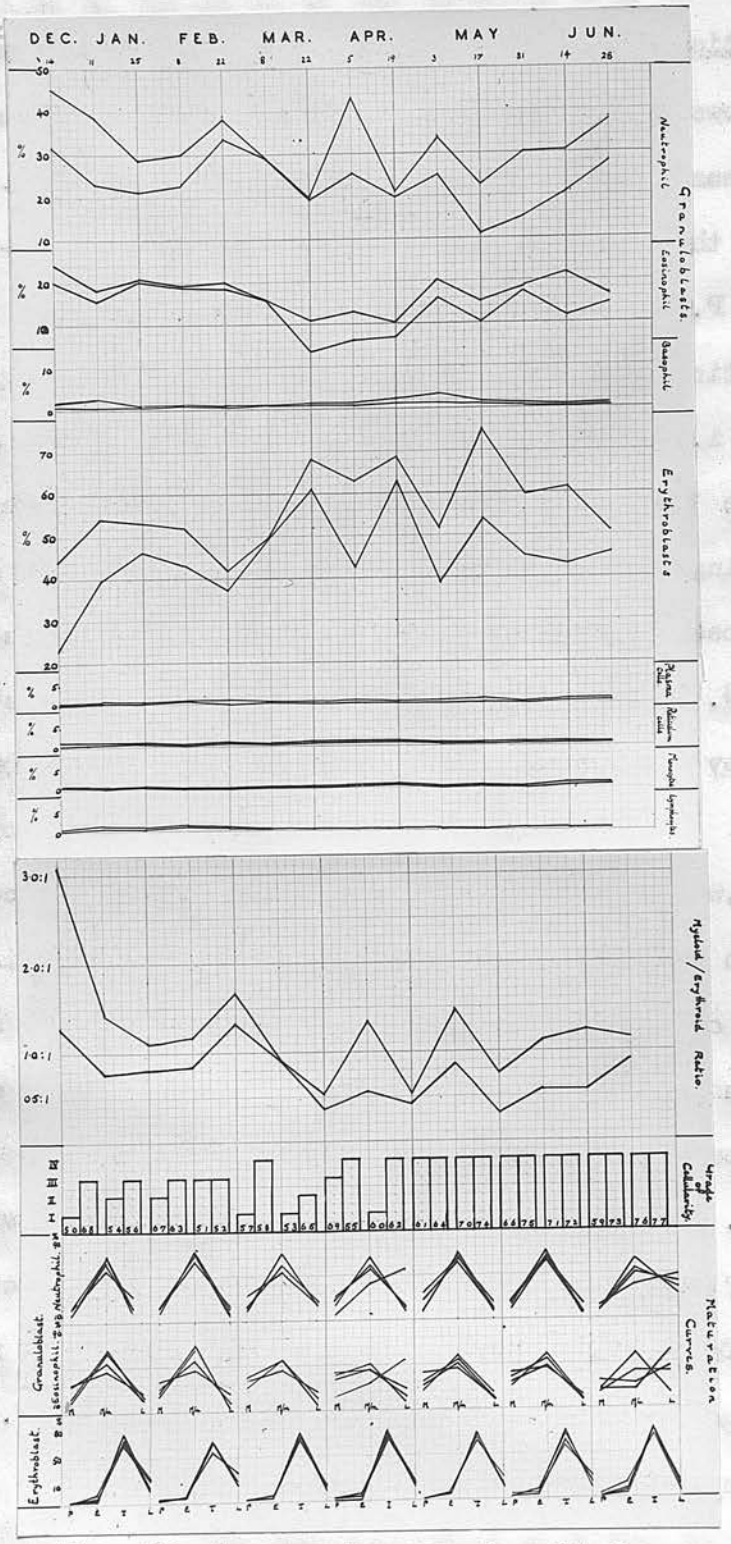


Fig. 37. Haemomyelograms for 27 sheep shown in diagramatic form.

1. Cellularity. The grading of the cellularity of the marrow spreads is shown in Fig. 37, from which it will be seen that in the 16 samples taken up to and including April 5th, four were classified as 'I', three as 'II', six as III, and three as IV. From April 19th to the end of the experiment all spreads were graded as 'IV'.

2. Haemomyelograms. Haemomyelograms for 27 of the 28 sheep are shown in Fig. 37 In the case of one sheep (53) a differential count was not possible, as in the preparation of the spread too much damage had occurred to the cells. Haemocytoblasts which were of very low incidence, ranging from 0.0 - 0.1 per cent., were omitted from the diagram. In the case of the erythroblasts and differentiated granuloblasts no subdivision according to age is shown but only the total incidence of the cells. The distribution of the cells according to stage of maturity is available by reference to the maturation curves. It will be seen from Fig. 37 that myeloblasts, promyelocytes, basophil granulocytes, plasma cells, reticulum cells, lymphocytes and monocytes were present in small and fairly constant numbers, so that the differences between the haemomyelograms of the sheep were due to variations in the erythroblasts and the neutrophil and eosinophil granuloblasts. This variation is best considered by reference to the M/E ratio and will be discussed under that heading. It is of interest to note however that the changes in incidence of neutrophil and eosinophil granuloblasts are independent of each other.

3, Myeloid/

3. Myeloid/Erythroid Ratio. The M/E ratio varied from 0.3 : 1 to 3.1 : 1. The variation is illustrated in Fig. 37 from which it will be seen that there was a tendency for the earlier samples to show a higher M/E ratio, i.e., a greater preponderance of granulocytic elements. Thus, in the ten samples taken up to and including March 22nd, only one showed a M/E ratio of less than 0.8 : 1, whereas after that date nine of the seventeen samples were found to have M/E ratios of less than 0.8.

4. Maturation Curves. The maturation curves for erythroblasts and neutrophil and eosinophil granuloblasts are shown in Fig. 37 The greatest consistency was observed in the case of the erythroblasts, whereas the greatest variability was in the eosinophil granuloblasts. The only gross departure from the general shape of the curves was in respect of those for sheep No. 60. The curves for this sheep showed a lower incidence of the more primitive elements in both granuloblasts and erythroblasts. The findings in respect of sheep No. 60 will be considered in greater detail at a later stage.

5. Mitosis. The variation seen in the mitotic activity of the marrows examined was not related to the time at which the samples were collected, nor could correlation with any other measurement be demonstrated. Division was more commonly seen in erythroblasts than granuloblasts, viz., 89% of the cells seen in mitosis were erythroblasts. Division was not observed beyond the stage of myelocyte in the case of the granuloblasts, but in the case of the erythroblasts, although the intermediate normoblast was the most common stage seen in mitosis, all ages of erythroblast were seen dividing/

dividing, including a few late normoblasts. Of the erythroblasts observed to be in mitosis, 1.9% were pro-erythroblasts, 21.0%, early normoblasts, 72.6% intermediate normoblasts, and 4.5% late normoblasts. In the case of the granuloblasts, 26.3% were myeloblasts, 68.4%, neutrophil myelocytes, and 5.3% eosinophil myelocytes. Table XXXVII. shows the relative frequency with which the stages of mitosis were seen in the two groups of cells.

	Prophase	Metaphase	Anaphase	Telophase
Granuloblasts	31.6%	21.0%	15.8%	31.6%
Erythroblasts	41.4%	35.6 %	11.5%	11.5%

Sheep No. 60.

As has already been stated, there are certain features which warrant consideration of the results for this sheep separately.

Blood. The P.C.V., Hb., and R.B.C. were the lowest recorded in the observations, being 23.0%, 6.9 gms. per 100 ml., and 7 million respectively. There was no difference between the M.C.V. and M.C.H.C. for this sheep and these indices for the remaining 27 sheep. No evidence of regeneration could be found in peripheral blood films.

Marrow. The marrow cellularity was graded as 'I', and the M/E ratio calculated to be 1.3 : 1. The maturation curves showed a low incidence of the more primitive cells in both granulopoietic and erythropoietic series. The figures for the maturation curves of granuloblasts and erythroblasts for this sheep are shown in the /

the Table below, with the ranges for these curves in respect of the other 26 sheep, for comparison.

Table XXXVII.

Maturation Curves for Marrow Cells in Sheep No. 60 compared with those for the remaining sheep in the observations.

	Sheep No. 60 %	Other 26 sheep.
Neutrophil Myelocytes	4.9	6.5 - 28.0
" Metamyelocytes	40.7	37.6 - 78.1
" Lobulates	54.4	8.1 - 48.9
Eosinophil Myelocytes	14.3	12.6 - 41.5
" Metamyelocytes	30.1	20.8 - 75.0
" Lobulates	55.6	9.6 - 43.1
Pro-erythroblasts	0.2	0.9 - 3.8
Early normoblasts	0.9	2.7 - 11.6
Intermediate normoblasts	78.8	57.7 - 78.0
Late normoblasts	20.0	9.2 - 32.7

The most striking feature in this comparison is the low incidence of pro-erythroblasts and early normoblasts, and the high incidence of lobulated neutrophils and eosinophils.

Discussion. It is an acknowledged fact that unless the erythrocyte levels are reduced to half their normal value, signs of regeneration in the peripheral blood are likely to be absent or of very low incidence. A sample taken from this sheep on December 13th showed the P.C.V. on that occasion to be 33.5%, so that the value recorded on April 5th (23.0%) does not represent a sufficiently marked /

marked reduction to lead to the appearance of regenerative signs in the peripheral blood. The marrow picture in this sheep is that described by Israels (1948) and Custer (1949) as occurring in hypoplasia leading on to aplasia. At post-mortem examination no lesions could be found in the carcase or viscera to account for the hypoplastic state of the marrow of this sheep, and it seems reasonable to postulate that the marrow changes represent a more extreme limit of the trend noted in most of the sheep sampled up to March 22nd.

Discussion of the nature of the Variations observed in the marrow of the 28 sheep.

The variation in the marrow picture of the sheep sampled was not great, and it is possible that it may do no more than represent the variation likely to be expected from sheep to sheep. There was a tendency however for the marrow of sheep sampled up to March 22nd to differ in some respects from that of sheep sampled after that date. Up to March 22nd the marrow showed a poorer cellularity and a relative paucity of erythropoietic elements. This was comparable with the changes which were noted to have developed at one stage of the experiment described in Section IV. when sheep were kept on an artificial diet. After March 22nd there was a general and marked improvement in marrow cellularity and an increase in most sheep , in erythropoietic tissue. The most striking exception to this rule was sheep No. 60, which showed a tendency towards a hypoplastic marrow. It is possible that the picture in this sheep may represent the extreme limit of the trend shown by the/

the sheep sampled up to March 22nd. The increase in erythropoietic tissue seen after March 22nd was not accompanied by any shift to the left in the maturation curves for the erythroblasts, as was seen in the sheep in Section III. as the result of the stimulation of the marrow caused by experimental blood loss.

The relation of the Variations in the Peripheral Blood and Marrow to Pregnancy, Worm Burden and Nutrition.

Pregnancy.

Lambing took place in this flock between April 14th and April 28th, and all sheep sampled up to and including April 19th were in varying stages of pregnancy. No significant difference could be shown between the erythrocyte levels of pregnant and non-pregnant sheep in the results recorded in Section I. In the sheep in this investigation the rise in erythrocyte levels started before lambing began, and there was no further rise after the sheep had lambed. Thus it was not possible to demonstrate any effect on blood or marrow due to any indirect influence pregnancy may exert by the demands it makes on nutritional intake.

Worm Burden.

Full details of the worm burden of these sheep are reproduced in the Appendix, pages [illegible] with the permission of Drs. Morgan, Parnell & Rayski. From these results it is seen that qualitatively the worm burden found in the sheep in this investigation was very similar to that encountered in the sheep sampled for the studies reported in Section I. in that the main genera responsible for the variation in the worm burden of these sheep were Ostertagia and Trichostrongylus. By the use of Morgan's 'Index for significance of infestation', details/

details of which are given in Section I. it was found that only two sheep were carrying infestations likely to produce clinical effects, and thus it is considered the worm burden may be said in the main to fall into the category of 'subclinical helminthiasis'.

Nutrition.

Owing to the poor pasture conditions of the Scottish hills in late winter and early spring, the nutritional status of hill sheep is acknowledged to be very low at that time of year. Unfortunately body weights for the sheep sampled in this investigation were not available, but Morgan et al (1951) have presented monthly weights for ewes on a Scottish hill farm, comparable to the farm from which the sheep in this experiment came. Their figures for the period November to April 1946 - 1947 show an average weight loss of from 7.2% to 11.4%, according to age - the younger ewes suffering the greater loss. There are two sources of indirect evidence of an improvement in pasture conditions in March on the hill from which the sheep in this experiment came. The first is in the form of meterological data supplied by the observatory at Eskdalemuir, which is situated only a few miles from the farm. Table XXXVIII.

Table XXXVIII.

Weather Data from Metereological Observatory, Eskdalemuir.

Month	Mean monthly temperature	Number of days		Total sun hours and 1/10ths
		Air Frost	Ground Frost	
1949 December	38.4	8	11	33
1950 January	37.1	14	15	34.3
February	35.4	18	18	68.7
March	41.3	12	9	90.4
April	40.2	13	13	112.8
May	48.2	3	4	144.3
June	55.2	0	2	185.9

From these data it may be seen that there was a rise in mean monthly temperature and hours of sunshine in March and that there was a reduction in the number of days on which air and ground frost were recorded. The second piece of evidence is related to the increase in worm burden. Hawkins (1945), as the result of observations on the effect of weather on untreated nematode infections in sheep, has shown that ewes become reinfected with the first onset of warm weather in spring. In view of these findings, the fact that the first high larval counts were found in sheep examined on March 22nd provides indirect evidence of a considerable improvement in weather about this time. There are thus strong indications of warmer weather conditions pertaining in March, and it is reasonable to assume that this improvement in weather also resulted in the growth of pasture of a higher nutritional value.

Before assessing the results of this investigation, the handicap of the fact that single samples were taken from different sheep throughout the period covered by the observations, must be acknowledged. As has already been stated, this undesirable feature was accepted in order to have available for each sheep the actual worm burden figures in preference to the less reliable worm egg counts, as the index of helminth infestation. The well recognised sampling errors inherent in differential marrow counts, the wide normal range in the blood picture of the sheep, together with the fact that gross deviations from the normal blood picture were not observed in these results, make it imperative that any conclusions reached should be no more than tentative indications for possible lines of further investigation on a much wider scale. While accepting these limitations it is obvious that no discussion of the variations observed/

Fig 38. Showing changes in worm burden, packed cell volume & percentage of erythroblasts in the marrow from Dec 14th to June 28th. The figures on the graph refer to the ear numbers of the sheep. The heavier lines connect the mean values calculated from the two measurements made on each of the fourteen occasions when sampling was carried out.

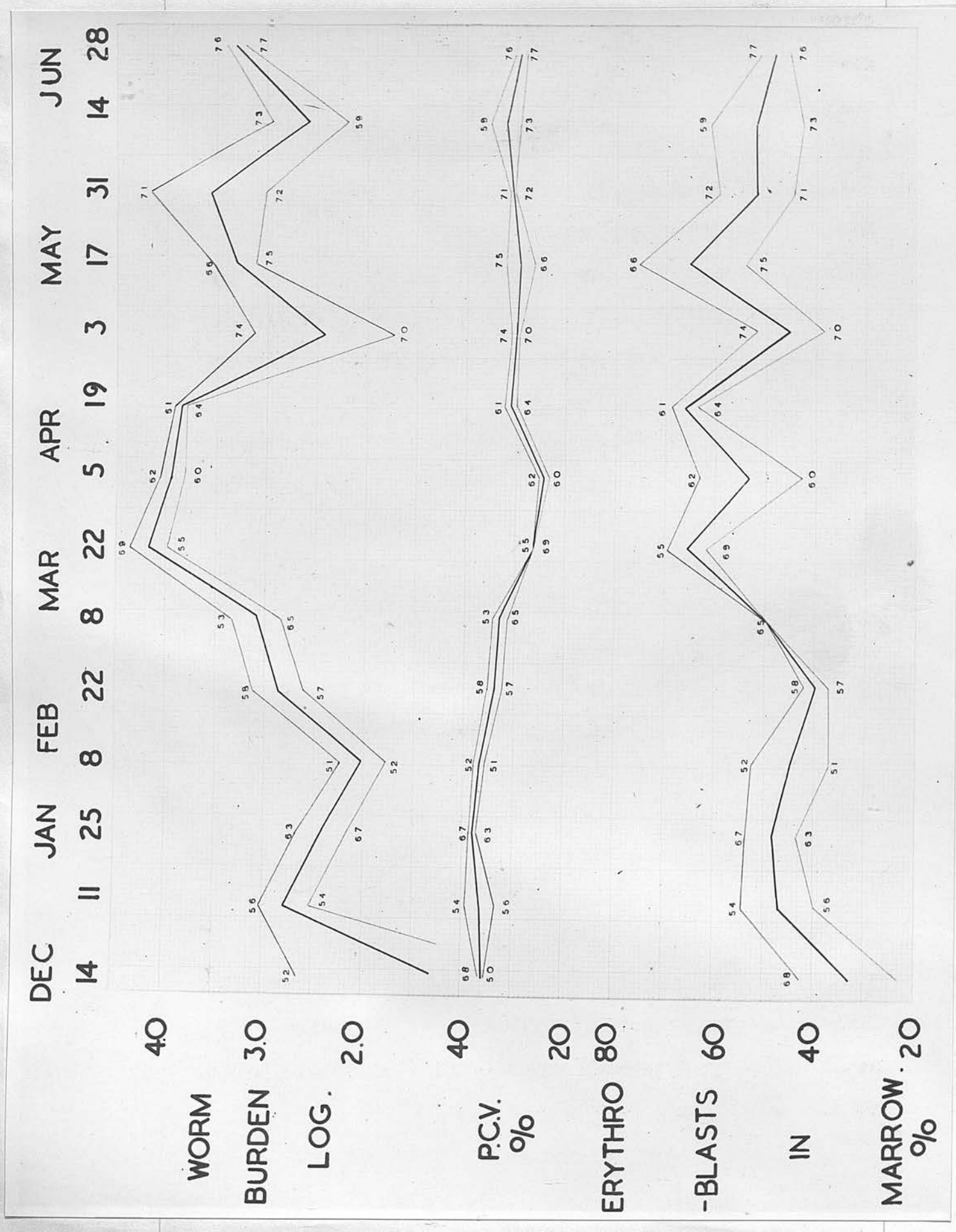


Fig. 38.

observed is possible, unless it is assumed that the two sheep sampled on any one day are to some degree representative of the whole group.

In Fig. 38 are shown the variations in P.C.V. and worm burden, together with the changes in the erythroblastic tissue of the marrow. In the case of the worm burden, as the figures ranged from 2 to 23,082, the log of the values has been taken to make presentation in graph form possible. From the results of the marrow examination an attempt has been made to express the M/E ratio in absolute terms by relating it to the grading for cellularity. Fig. 39.

From Fig. 38 it is seen that the changes in worm burden are reflected by inverse changes in P.C.V. This relationship is most marked up to April 19th; after that date there are some discrepancies in the relationship, e.g., in the case of sheep 71 the worm burden was 14,933, but the P.C.V. was 31.0%. This is in general agreement with the findings reported in Section I. where a closer correlation was found between worm burden and P.C.V. for the sheep sampled in January and April than was the case with the sheep sampled in June and July. The results of the experiments described in Section IV. showed that low nutrition alone did not affect the blood picture, and this was shown to be the case in the results under discussion, in that up to February 22nd although the sheep were in a state of low nutrition no effect on erythrocyte levels was seen until the rise in helminth burden occurred. Thus, the worms may be regarded as the exacerbating factor exerting its effect on a tissue which is probably already under considerable strain to maintain physiological levels.

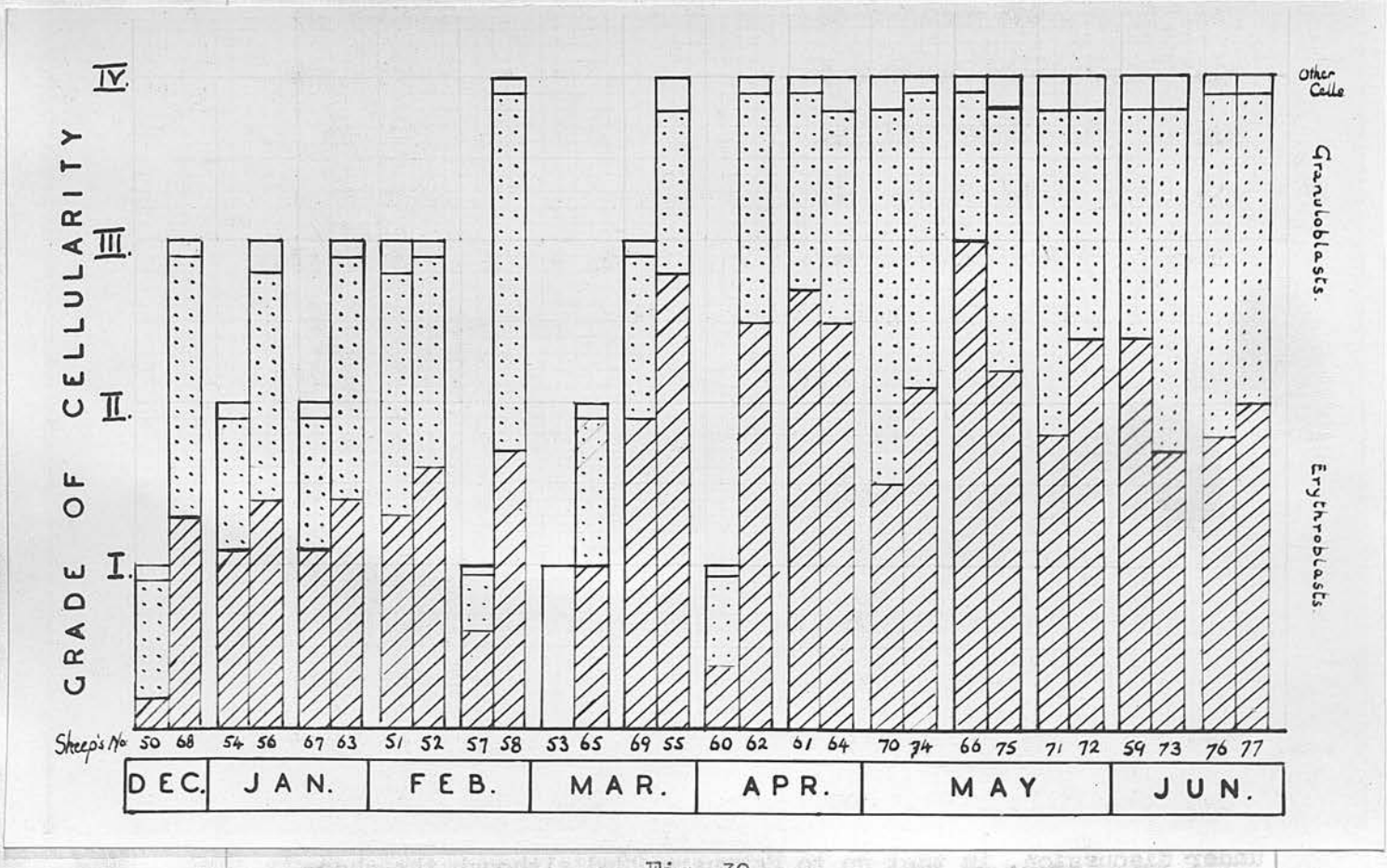


Fig. 39.

Incidence of marrow cells related to the cellularity of marrow spreads.

From Figs. 38 & 39 it is seen that in the sheep sampled on and after March 22nd there was an increase in the erythroid tissue of the marrow as compared with those sampled before that date. It has been shown that this increase in erythroblasts was not accompanied by any alteration in maturation curves, and was therefore more in the nature of a proliferation of tissue. It is suggested that this change was related to the improved pasture conditions which occurred at this time. It is not possible to state whether the tendency for the marrow to have less erythroid tissue before March 22nd can be ascribed to the low nutrition obtaining at this time. If it is assumed that the marrow picture in sheep 60 represents a more advanced stage of the general marrow picture shown in the sheep sampled before March 22nd, it would appear that there was a tendency for erythroid hypoplasia to develop during the late winter and early spring months. There was a tendency for a similar state to develop in the sheep on controlled diets in Section IV. where it was concluded that, as the sheep on the poorer diet did not show as marked a change as those on the better diet, the cause for the changes did not lie in the diet being deficient in amount. It is possible that the diet of both the sheep in Section IV. and thosesampled before March 22nd in this experiment contained insufficient amounts of an undetermined factor necessary for erythropoiesis, but until more is known of the requirements for blood formation in the sheep no conclusions as to its possible nature can be drawn.

It is of interest from an ecological point of view to notice that the warmer weather conditions which favoured the rise in worm burden also produced an improvement in pasture, which in turn helped/

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It is of interest from an ecological point of view to notice that the warmer weather conditions which favoured the rise in worm burden also produced an improvement in pasture, which in turn helped/

helped the host to combat the effects of the parasites and facilitated the occurrence of the phenomenon of 'self cure' (Ross & Gordon, 1936). Thus, as far as the blood picture is concerned, a state of equilibrium was restored, in which helminth burden was reduced and erythrocyte levels rose again, although they did not reach those seen earlier in the year when the worm burden was at its lowest level.

General Discussion.

The results of the observations recorded in this thesis show the relationship of the worm burden and erythrocytic properties in the blood picture of Scottish hill sheep from January to June. During the late winter and early spring months when the sheep were on a low level of nutrition this association was most closely marked but even after the nutrition improved as a result of the growth of 'spring pasture', the presence of the parasites probably continued to exert some influence on erythrocyte levels, as these were generally lower than those found in January, when the worm burden was at its lowest. The role of nutrition in relation to helminth parasites is well recognised. Gordon (1948) has summarised it by stating that adequate nutrition not only favours resistance to the initial establishment of infestation, but also assists the animal to withstand the effects of an established infestation. Thus it is possible for a comparatively heavy worm burden to be present in a well nourished sheep without causing any ill-effect (Kauzal, 1933).

Although the method by which helminth parasites influence the blood picture does not fall within the scope of this thesis, it is desirable that some attempt should be made to show how my findings fit/

fit into the generally accepted views on helminth pathogenicity for blood and blood-forming tissues. Wintrobe (1946), discussing the ability of parasite infestations to produce anaemia in the human, makes the general statement that with the exception of infestations by Diphyllobothrium latum and Schistosoma mansoni, anaemia does not occur unless nutrition is impaired, blood is lost, or certain organs are invaded. This statement may be taken as generally true as far as the domestic animals are concerned. Thus, it has been conclusively shown that the anaemia associated with Haemonchus contortus is due to chronic blood loss (Fourie, 1931; Broughton & Hardy, 1934). This has also been shown to be true, at least to some degree, in the case of Bunostomum trigonocephalum (Lucker & Neumayer, 1946). There are, however, a number of nematode worms commonly affecting sheep other than these two species, the commoner ones being members of the families Strongylidae and Trichostrongylidae. The latter family are acknowledged to be blood suckers (Mönnig, 1947), but in the case of the Strongylidae, although the larvae of Chabertia ovina have been shown to suck blood (Wetzal, 1931) and (Gordon & Graham, 1933), the adult worms of this family are not considered to exert their influence on the blood picture in this way. Even in Trichostrongylidosis it is not believed that the reduction in erythrocyte levels can be entirely explained on the basis of blood loss. The exact method by which anaemia is produced in association with these two families is as yet not properly determined, but a number of theories have been advanced. For example, Fourie (1936), as the result of a study of oesophagostomiasis in sheep, postulated a toxic action of the parasite, which produced an atrophy of haematopoietic tissue. Holman & Pattison (1941) from their/

their findings in an outbreak of parasitic gastritis in lambs due to H. contortus and Ostertagia circumcincta, concluded that although haemorrhage was partly responsible for the low erythrocyte levels, the parasites also exerted their effect by inhibiting the anabolism of iron to haemoglobin, and in certain cases by interrupting the formation of the haematopoietic principle. Gibson (1947), studying the pathogenicity of Trichostrongylus axei in sheep, attributed the oligocythaemia which he recorded to a disturbance of the metabolic processes brought about perhaps by the production of anti-enzymes by the parasite - a theory which was first advanced by Stewart (1933) and Shearer & Stewart (1933).

My findings confirm the now generally accepted view that parasites exert their maximum effect on sheep on a low plane of nutrition. It would appear that this effect is probably achieved by the interference with synthesis or absorption of a factor necessary for normal erythropoiesis. This factor is related to nutrition, and when nutrition is at a high level the effect of the worms is at least partly offset, suggesting that it is then present in more than adequate amounts. Even in sheep on a low plane of nutrition it is still present in adequate amounts in the absence of worms, but the effect of even small helminth infestations on its availability (as seen by the reduction of erythrocyte levels they can cause) point to its being present in minimal amounts when nutrition is low. The exact nature of this important factor cannot be suggested, but it is possible that it may be included among the 'specific nutritive factors', the existence of which was postulated by Stoll (1946) in a discussion of the 'break through in immunity' to parasites caused by poor nutrition.

My findings confirm Holman's contention that 'if sheep suffering from deficient diet and worm infestation could be eliminated from among apparently healthy sheep, the normal range for some, perhaps most, blood constituents could be reduced.' Under field conditions the complete elimination of these two factors will rarely be possible, but it is clear from my results that in the evaluation of blood and bone marrow findings regard must be paid to the level of nutrition and parasite burden obtaining in the flock at the time of year at which sampling is carried out.

SECTION VI.

A study of the Blood and Bone Marrow of fifteen lambs in a debilitated state following an attack of the acute form of Contagious Pustular Dermatitis.

Contagious pustular dermatitis is described as an infectious exanthematous disease common to the sheep and goat, and caused by a filter passing virus. Clinically it may occur in two forms: a benign form in which the lesions are limited to a peristomatitis, and a malignant form in which the lesions extend to invade the buccal cavity, with a possible further spread to other parts of the skin of the body, particularly in the region of the coronet, axilla, thigh and under-surface of the tail. No recognisable lesions are found in the internal organs, but secondary bacterial infection may increase the severity of the skin lesions. The disease is common in lambs under one year old and may cause considerable loss in condition, probably due to the difficulty in prehension. There may be added to this effect, in the malignant form, the disability of lameness due to foot lesions, and the systemic disturbance associated with the septic processes caused by the secondary bacterial invaders.

(Report on Diseases of Farm Livestock. Section II. Diseases of Sheep, published by the National Veterinary Medical Association. 1944)

Early in November 1950, fifteen eight-month-old cross bred lambs, which had contracted the malignant form of this disease in mid-September, were made available to the writer for study. The lambs had passed through the acute phase of the disease, but as the result of the infection they were under-sized and in poor bodily condition. They had been culled from the flock by the owner, because in his experience/

Table XXXIX.

Results of Preliminary General Examination.

Lamb's Number	Weight in lbs.	Worm egg count per gm.faeces	P.C.V. %	Body condition	Result of clinical examination.
51	-	10,400	19.0	V. poor	H.L.
52	34	700	25.0	V. poor	A.L. hind feet, lame
45	34	2,800	32.0	V. poor	A.L. ears.
E.63	48	1,100	27.5	Poor	H.L.
V.35	38	1,400	28.0	Fair	H.L.
48	56	2,500	31.0	Poor	H.L.
G. 2	55	-	-	F. good	H.L.
J.52	58	2,200	35.5	F. good	H.L.
O.45	52	3,000	35.0	Poor	H.L. Abscess left hind foot, lame.
X.22	50	900	35.5	Fair	H.L.
60	65	800	35.5	Fair	H.L. Abscess right foot and knee.
50	58	5,400	38.0	Fair	H.L.
32	35	2,100	32.5	Fair	H.L.
D.65	56	800	28.0	Poor	H.L.
P.44	48	200	26.5	V. poor	H.L. Abscess right foot.
Means	49		28.6		

H.L. = Healed lesions of contagious pustular dermatitis.

A.L. = Active " " " " "

experience lambs showed such a very slow rate of recovery from this debilitated state that their reqring was uneconomic, and they were presented to the College for study. It was therefore decided by the writer to study the blood and bone marrow of these lambs with a view to assessing the interference in haematopoiesis associated with the debility arising from the malignant form of pustular dermatitis.

Plan of Investigation.

The investigation was divided into three parts as follows:-

a. Preliminary general examination.

b. Detailed examination of blood and bone marrow.

c. Re-examination of blood and bone marrow of lambs showing abnormal marrow pictures after the elapse of an interval of time allowed for recovery.

a. Preliminary general examination.

On arrival at the College the lambs were earmarked and weighed and a clinical examination was made of each lamb. At the same time blood samples were collected for the estimation of P.C.V., and worm egg counts were carried out on faeces samples. The P.C.V. estimation was by the method already described in Section I. and worm egg counting was by the Gordon Whitlock technique (Gordon & Whitlock, 1939).

Results.

A summary of the results of the preliminary examination are shown in Table XXXIX.

The mean body weight for these lambs was 49 lbs. This is considerably below the normal average weight given by Fraser (1949) for Scottish lambs, at 68 days old. The lambs in this investigation were approximately 240 days old at the time of weighing.

From a clinical examination of the lambs it was evident that they had all recently suffered from the malignant form of contagious pustular dermatitis. There was evidence of healed and healing lesions on the ears and round the coronets of all the lambs examined. Suppurative lesions were found on four of the lambs, 52, 45, 60 and P. 44.

Eight of the lambs were described as in poor or very poor condition, five as fair, and in only two could the condition be classed as fairly good. The worm egg counts, with one exception, fell within the range suggested by Taylor (1939) as representing what he describes as the "broad border line" in which clinical significance is in doubt. The exception was No. 51, which had the highest worm egg count and the lowest P.C.V. and in which the condition was classed as "very poor". The remaining lambs showed no association between their worm egg count on the one hand, and P.C.V. and body condition on the other. From these findings it was considered justifiable to regard the helminth burden as a minor contributory cause of the debilitated state of these lambs.

In the course of the worm egg counts oocysts were observed in the case of faeces from some of these lambs. According to Dikmans & Shorb (1942), coccidia occur in practically all lambs and adult sheep and cause little or no disturbance. In the absence of symptoms of enteritis in any of the lambs, it was considered that the light coccidial infection indicated by the findings of small numbers of oocysts in the faeces was not significant.

The mean value for P.C.V. in these lambs was 28.6%. This is considerably lower than the mean of 38.1% given in Section I. for ten/

ten sheep under one year old and sampled in January, and as the standard deviation for the P.C.V. of these latter sheep was ± 2.55 a P.C.V. reading would be regarded as "possibly pathological" if it was below 33.0% (38.1 - 5.1) and below 30.45% as "definitely pathological" *. On this basis in six of the lambs the P.C.V. level was "definitely pathological" and in three of the remaining nine lambs the reading was "possibly pathological". Thus 60% of the lambs were showing some degree of anaemia.

b. Detailed Examination of Blood and Bone Marrow.

During the four weeks following their arrival, blood and bone marrow samples were examined from all of the fifteen lambs.

The examination of the blood included P.C.V. and Hb. estimations, erythrocyte and leucocyte counts and differential leucocyte count. The marrow examination, which was carried out on material obtained by sternal puncture biopsy, consisted of an assessment of cellularity of spread preparations, and a differential marrow cell count. The techniques of sampling and examination were those already described in previous sections.

Results.

The results of these examinations have been summarised in the form of Tables XL. and XLI. and Figures 40-42. The full data of the results appear in the Appendix, pages A.42 to A.47.

In the interpretation of the findings in this investigation use has been made of standards calculated from observations reported earlier in this thesis.

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* The terms "definitely pathological" and "possibly pathological" are used according to Holman in his discussion of the Interpretation of the Leucocytic Picture in the chapter on Clinical Haematology in "Diagnostic Methods in Veterinary Medicine", by Geo. F. Boddie, 3rd edition, 1950.

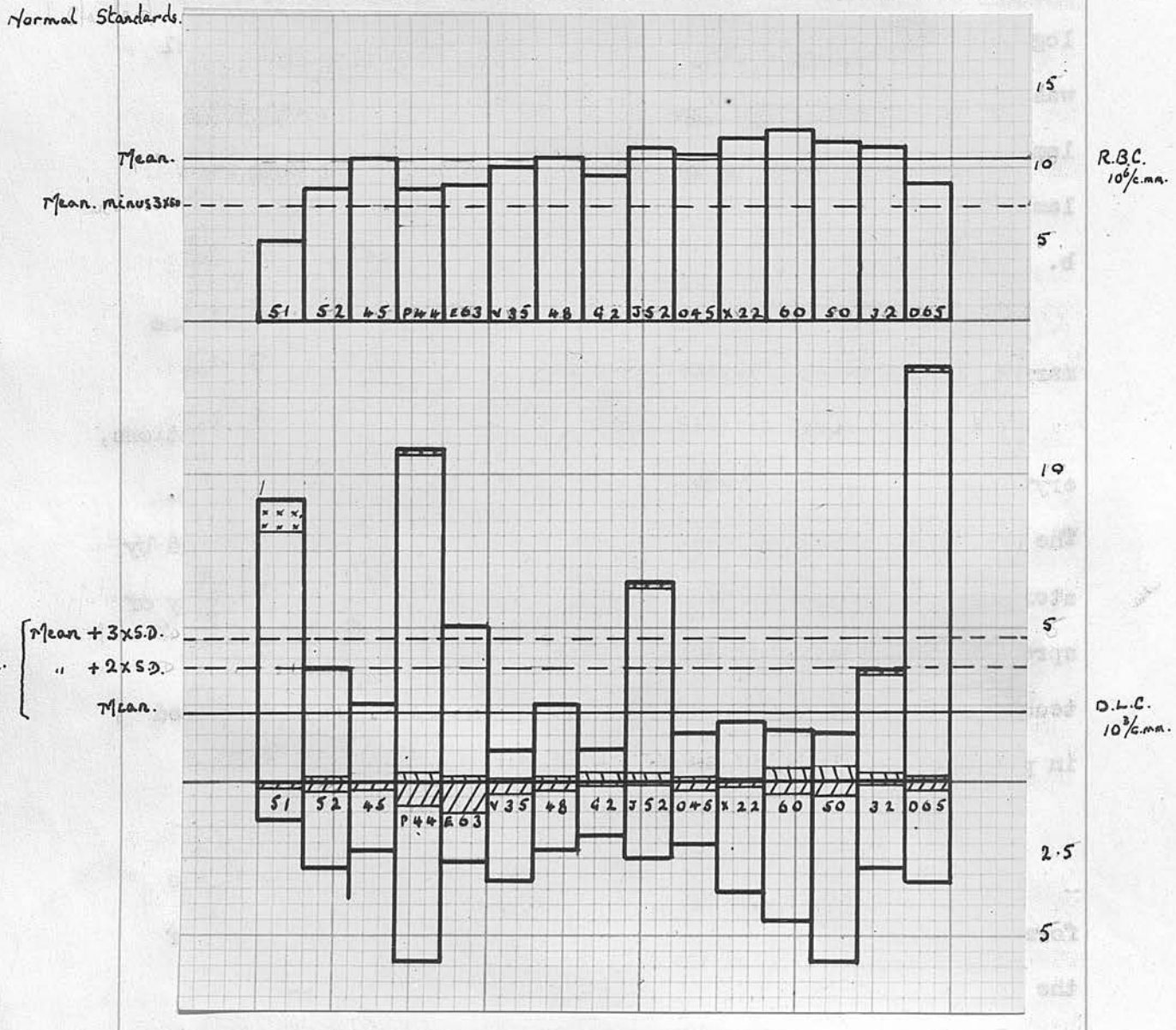

Fig. 40. Haemogram showing erythrocyte and differential leucocyte counts. The sheeps' numbers are shown on the figures.

Peripheral Blood.

In the case of the peripheral blood the mean and standard deviation (S.D.) of the sheep under one year old which were sampled in January (Section I.) provided the normal standard. These standards were chosen as the sheep from which they were compiled were of approximately the same age as those in this investigation, and had also been shown to be carrying an insignificant worm burden.

	Mean	S.D.
Erythrocyte count 10/cu.mm.	10.72	± 1.13
M.C.V. cu.	36.20	± 2.49
M.C.H.C. %	29.10	± 1.20
Leucocytes/ cu. mm.		
Neutrophil Stab.....	35	∓ 44.8
Neutrophil Polys.....	2274	∓ 781.8
Eosinophils	96	∓ 80.0
Lymphocytes.....	4881	∓ 924.1
Monocytes	386	∓ 198.5

Values found to be above or below the mean ∓ twice the standard deviation were considered as probably abnormal, those above or below the mean ∓ three times the standard deviation were regarded as definitely abnormal.

The results of the peripheral blood examinations of the lambs in this investigation are shown as a haemogram in Fig. 40.
Using the above figures as a standard, it was found that a number of the lambs had blood pictures abnormal in some respect.

In lamb 51 the erythrocyte count showed the existence of an anaemia/.

anaemia. From the M.C.V. and M.C.H.C. the anaemia in this lamb was classified as normochromic normocytic. No regenerative forms were demonstrated in the examination of 10,000 erythrocytes in the blood film.

Five lambs showed a neutrophilia which was definitely abnormal. These were J. 52, E. 63, P. 44, D. 65 and 51. In the case of P.44, D. 65, and 51 the neutrophilia was accompanied by a shift to the left. Both P. 44 and 51 also showed an eosinophilia which was above the standard mean + twice S.D.

The eosinophil count exceeded the 'Normal' mean + three times the S.D. in the case of lambs G.2, 50, 60 and 32.

The monocyte count in lamb E. 63 was above the level of the 'normal' mean + 3 X S.D.

Thus, nine of the fifteen lambs showed abnormal leucocyte counts. These findings were regarded as representing various stages in the leucocyte reaction to infection described by Schilling (1929).

Bone Marrow.

For the interpretation of the results of the marrow examination use was made of the M/E ratio and cellularity gradings found in the three sheep kept on a diet providing full maintenance and production (Section IV.) These sheep were chosen as being approximately the same age and as being sampled under similar conditions to the lambs in this investigation.

In the table below the M/E ratio and the cellularity grading for the spreads are shown for the fifteen lambs sampled in this study/

study. In the same table the ranges in M/E ratio and cellularity found in the 21 samples collected from the three normal sheep (Section IV.) are shown for comparative purposes.

Table XL.

M/E Ratio and Cellularity grading of Marrow Spreads of fifteen Lambs.

Normal Standard 0.3 : 1 - 2.5 : 1					
Lamb No.	M/E Ratio	Cellularity	Lamb No.	M/E Ratio	Cellularity
60	0.7 : 1	II.	50	1.5 : 1	IV.
32	1.5 : 1	III.	D.65	1.4 : 1	IV.
51	37.3 : 1	IV.	52	9.3 : 1	IV.
45	18.7 : 1	IV.	P.44	5.8 : 1	IV.
E.63	15.2 : 1	III.	V.35	10.8 : 1	III.
48	2.1 : 1	III.	G.2	1.8 : 1	IV.
J.52	1.4 : 1	III.	045	0.9 : 1	IV.
X.22	1.5 : 1	II.			

The grading of cellularity shows that no deficiency of cells existed in any of the spreads examined, and thus there was no tendency towards general marrow hypoplasia. However, six of the fifteen marrows had a M/E ratio above the normal range, showing that in these preparations there was a marked preponderance of granuloblastic tissue. The lambs showing this abhormality were 51, 52, 45, P.44, E.63 and V.35. Three of these lambs have also been shown to have a neutrophilia, i.e., 51, P.44 and E.63.

The/

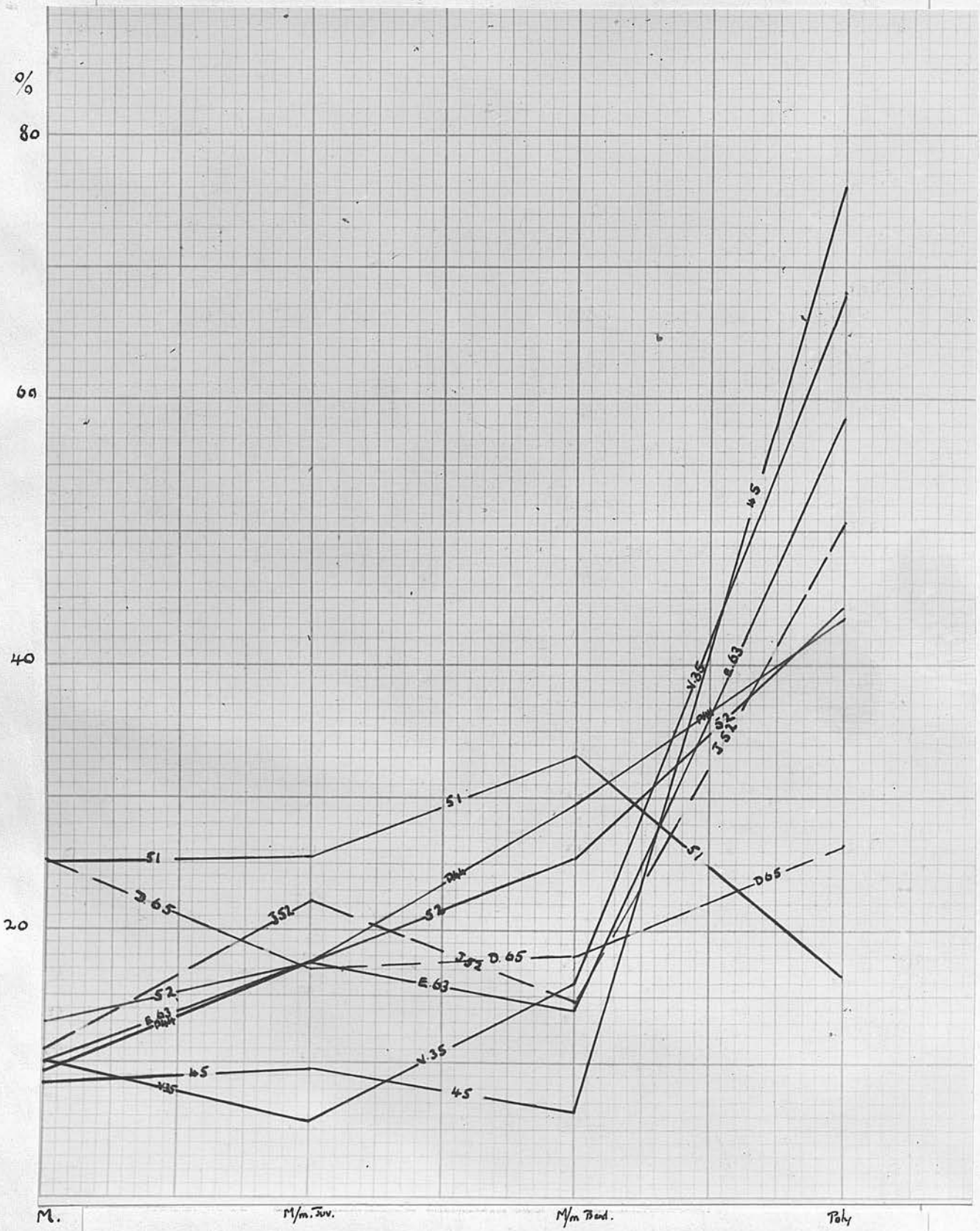

Fig. 41. Maturation curves for neutrophil granuloblasts for the sheep which were found to have either a neutrophilia or an abnormally high M/E ratio.

The highest M/E ratio was in the case of lamb 51. Examination of the marrow spread from this lamb showed the erythroblasts to be of very low incidence, and it is suggested that the normochromic normocytic anaemia found in this lamb was due to erythroblastic hypoplasia.

In Figs. 41 and 42 maturation curves have been drawn in respect of the neutrophil granuloblasts. Fig.41 shows curves for the sheep which were found to have either a neutrophilia or an abnormally high M/E ratio, viz., D. 65, 51, J. 52, E. 63, P. 44, 45 and V. 35. Fig.42 shows the curves for the sheep in which both the neutrophil count and M/E ratio were normal. It will be seen that there is a marked tendency for the curves in Fig. 41 to show a higher percentage of juvenile metamyelocytes than is the case in Fig.42.

Great variation was observed in the maturation curves of the eosinophil granuloblasts, but in the case of the lamb G.2, in which there was an eosinophilia, there was also a preponderance of eosinophil myelocytes, these cells comprising 61% of the eosinophil granuloblasts counted.

C. Re-examination of Blood and Bone Marrow of Lambs showing an abnormal marrow picture.

From four of the six lambs showing abnormal M/E ratio further blood and marrow samples were collected after the lapse of approximately eight weeks from the first sampling. The lambs so examined were P. 44, E. 63, V. 35, and 45, and the results of the first and second examinations are shown below. Re-sampling was not possible in the case of lambs 51, and 52, as both the lambs died soon after arrival from Enterotoxaemia (Clostridium welchii Type D.), which was associated with the rapid change to a more nutritious diet.

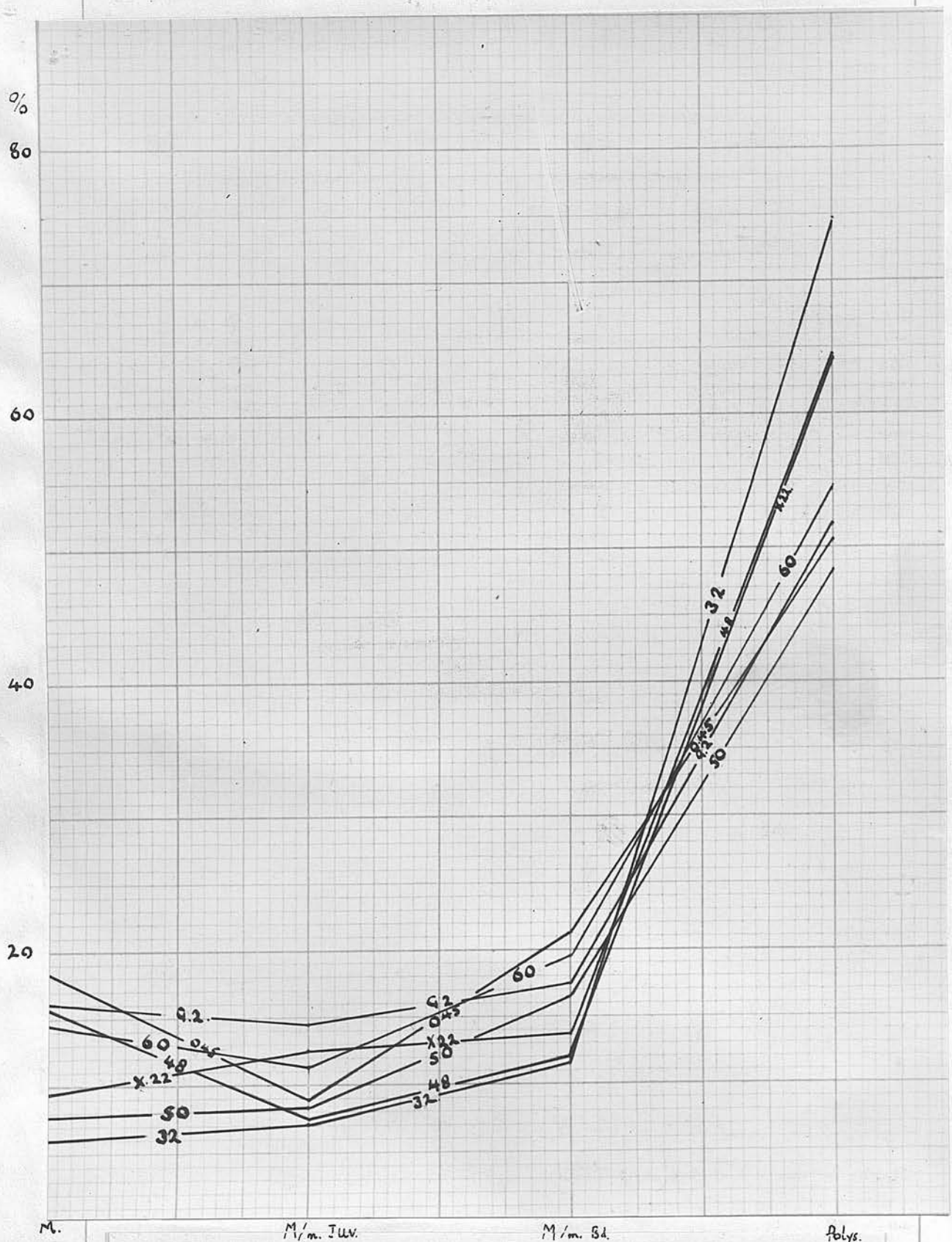

Fig. 42. Maturation curves for neutrophil granuloblasts in the sheep in which both neutrophil count and M/E ratio were normal.

Table XLI.

Erythrocyte Count, Differential Leucocyte Count, and Myeloid Erythroid Ratio at first and second sampling.

Lambs No.	P.44		E. 63		V.35		45	
Erythrocyte Count 10^6/cu.mm.	8.7	12.3	9.2	12.1	10.0	11.8	10.8	6.5
D.L.C./cu.mm.								
Neutrophil Band	186	15	90	0	48	0	0	16
Neutrophil Polys.	11532	3319	5040	1155	1080	260	2525	1914
Eosinophils	279	192	45	665	24	260	0	0
Basophils.	0	0	90	70	0	65	0	16
Lymphocytes.	5859	2183	2610	4655	3336	5460	2295	1287
Monocytes	744	192	1125	455	360	455	2281	66
M/E Ratio: 1	5.8	1.4	15.2	3.4	10.8	2.9	18.7	-

Lambs P. 44, E. 63, and V. 35.

The leucocyte counts of P. 44 and E. 63 both showed a return to normal levels, with the exception in the case of E. 63 in that the eosinophils were above the normal range.

In P. 44, E. 63 and V. 35 the erythrocyte counts all rose, confirming that some degree of oligocythaemia had existed in the first samples taken from these lambs.

Although there was a considerable reduction in the relative incidence of granuloblasts in the marrows of all three sheep, only in P. 44 did the M/E ratio fall below the maximum limit of the ranges found in the three sheep which formed the normal basis for comparison in this investigation.

Lamb 45.

In lamb 45 it was found at the second sampling that an anaemia had developed, which from the erythrocyte indices was classified as normochromic and normocytic. No signs of regeneration were found in the peripheral blood films. The hypoplastic nature of the anaemia was confirmed by the results of the marrow examination. The spreads prepared from the second sampling were graded at 0 for cellularity. They consisted of fat and reticular stroma with the cellular content restricted to occasional polymorphonuclear leucocytes, late normoblasts and haematogones. There were too few cells to permit of a differential cell count.

It is of interest to notice that although in the initial sample collected from this lamb the granuloblasts were very predominant (M/E ratio, 8.7 : 1), there was no shift to the left in the neutrophil granuloblasts and, on the contrary, over half the cells counted in the marrow were polymorphonuclear neutrophils, although no neutrophilia was evident in the peripheral blood. This finding suggested some degree of hypoplasia existing at the time of the first sampling.

Four days after the second samples had been collected, the lamb was found in a collapsed state; it was slaughtered and a post-mortem examination carried out. No cause could be found for the underdevelopment and emaciation which was observed. Histological examination of sections of sternal and femoral marrow showed it to consist mainly of "fat-free" adipose tissue with only very slight haemopoietic activity on the borders. This finding confirmed the diagnosis of aplasia made from the examination of the marrow obtained by biopsy sampling.

Discussion and Conclusions.

The owner's history of the outbreak and the results of the preliminary clinical examination, indicate that the lambs in this investigation were in the early stages of recovery from the malignant form of contagious pustular dermatitis. The differences in the bodily condition of the lambs showed that some had made greater progress towards complete recovery than others. Thus, the condition in eight of the lambs was described as "good" or "fairly good", but in seven as "poor" or "very poor". Of the six abnormal marrows found in the investigation, five occurred among the seven sheep in poor or very poor condition. The main abnormality demonstrated in the marrow was a granuloblastic hyperplasia with which a neutrophilia was associated in most cases. There was some evidence to suggest that the paucity of the erythroid tissue of the marrow was not merely relative to the granuloblastic hyperplasia, but rather that there was a tendency towards the development of a hypoplastic state in the erythroblastic tissue.

Evidence of leucocyte reaction persisted in both blood and bone marrow of some of the lambs, although the skin lesions were either healed or of limited extent. This fact is an indication of the severity of the infection to which these lambs had recently been exposed. In children severe infections are acknowledged to be a common cause of hypoplasia (Fallon, 1938), and it is possible that the tendency for hypoplasia to develop in these lambs played some part in prolonging their debility. Further observations on the blood and bone marrow of lambs during the acute stages of the disease and in the recovery period are needed to confirm this hypothesis.

SECTION VII.

The Blood and Bone Marrow of six Sheep suffering from Cachexia.

The object of the investigation was the study of the nature of the anaemia found in six cachetic sheep by a simultaneous examination of blood and bone marrow. The sheep had been culled from the flocks from which they came as being representative examples of that particular form of chronic debility which was causing recurrent losses in the flock concerned. Five of the six sheep came from hill farms, the sixth coming from a small flock at a zoological park. The animals had been submitted to the Department of Pathology of the Royal (Dick) Veterinary College for post-mortem examination, but before slaughter samples of blood and bone marrow were examined by the writer, using the techniques described in Sections I. and II. respectively. The examination of the blood comprised P.C.V., R.B.C., Hb., W.B.C. and D.L.C. with an examination of stained films for evidence of regeneration. Values for the indices M.C.V. and M.C.H.C. were calculated from the results of the examination of the erythrocytic properties. From the marrow samples, spreads were prepared. The grade of cellularity of the spreads was estimated and haemomyelograms and maturation curves constructed from the results of a differential cell count. Sections were cut from aggregations of the marrow flecks collected at biopsy sampling. At the time of sampling a clinical examination of each animal was carried out. All six sheep were found to be in an emaciated condition, and all showed clinical symptoms of anaemia in the form of pallor of visible mucous membranes and increased pulse and respiratory rates on slight exertion. The sheep were then slaughtered and a post-mortem examination/

examination was conducted by I.S. Beattie, B.Sc., M.R.C.V.S., of the Department of Pathology, and it is with his permission that a summary of the findings is reproduced here for correlation with my results.

Results.

In the interpretation of the results of the examination of the blood and bone marrow, use was made of standards calculated from observations made in previous Sections in this thesis. For the peripheral blood measurements the standards used were those calculated from the sheep sampled in Section I. In the case of the marrow findings comparison was made with the ranges found in the results of the examination of the twenty-seven ewes which comprised the material for the investigation described in Section V. On these bases the results of the blood and bone marrow examination of the six sheep in this study are shown in Table XLII. The data from which this table is compiled are shown on Appendix pages A.48 and A.49.

Histological Sections.

The photomicrographs shown as Figs. 43 - 46 are taken from sections made from marrow flecks obtained at the biopsy of sheep L.20, H. 613, H. 911 and RR. 200.

From the grading of the cellularity of the spreads, L. 20 and H.613 were shown to have hypercellular marrows. In the case of H. 911 and RR. 200 the grading was 0 and a state of advanced hypoplasia was diagnosed. This difference in marrow activity is shown in the sections of marrow flecks appearing as Figs. 43, 44, 45 and 46.

From the Table it will be seen that in 99/51, L.20 and H. 613 the anaemia was associated with a hypoplastic condition of the marrow; In H. 911 and RR. 200 advanced hypoplasia was found, and in the case of/

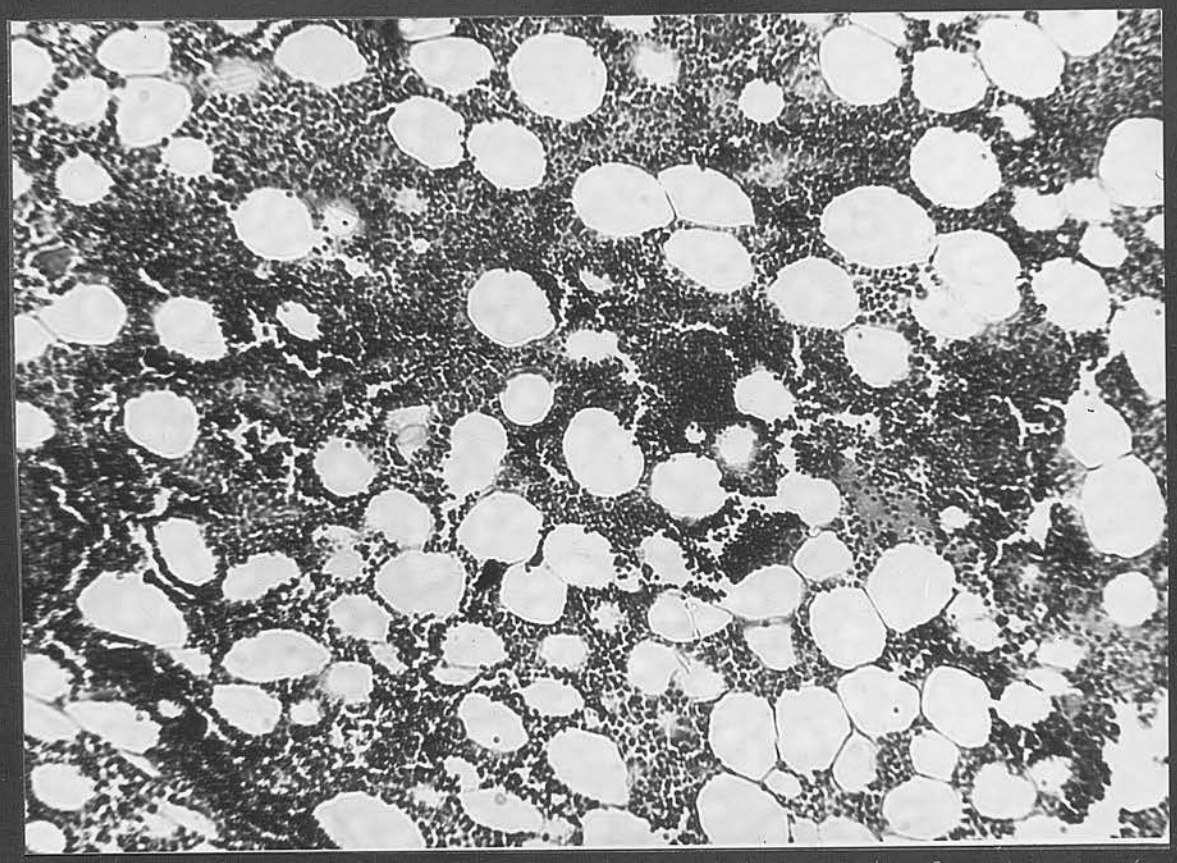

Fig. 43. Section of marrow flecks obtained by sternal puncture from sheep L.20 (x250)

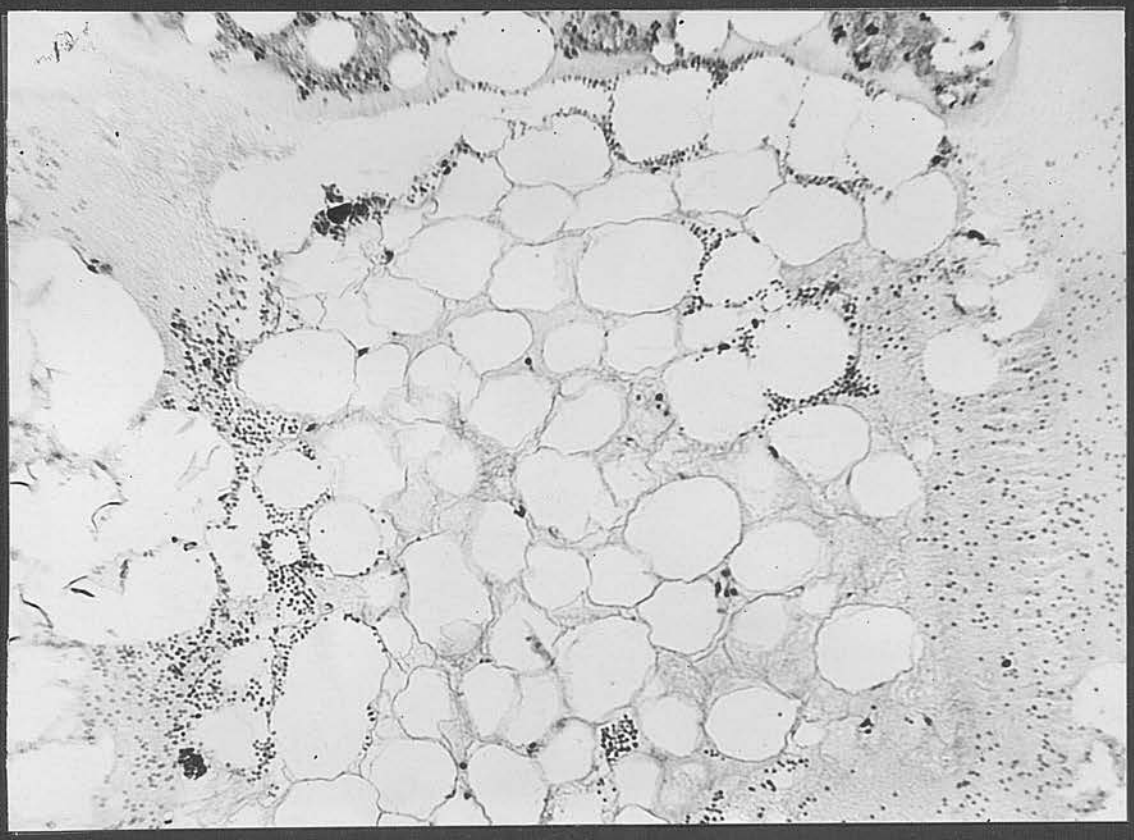

Fig 44. Section of marrow flecks obtained by sternal puncture from sheep R.R.200 (x200)

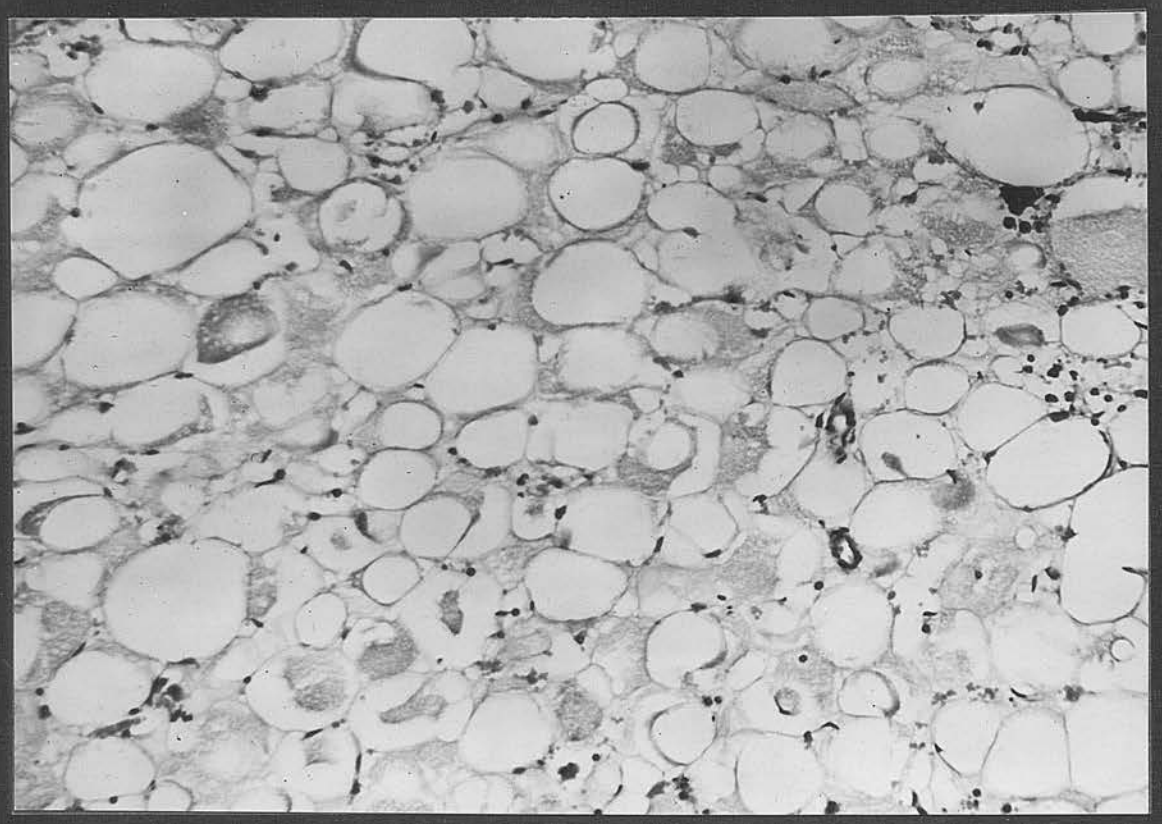

Fig 45. Section of marrow flecks obtained by sternal puncture from sheep H.9.11. (×250)

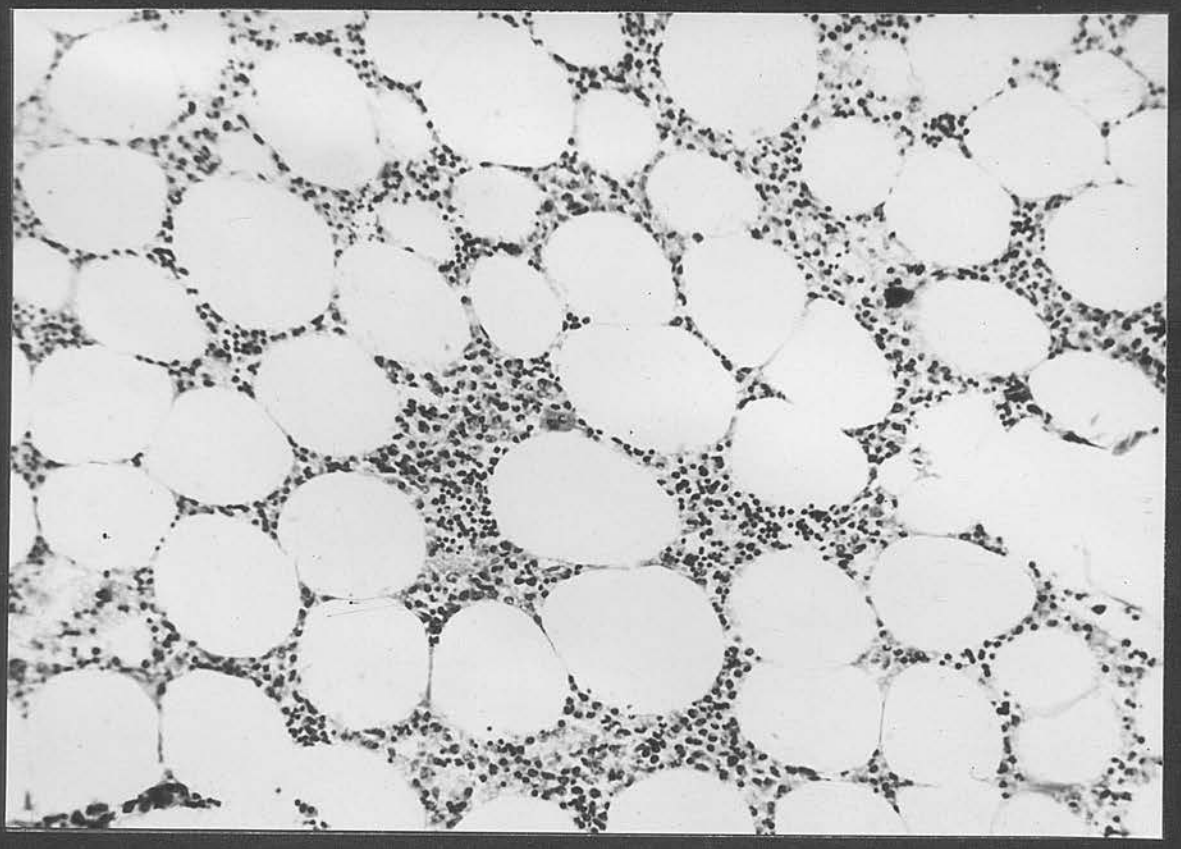

Fig. 46. Section of marrow flecks obtained by sternal puncture from sheep H613 (×250)

Table XLII.

Showing the results of the examination of the blood and bone marrow of the six sheep and a summary of the post-mortem findings.

– = Normal.

Sheep's No.		99/51	L. 20	H. 613	H. 602	H. 911	R.R. 200
Age and Sex		2½ y.o ewe	2 y.o. ewe	2 y.o. ewe	2 y.o. ewe.	5 y.o. ewe	Aged ram
Erythron	Peripheral blood	Normochromic, normocytic anaemia without regeneration	Normocytic normochromic anaemia without regeneration.	Hypochromic normocytic anaemia without regeneration.	Macrocytic hypochromic anaemia with regeneration	Normal	Normochromic normocytic anaemia without regeneration.
	Marrow	Erythroblasts reduced in numbers	Erythroblasts increased in numbers and some shift to the left	Erythroblasts increased.	Erythroblasts increased	Advanced hypoplasia	Advanced hypoplasia
Leukon	Peripheral blood	Slight neutrophilia.	–	–	Marked neutrophilia with shift to the left.	Neutrophilia with shift to the left.	Marked neutrophilia with shift to the left.
	Marrow	Granuloblasts predominate	–	Granuloblasts increased	Granuloblasts predominate	Advanced hypoplasia	Advanced hypoplasia.
Other marrow cells		–	–	–	Plasma cells increased	–	–
Cellularity		Normal	Hypercellular	Hypercellular	Hypercellular	Almost acellular	Almost acellular
M/E Ratio		15 : 1	0.2 : 1	1.4 : 1	4.6 : 1	Differential count not possible.	Differential count not possible
Diagnosis of haemocytological picture.		Hypoplasia of erythroblastic tissue.	Hypoplasia due to interference with maturation in the erythroblasts	Hypoplasia due to interference with maturation in the erythroblasts.	Hyperplasia of both erythroblastic and granuloblastic tissue.	General advanced hypoplasia	General advanced hypoplasia.
Post-mortem findings		Heavy infestations with ostertagia & trichostrongylus spp. Johne's disease & advanced fascioliasis.	No significant findings.	Heavy infestations with ostertagia & trichostrongylus spp.	Heavy infestations with Haemonchus, ostertagia & trichostrongylus spp.	Heavy infestations with ostertagia & trichostrongylus spp. Johne's disease	No findings other than advanced cachexia.
Marrow sections.		Normal Cellularity	V. cellular	V. cellular	V. cellular	Almost acellular.	Almost acellular.

of the remaining sheep, H. 602 hyperplasia of the erythroblastic tissue was demonstrated.

In sheep 99/51, L. 10 and H. 613 two different forms of hypoplasia were recognised. In 99/51 the change took the form of a simple reduction in erythroblastic tissue. That this was not merely relative to an increase in the granuloblastic elements is shown by the fact that the marrow was not found to be hypercellular, and no shift to the left could be demonstrated in the granuloblasts. In the case of L. 20 the marrow cellularity was increased, and this was found to be due to an increase in the erythroblastic tissue, in which some shift to the left was found. A similar increase in cellularity was found in the marrow from H. 613, both erythroblastic and granuloblastic tissues contributing to the increase. The fact that in spite of highly active erythroblastic tissue in the marrow in both L. 20 and H. 614 neither showed a corresponding increase in regenerative forms in the peripheral blood, suggests that the anaemia in these sheep was attributable to a 'blocking' of erythroblastic maturation in the marrow.

The anaemia in the case of ram R.R. 200 was found to be associated with a state of advanced hypoplasia, and as the ram was in a state of collapse at the time of sampling the neutrophilia found in the peripheral blood may be assumed to be part of a terminal leucocytosis. The findings in respect of sheep H. 911 requite special consideration. Although there were clinical symptoms of anaemia, the blood picture was normal and gave no indication of the state of the marrow, and it was only as a result of the examination of marrow by sternal biopsy that a state of advanced hypoplasia was found. In view of the possible criticism that the

material/

material obtained by bioptic sampling may not, in this case, have been representative of the true marrow picture, it should be stated that sections of marrow made from a number of sites confirmed the state of advanced hypoplasia diagnosed from the examination of material obtained by sternal puncture.

The findings in respect of sheep H. 602 offer a direct contrast to the other five sheep. This animal was found to be suffering from macrocytic hypochromic anaemia with evidence of regeneration in the peripheral blood. There was also a marked neutrophilia and shift to the left. These changes in the peripheral blood were reflected in the marrow by the presence of a general hyperplasia. The post-mortem examination showed a heavy infestation with Haemonchus contortus. and it is suggested that the anaemia was predominantly that of blood loss due to the activities of this worm (Fourie, 1931). The existence of pulmonary abscess formation provided a possible cause for the granuloblastic stimulation, the effects of which were seen in both peripheral blood and marrow. The exact significance of the increase in plasma cells in this sheep's marrow is not known.

The post-mortem examinations conducted on the five sheep showing varying forms of hypoplasia did not indicate the exact cause of the anaemic states found. Heavy infestations with Ostertagia and Trichostrongylus spp. were discovered in three of the five sheep, and in two of these Johne's disease was shown to co-exist. As the pathogenicity of these worms for blood forming tissue is not understood, the exact role they played in the production of the findings cannot be definitely stated. These results then are presented primarily/

primarily to show the marrow changes likely to be encountered in advanced anaemia in the sheep. It is of interest therefore to note the different forms of hypoplasia which are represented. These ranged from a simple reduction in erythroblastic tissue to a state of advanced general hypoplasia, such as is known to occur as a terminal phase in anaemia due to maturation deficiencies, or exhaustion of marrow tissue by marked sepsis, etc. (Whitby & Britton). In two of the sheep the existence of an aregenerative anaemia in the presence of a hyperplastic marrow suggests that in these cases either some factor necessary for maturation was lacking, or a factor inhibiting maturation was active.

Conclusions and a Note on the Possible Value of Sternal Puncture Marrow Biopsy based on the work recorded in this Thesis.

Up to the present time little use has been made of the technique of marrow biopsy in the study of disease processes in the domestic animals, but reports have been published of its employment for this purpose in the horse, (Hjärre & Berthelsen, 1938); cow (Holzel, 1939) (Blakemore & Venn, 1950), and dog (Bloom, 1945).

The technique which has been devised and developed by the writer for the sampling of marrow during life in the sheep has been shown to be both simple and effective. Furthermore, it is considered that the qualitative and quantitative standards which have been reported in this thesis from the examination of material obtained using the technique should form a basis for the future use of the procedure in the study of disease conditions in which changes in the blood and blood-forming tissue occur. It has also been shown that bioptic sampling of marrow may be repeated at frequent intervale in the same animal/

animal with safety and thus it will be possible to examine the changes which take place in the marrow over a period of time.

Until there is a clearer definition of the requirements for blood formation in the sheep, the nature of many of the anaemias which occur in this species will remain obscure. An example of such an anaemia is that which is recognised to occur in association with diets deficient in cobalt and copper. There is evidence to suggest that these elements are concerned with the maturation of erythroblasts in the marrow. If this is the case it might be possible to demonstrate abnormalities in nuclear structure and haemoglobination of cytoplasm of the erythroblasts in the marrow in animals suffering from a deficiency of these elements. In any investigation of this hypothesis the examination of serial samples of marrow must play an indispensible part, and for this purpose the techniques described in this thesis are well suited.

Similarly, although anaemia is a well recognised symptom in many forms of helminthiasis, the pathogenesis of the anaemia in the case of many of the helminth species is imperfectly understood. By the use of bioptic marrow sampling in the study of artificially induced parasitism much valuable information might be obtained from the nature of the marrow response to the anaemia, and this should lead to a better understanding of the processes by which the parasites influence the blood-forming tissue and the blood.

The use of marrow biopsy must form an integral part of fundamental investigations such as those described above and will greatly assist the study of any disease in which changes have been shown to occur in the peripheral blood.

SUMMARY.

1. Blood samples were collected from Scottish hill sheep ranging in age from a few weeks to seven years old on four separate occasions between January and July. The number of animals sampled and the dates of sampling were as follows:- January, 11th, 44 sheep; April 5th, 49 sheep; June 7th, 64 sheep; and July 12th, 37 sheep. The measurements included packed cell volume, haemoglobin estimations, erythrocyte and leucocyte counts and differential counts. Values for mean corpuscular volume and mean corpuscular haemoglobin concentration were calculated. From the results significant variations in the erythrocytic properties were demonstrated. The sheep under two years of age were shown to have lower values for packed cell volume and haemoglobin and higher mean corpuscular haemoglobin concentration indices than sheep over that age. The erythrocyte levels, as estimated by packed cell volume and haemoglobin measurements for sheep sampled in January, April and June differed from each other significantly. The highest levels were recorded in January, the lowest in April, the values obtained in June occupying an intermediate position. The mean corpuscular haemoglobin concentration for sheep sampled in January was significantly lower than in sheep sampled in April and June. The significant association which was shown to exist between packed cell volume and worm burden in the sheep sampled in January and April suggested that the lower erythrocyte levels encountered in April were related to the higher worm burdens found at that time. Standards have been presented in the form of means and standard error, for the erythrocyte and leucocyte properties in the 194 sheep examined.

2. A technique of marrow biopsy for the sheep by sternal puncture has been described. From material obtained by this technique a description of the histology of the marrow has been presented. In the subsequent use of bioptic marrow sampling the examinations consisted of differential cell counts and estimation of the incidence of mitosis among the cells of the marrow. From the results of the differential cell counts, haemomyelograms and maturation curves have been constructed. The relative proportions of erythroblastic and granuloblastic tissue are expressed as a myeloid erythroid ratio. A scheme for the grading of marrow preparations has been devised to provide a method of quantitative measurement in the examination of spread preparations.

3. An anaemia was induced in three sheep by bleeding. From an examination of serial samples of marrow obtained by sternal puncture, it was possible to demonstrate the development of erythroblastic hypoplasia in response to the blood loss. The examination of the peripheral blood included an estimate of regenerative changes, as shown by the presence of immature forms. There was some evidence that the marrow response to the stimulus of blood loss included a speeding up of the maturation of the erythroblastic tissue.

4. Blood and bone marrow samples were examined at weekly intervals for seven weeks from six sheep on measured diets. The bone marrow was obtained by sternal puncture. The helminth infestation was maintained at a negligible level. Three of the sheep received a diet providing full maintenance, and three a diet providing half maintenance. No significant difference could be demonstrated in the ranged of variation recorded in the two groups. The results indicate/

indicate the range of variation likely to be encountered when successive samples from the same individual sheep are examined under artificial conditions of feeding and management.

5. The blood and bone marrow of two sheep were examined at fortnightly intervals from December to June. Two different sheep were sampled on each occasion, and the results of worm burden estimations carried out after slaughter were available for correlation with blood and bone marrow findings. The highest levels recorded in the erythrocytic properties of the peripheral blood were in the eight sheep sampled up to and including February 8th. From then until April 5th there was a gradual fall, the lowest readings occurring at the latter date. Thereafter there was an abrupt rise, but this rise was not sufficient to constitute a general return to the levels recorded prior to February 8th. These changes in the erythrocyte level were accompanied by inverse changes in the worm burden. There was an increase in the erythroblastic tissue of the marrow of the sheep sampled from March 22nd to May 17th as compared with the marrow of the sheep sampled before and after this period. The influence of the helminth burden and nutrition on the erythron is discussed. Evidence is advanced to show that the reduction in the erythrocyte levels observed in February, March, and early April was related to the increase in worm burden which occurred at this time. The relative paucity of erythroblastic tissue in the marrow was considered to have facilitated this effect. The increase in erythroblastic tissue, which coincided with the onset of weather conditions favourable to the growth of pasture assisted the return of erythrocyte values to higher levels.

6. An examination of the blood and bone marrow was carried out on fifteen lambs in a debilitated condition following an attack of the malignant form of contagious pustular dermatitis. It was possible to show that the anaemia found to exist in a number of the lambs was associated with some degree of erythroblastic hypoplasia.

7. A study of the blood and bone marrow was carried out in six cachetic sheep which showed symptoms of anaemia. In five of the sheep varying degrees of erythroblastic hypoplasia were demonstrated, and in the sixth, a state of hyperplasia of the erythroid tissue was shown to exist.

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APPENDIX.

Group B. Sampled 11/1/49.

Sheep's Number	Year of Birth	P.C.V. %	Hb gms/100 ml.	R.B.C. 10^6/cu.mm.	M.C.V. cu.μ	M.C.H.C. %	Total Worm Burden
801	1948	35.0	10.5	10.24	34.5	29.5	7828
803	"	39.5	11.5	10.90	37.0	28.5	3381
805	"	37.0	10.5	9.91	37.0	28.5	2127
811	"	36.0	10.9	10.52	35.0	30.0	1911
813	"	36.0	11.2	9.48	38.5	30.0	3235
816	"	36.5	10.4	9.71	37.5	28.0	1484
821	"	37.5	11.5	10.90	35.0	30.0	3962
833	"	43.0	11.6	12.93	34.0	27.0	1543
842	"	41.0	12.0	10.18	41.0	28.5	1827
845	"	39.5	12.6	12.40	32.5	31.0	610
726	1947	37.0	12.6	11.00	34.0	34.0	1157
732	"	40.5	12.2	9.43	43.0	29.5	614
738	"	40.5	11.7	10.68	38.0	28.5	3191
739	"	40.5	10.7	10.73	38.5	26.5	4810
743	"	37.0	10.8	10.88	35.0	28.5	1451
671	1946	41.0	11.2	11.05	38.0	27.0	254
675	"	44.5	12.2	12.62	34.5	28.0	112
679	"		12.0	10.27			360
680	"	41.0	12.0	13.38	31.0	28.5	1054
684	"	41.5	11.8	10.80	39.0	28.0	1904
693	"	38.0	11.1	11.60	33.5	29.0	5382
695	"	41.0	12.0	11.32	37.0	29.0	1108

Continued/

Group B. Sampled 11/1/49.

Sheep's Number	Year of Birth	P.C.V. %	Hb gms/100 ml.	R.B.C. 10^6/cu.mm.	M.C.V. Cu. μ	M.C.H.C. %	Total Worm Burden
697	1945	43.0	11.8	10.93	40.0	26.5	20
698	"		10.9	11.65			106
701	"	36.5	10.5	9.20	40.0	28.5	518
716	"	39.5	11.8	11.88	34.0	29.0	2262
717	"	36.0	11.5	9.87	37.0	31.5	578
720	"		11.9	10.10			20
703	"	45.0	12.2	12.14	38.0	26.0	4771
744	1944	40.0	11.9	11.20	36.0	29.0	889
749	"	40.5	11.8	11.58	36.0	28.5	539
759	"	43.5	12.5	10.60	41.0	28.5	865
761	"	46.5	12.5	12.10	39.5	26.5	4562
766	"	42.0	11.9	12.28	35.0	28.0	760
654	1943	40.0	11.8	9.60	42.0	28.5	1001
655	"	41.0	10.6	11.10	37.5	25.5	427
657	"	41.0	12.2	10.38	40.0	25.0	1281
658	"	37.0	11.3	9.40	39.5	30.0	1907
664	"	46.5	13.8	14.15	33.0	29.0	552
769	1942	46.0	14.2	10.90	43.0	30.0	1858
771	"	43.0	11.9	10.10	42.5	27.0	69
772	"	31.0	9.8	8.68	36.0	31.5	1820
786	"	51.8	14.0	14.13	34.0	27.0	600
789	"	44.0	12.6	11.60	38.0	28.0	300
790	"	39.0	11.3	10.00	40.0	28.5	103
791	"	41.0	11.9	10.50	39.5	28.5	505
794	"	36.0	11.1	10.02	36.5	30.5	1329

Section I.

Group B.		LEUCOCYTES IN THOUSANDS PER CU. MM.						
Sheep's Number	Year of Birth	Total	Neutrophil Bands	Neutrophil Polys.	Eosinos.	Basos.	Lymphos.	Monos.
801	1948	10.5		3990	105		5675	735
803	"	7.9		2726	40		4780	356
805	"	8.9		1994	89		6266	552
811	"	5.6	120	1641	241		3554	241
813	"	7.2		1152	216		5616	216
816	"	6.9	76	1780	76		4595	373
821	"	6.3	32	2318	0		3679	271
833	"	6.5	33	1918	98		4388	65
842	"	7.9		2520	0		4874	506
845	"	9.0	90	2700	90		5580	540
726	1947	10.5		4137	116		5922	326
732	"	6.9	138	2346	207		3933	276
738	"	5.8	58	1873	58		3515	296
739	"	5,6	56	2173	230		2974	168
743	"	3.3		1056	66		2079	99
671	1946	7.7	154	2233	154		4312	847
675	"	5.1	51	1770	102		3019	158
679	"	3.5		2135	70		1190	105
680	"	6.8		2808	292		3325	374
684	"	7.8		3120	234		4212	234
693	"	6.8		2149	218		4080	354
695	"	16.7		2355	167		13327	852

Continued/

Group B. LEUCOCYTES IN THOUSANDS PER CU. MM.

Sheep's Number	Year Of Birth	Total	Neutrophil Bands	Neutrophil Polys.	Eosinos.	Basos.	Lymphos.	Monos.
697	1945	4.9	98	1960	98	49	2597	98
698	"	4.4	44	1628	88		2464	176
701	"	7.7	77	3049	485		3850	239
716	"	7.1						
717	"	7.9		3634	79		3871	316
720	"	4.4		1778	44		2354	224
703	"	9.5	105	5007	105		4190	95
744	1944	6.1		1464	384	31	4002	220
749	"	8.3	83	1494	332		6225	166
759	"	7.5		2678	75		4515	233
761	"	8.6		2081	301		6132	86
766	"	5.8		1682	116		3596	406
654	1943	6.3		2993	284		2835	189
655	"	7.2	72	1987	511		4334	295
657	"	6.1		1830	122		3904	244
658	"	8.2	82	2927	164		4690	336
664	"	8.7		3132	174		4959	435
769	1942	6.1	31	2123	92		3648	201
771	"	7.4		2664	370		4218	148
772	"	3.9	78	1638	39		1989	156
786	"	7.3	73	1971	438		4526	292
789	"	6.4		2880	64		3072	384
790	"	8.9	89	4076	463		3809	463
791	"	5.5	55	2948	116		2156	226
794	"	5.4		2430	108		2484	378

Group C. Sampled 5/4/49.

Number	Year of Birth	P.C.V. %	Hb. Gms/100 ml.	R.B.C. 10^6/cu.mm.	M.C.V. Cu. μ	M.C.H.C. %	Worm Burden
809	1948	27.5	8.4			30.5	25174
815	"	23.5	8.1			35.0	22646
817	"	21.5	7.6			36.0	6386
820	"	26.0	8.7	8.20	31.5	34.0	6511
826	"	28.0	10.0	5.35	52.5	36.0	9801
829	"	23.5	7.7			34.5	23193
834	"	25.0	8.0			32.0	11377
835	"	28.0	9.5			34.0	19421
838	"	30.0	8.8	4.90	61.0	30.0	19627
844	"	23.0	7.8			34.0	20474
728	1947	24.0	7.8			32.5	1992
730	"	25.0	8.1			32.5	10012
734	"	18.0	5.7	4.21	43.0	32.0	8322
736	"	23.0	7.6			33.5	3487
740	"	30.0	9.8	10.80	28.0	32.5	17848
668	1946	28.0	8.4	7.01	40.0	29.5	11820
674	"	28.0	8.4	5.86	47.5	29.5	13717
677	"	29.0					9867
685	"	34.0	9.1	7.27	47.0	27.0	6561
687	"	32.5	9.2			28.5	8217
688	"	33.5	9.8			29.5	3874
690	"	32.0	9.7			30.0	1589
691	"	31.0	9.2			29.5	7746

Continued/

Group C. Sampled 5/4/49.

Number	Year of Birth	P.C.V. %	Hb. Gms/100 ml.	R.B.C. 10^6/cu.mm.	M.C.V. Cu. μ	M.C.H.C. %	Worm Burden
700	1945	36.0	9.4			26.0	1590
704	"	28.0	9.1	4.50	62.0	32.5	24773
705	"	29.0	7.7	6.70	43.0	33.5	16494
708	"	30.5	9.0			29.5	1096
711	"	33.5	9.5			28.5	3515
715	"	34.0	9.4	6.90	49.0	27.5	14441
722	"	31.0	9.5			30.5	1213
750	1944	28.0	8.8	8.06	35.0	31.5	1960
756	"	30.5	8.7	7.15	42.5	28.5	1691
757	"	31.0	9.5			30.0	9905
758	"	30.0	9.7	6.65	45.0	32.5	8864
764	"	25.0	8.1	4.79	52.0	33.0	2616
767	"	35.5	10.4	7.41	48.0	29.5	1753
652	1943	34.0	9.1			26.5	2511
653	"	26.5	8.0	5.37	49.5	30.0	5486
659	"	27.0	8.3	7.73	35.0	31.0	8645
661	"	29.5	8.5			28.5	11603
776	1942	33.5	9.1			27.0	4895
777	"	26.0	8.3			32.7	4020
780	"	24.0	8.0	6.69	36.0	34.0	7414
782	"	34.5	9.5			27.5	2081
783	"	30.0	8.8	9.60	31.5	29.0	6884
792	"	33.5	9.1			33.0	4354
793	"	27.0	9.1	5.80	46.5	34.0	18743
797	"	26.0	8.1	6.62	39.5	31.0	5625
798	"	29.5	8.7			29.5	1972

Group C.		LEUCOCYTES IN THOUSANDS PER CU. MM.						
Sheep's Number	Year of Birth	Total	Neutrophil Bands	Neutrophil Polys.	Eosinos.	Basos.	Lymphos.	Monos.
820	1948	12.0						
826	"	9.6	96	4560	150		4224	576
838	"	12.0		4440	0		6840	720
734	1947	6.2		2356	0	31	3255	558
740	"	9.0	45	4005	0		4500	450
668	1946	6.2		1798	31		3565	806
674	"	7.4		2923	37		3404	1036
685	"	11.5	58	3910			6555	978
704	1945	6.3		2583	315		3024	378
705	"	6.8		2448		68	3332	952
715	"	9.6		4992			3774	864
750	1944	9.0						
756	"	2.8						
758	"	5.4		2052			3078	270
764	"	3.6		1548			1854	198
767	"	12.8		4608			6656	1536
653	1943	7.6		2432	152		4028	988
659	"	5.7		1910	57		3078	656
661		7.0	70	3920	140	35	2450	385
777	1942	6.2		2480	124	62	3038	496
780	"	2.0						
782	"	3.9		1638	117		1794	351
783	"	7.5		2775			4050	675
792	"	8.1		2957	243		4091	810
793	"	7.7						
797	"	7.4		3700	259		3034	407

Group D. Sampled 7/6/49.

Number	Year of Birth	P.C.V. %	Hb. Gms/100 ml.	R.B.C. 10^6/cu.mm.	M.C.V. Cu.μ	M.C.H.C. %	Worm Burden
898	1949	29.0	8.8			30.0	101
878	"	29.0	8.7			30.0	404
880	"	31.0	9.1			29.0	400
883	"	36.0	10.8	10.04	36.0	30.0	56
884	"	30.0	9.0	10.24	29.0	30.0	100
886	"	32.0	9.5			29.5	100
891	"	28.0	8.4	6.72	41.6	30.0	50
895	"	38.0	11.3			30.0	152
4	"	29.0	8.5	9.00	32.0	29.0	150
20	"	21.0	7.0			34.0	0
11	"	32.5	9.8	10.84	30.0	30.0	51
26	"	20.0	6.2			31.0	0
21	"	31.0	9.0	9.93	31.0	29.0	51
13	"	30.5	9.5	10.27	30.0	31.0	0
810	1948	32.5	10.2	9.39	34.5	31.5	2415
818	"	31.0	9.0			29.0	3453
819	"	32.0	9.5			29.5	2324
822	"	28.5	9.5			33.5	7618
831	"	30.5	10.1			33.0	6663
836	"	30.0	9.5			31.5	1177
840	"	29.0	9.0	8.41	34.5	31.0	7497
843	"	28.5	8.7	7.20	39.5	30.5	4972
846	"	34.5	9.5	10.00	34.5	27.5	2973
847	"	27.0	8.4			31.0	1153

Continued/

Group D. Sampled 7/6/49.

Number	Year of Birth	P.C.V. %	Hb. Gms/100 ml.	R.B.C. 10^6/cu.mm.	M.C.V. Cu.μ	M.C.H.C. %	Worm Burden
724	1947	32.5	10.2			31.5	4823
731	"	31.0	11.1	5.80	53.5	36.0	2638
735	"	29.0	9.7			34.0	443
737	"	32.0	9.1			29.0	2500
742	"	31.5	10.4	7.85	40.0	33.0	765
669 a	1946	27.5	8.8			33.5	5188
673	"	33.5	9.4			28.0	719
681	"	32.5	10.1			31.5	1523
682	"	33.5	10.4			31.0	652
686	"	32.0	10.4			32.5	4519
689	"	27.5	9.4	7.23	38.0	34.5	8044
692	"	32.5	9.8			30.0	5627
694	"	31.5	9.9			31.5	2892
669 b	1945	36.5	11.2			31.0	5891
706	"	31.0	10.1	5.94	52.0	34.5	1378
709	"	34.0	10.1			29.5	673
710	"	29.5	9.5	9.61	31.6	33.5	1942
712	"	33.0	9.1			27.5	3687
713	"	30.0	8.5	6.49	46.0	28.0	2617
721	"	35.5	11.1			31.0	1188
723	"	36.0	10.1			28.0	2427

Continued/

Group D. Sampled 7/6/49.

Number	Year of Birth	P.C.V. %	Hb. Gms/100 ml.	R.B.C. 10^6/cu.mm.	M.C.V. Cu.μ	M.C.H.C. %	Worm Burden
747	1944	32.0	8.7			27.0	3506
751	"	36.0	9.5			26.5	4622
753	"	39.0	9.7			25.0	3139
755	"	29.5	8.8	5.81	51.0	30.0	3397
765	"	34.5	9.1			26.5	6834
768	"	33.5	9.4			28.0	197
651	1943	29.0	9.1	7.00	41.5	31.5	14120
656	"	32.0	10.2			32.0	9628
662	"	31.0	9.5			30.5	21168
663	"	30.0	9.7	7.13	42.0	32.5	4841
666	"	32.0	10.5			33.0	1133
775	1942	32.0	10.2			32.0	4178
778	"	33.0	9.5	7.40	44.5	29.0	716
784	"	28.5	9.4	5.80	49.0	33.0	4461
785	"	30.0	9.1	5.80	52.0	30.0	12715
787	"	30.0	8.5			28.0	147
788	"	38.0	10.5			28.0	6042
795	"	35.0	9.7			28.0	8147
796	"	31.5	9.9	8.81	35.5	31.5	1760

Group D. LEUCOCYTES IN THOUSANDS PER CU. MM.

Sheep's Number	Year of Birth	Total	Neutrophil Bands	Neutrophil Polys.	Eosinos	Basos.	Lymphos.	Monos.
883	1949	11.0		6490			4125	385
884	"	8.2		2542	82		5084	492
891	"	5.6		1120			4088	392
4	"	6.0	30	2580	30		2880	480
20	"	8.8		3608			4752	440
11	"	7.6		1824			5168	608
26	"	11.3		5085			5763	452
21	"	9.0		2970			5400	630
13	"	6.0		0	60		5340	600
810	1948	11.9		6188	119		5296	298
822	"	15.35	154	5066	614		8289	1228
840	"	10.1		4444	202		4949	505
843	"	12.5		5250	375		6000	875
846	"	15.7		6123	157		8792	628
731	1947	6.3		7666			4884	630
735	"	9.1		3094			5460	546
742	"	7.4		1998	444	148	4514	296
689	1946	8.2		2624	82		4920	574
694	"	7.5		1950	75		4950	525
669,	1945	7.7		2079	539		4620	462
706	"	8.4		3360	252		4200	588
710	"	7.2		3204	144		3492	360
713	"	7.3	110	2920	73		3687	511
755	1944	12.8		4224	640	128	7168	640
651	1943	8.2		4920	246		2706	328
662	"	12.8		4992	384		6720	704
663	"	9.8		5390	490		3528	392

Group R. Sampled 12/7/49.

Lambs born in 1949.

Number	Month of Birth.	P.C.V. %	Hb. Gms/100 ml.	R.B.C. 10^6/cu.mm.	M.C.V. Cu.μ	M.C.H.C. %	Worm Burden
877	April	36.4	11.1			31.0	2,940
881	"	29.5	9.7	9.92	30.0	33.0	731
882	"	29.0	9.4	9.00	32.0	32.5	955
885	"	25.5	8.4	9.50	27.0	33.5	1,862
887	"	34.5	11.3			33.0	499
888	"	30.0	9.9			33.0	695
893	"	35.0	10.9	11.39	30.5	31.0	1,619
899	"	29.0	9.5	8.68	33.5	33.0	255
900	"	31.0	9.8	8.85	35.0	32.0	1,068
2	"	26.0	8.1	8.00	32.5	31.5	2,210
8	"	30.0	9.5	10.20	29.5	31.5	1,660
9	"	33.0	10.9	10.02	33.0	33.5	581
12	"	30.5	9.8	10.82	28.0	32.0	1,463
16	"	31.0	9.8			31.5	4,029
18	"	30.5		11.25	27.0		1,558
19	"	33.0	9.5	10.03	33.0	28.5	1,010
23	"	32.0	9.5			29.5	456
892	"	35.0		12.15	29.0		1,962
25	May	31.0		12.58	25.0		654
27	"	33.0	10.1			30.5	105

Group R. Sampled 12/7/49.

Sheep's Number	Year of Birth	P.C.V. %	Hb. Gms/100 ml.	R.B.C. 10^6/cu.mm.	M.C.V. c.μ	M.C.H.C. %	Worm Burden
804	1948	30.0	9.7	9.86	30.5	32.5	620
806	"	30.0	9.2			30.5	1,878
823	"	31.5	10.5			34.5	758
824	"	34.5	11.3			32.5	1,988
830	"	30.5	9.1	8.40	36.5	29.5	1,238
839	"	35.0	10.6			30.0	315
729	1947	31.0	10.1			32.5	10,234
741	"	33.5	9.8			29.0	1,717
667	1946	31.5		8.26	38.0		2,759
683	"	33.0	10.9			33.0	1,635
714	1945	32.0	9.5	10.36	31.0	29.5	1,252
718	"	30.0	9.5	9.00	33.0	31.5	1,570
754	1944	35.0	10.8			31.0	1,163
763	"	34.0	10.1			29.5	3,780
660	1943	30.0	9.8	7.94	38.0	32.5	1,847
773	1942	32.0	10.5			33.0	386
779	"	35.0	11.5			33.0	937

Group R. LEUCOCYTES IN THOUSANDS PER CU. MM.

Sheep's Number	Year of Birth	Total	Neutrophil		Eosinos.	Basos.	Lymphos.	Monos.
			Bands	Polys.				
881	1949	6.8		2448	68		3910	374
882	"	8.3		4399	0		3486	415
885	"	9.2		3220	184		5520	276
893	"	9.9		1535	149		7673	545
899	"	4.6		552	0		3818	230
900	"	5.3		1034	0		3975	292
2	"	5.7		1482	0		3933	285
8	"	6.2		3906	0		2046	248
9	"	5.4		837	162		3969	432
12	"	5.4		918	0		4158	324
16	"	5.0	50	800	0		3750	400
18	"	6.6		2376	0		3762	264
19	"	7.0		2240	0		4480	315
23	"	6.4		1856	320		4224	288
25	"	6,4		2368	64		3680	288
27	"	9.0		2745	450		5760	450
892	"	7.2		1872	72		4824	432
804	1948	8.4		4746	168		3234	252
806	"	10;3		5665	0		4120	515
830	"	8.9		4139	178		4139	445
741	1947	7,2	36	3528	72		3204	360
667	1946	8.0		2960	440		4240	360
714	1945	8.4		1848	462		5586	504
718	"	5.2		2184	520		2704	260
754	1944	10.1		3586	556		5404	556
660	1943	5.2		2678	156		2106	260
779	1942	4.0	40	1580	180		1980	220

Analysis of Variance of Packed Cell Volume, Haemoglobin and Mean Corpuscular Haemoglobin Concentration in sheep of different ages in Groups B, C & D.

1. Packed Cell Volume.

Table of Means for P.C.V. for different ages in Groups B, C & D.

Groups	Ages in Years							Means for groups over all ages
	Under 1	1 - 2	2 - 3	3 - 4	4 - 5	5 - 6	6 - 7	
B.	38.10	39.10	41.17	40.00	42.50	41.10	48.48	40.30
C.	26.50	24.00	31.00	31.71	30.00	29.25	29.33	28.71
D.	29.70	30.35	31.30	31.31	33.19	34.08	30.80	31.27
Means for years over 3 groups	31.00	30.95	34.26	33.63	34.63	35.13	34.08	33.10

Analysis of Variance of P.C.V. in sheep of different ages in Groups B, C & D.

	D. F.	Sums of Squares	Mean Square	Ratio F.	Result
Due to Age	6	401.37	66.90	5.83	Sig. @ 1%
Due to Groups	2	3142.87	1706.44	148.81	Sig. @ 1%
Interaction between Age & Group	12	142.33	11.86	-	
Error	128	1467.75	11.47		
Treatments Total	148	5424.32			

Subdivision of Ages	D. F.	Sums of Squares	Mean Square	Ratio F.	Result
Within 1 yr. & 2 yrs.	1	0.04	0.04	0.00	Insig.
Within 3 yrs. & 7 yrs.	4	22.66	5.67	0.49	Insig.
1 & 2 versus 3 to 7	1	378.66	378.67	33.022	Sig. @ 1%
Treatments Total	6	401.37			

Subdivision of Groups	D. F.	Sums of Squares	Mean Square	Ratio F.	Result
Within Groups B & D	1	2011.10	2011.10	175.38	Sig. @ 1%
Within Groups C & D.	1	170.40	170.40	14.86	Sig. @ 1%
Group C. vs. B & D	1	1401.73	1401.73	122.24	Sig. @ 1%
Treatments Total	3	3583.23			

2. Haemoglobin.

Table of Means for Haemoglobin for different ages in Groups B, C & D.

Groups	Ages in Years							Means for groups over all ages
	Under 1	1 - 2	2 - 3	3 - 4	4 - 5	5 - 6	6 - 7	
B.	11.27	11.60	11.76	11.51	12.12	11.94	12.10	11.72
C.	8.46	7.80	9.11	9.09	9.20	8.48	8.74	8.73
D.	9.34	10.10	9.78	9.96	9.20	9.80	9.60	9.49
Means for years over 3 groups	9.50	9.52	10.35	10.11	10.29	9.92	10.20	9.94

Analysis of Variance of Haemoglobin in sheep of different ages in Groups B,C & D.

	D. F.	Sums of Squares	Mean Square	Ratio F.	Result
Due to Age	6	17.90	2.98	4.10	Sig. @ 1%
Due to Groups	2	230.49	115.25	158.35	Sig. @ 1%
Interaction between Age & Group	12	4.53	0.38		
Error	130	94.61	0.73		
Treatments Total	150	347.53			

Subdivision of Ages	D. F.	Sums of Squares	Mean Square	Ratio F.	Result
Within under 1 & 1-2	1	0.01	0.01	0.01	Insig.
Within 2 - 7	4	1.93	0.43	0.59	Insig.
Under 1 & 1-2 vs. 2-7.	1	15.97	15.97	21.94	Sig. @ 1%
Treatments Total	6	17.91			

Subdivision of Groups	D. F.	Sums of Squares	Mean Square	Ratio F.	Result
Within Groups B & D	1	126.4	126.4	173.67	Sig. @ 1%
Within Groups C & D.	1	14.49	14.49	19.90	Sig. @ 1%
Group C vs. B & D.	1	104.09	104.09	143.01	Sig. @ 1%
Treatments Total	3	244.98			

3. Mean Corpuscular Haemoglobin Concentration.

Table of Means for Mean Corpuscular Haemoglobin Concentration for different ages in Groups B, C & D.

Groups	Ages in Years							Means for groups over all ages
	Under 1	1 - 2	2 - 3	3 - 4	4 - 5	5 - 6	6 - 7	
B.	29.10	29.40	28.25	28.30	28.10	27.60	28.88	28.60
C.	33.60	32.60	29.07	29.71	30.83	29.00	30.78	31.01
D.	30.18	30.80	32.70	31.56	30.38	27.17	31.90	30.57
Means for years over 3 groups	30.87	30.90	29.81	30.10	29.92	27.80	30.34	30.13

Analysis of Variance of Mean Corpuscular Haemoglobin in sheep of different ages in Groups B, C & D.

	D. F.	Sums of Squares	Mean Square	Ratio F.	Result
Due to Age	6	115.52	19.25	5.37	Sig. @ 1%
Due to Groups	2	150.81	75.41	21.02	Sig. @ 1%
Interaction between Age & Group	12	144.73	12.06	3.36	Sig. @ 1%
Error	127	455.56	3.59		
Treatments Total	147	866.62			

Subdivision of Ages	D. F.	Sums of Squares	Mean Square	Ratio F.	Result
Within under 1 & 1-2	1	0.01	0.01	0.00	Insig.
Within 2 - 7	4	67.52	16.88	4.71	Sig. @ 1%
Under 1 & 1-2 vs. 1-7	1	47.99	47.99	13.38	Sig. @ 1%
Treatments Total	6	115.52			

Subdivision of Groups	D. F.	Sums of Squares	Mean Square	Ratio F.	Result
Within Groups B & D	1	95.54	95.54	26.64	Sig. @ 1%
Within Groups C & D.	1	4.97	4.97	1.38	Insig.
Group C vs. B & D.	1	55.28	55.28	15.41	Sig. @ 1%
Treatments Total	3	155.79			

Section III.

Sheep V. 35.

600 ml. blood removed on day 0.

Day	0	1	2	3	4	5	43
P.C.V. %	42.5	31.5	26.5	27.5	27.5	30.0	34.0
Hb. gms./100 ml.	12.0	8.5	7.8	8.4	8.3	8.5	10.2
R.B.C. 10^6/cu.mm.	11.8	8.9	7.1	7.2	6.8	9.1	10.1
M.C.V. cu. μ.	36.0	36.0	38.0	38.5	41.0	34.0	41.0
M.C.H.C. %	28.5	27.0	29.5	30.0	30.0	28.0	30.0
Reticulocytes %	0.00	0.00	0.00,	0.00	0.00	0.00	0.00
Polychromasia %	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Punctate Basophilia %	0.00	0.10	0.00	0.00	0.00	0.10	0.00
S.G. Blood	1.0537	1.0464	1.0421	1.0437	1.0426	1.0450	1.0491
" Plasma	1.0295	1.0245	1.0256	1.0256	1.0255	1.0262	1.0254
W.B.C. 10^3/cmm	6.5	7.8	7.7	6.2	7.5	6.0	5.6
D.L.C./cu.mm.							
Neut. Polymorphs	260	1,092	847	868	863	60	840
Eosinophils	260	1,326	385	620	150	240	112
Basophils	65	480	154	248	113	180	0
Lymphocytes	5,460	4,836	6,006	4,216	5,888	5,280	4,144
Monocytes	455	546	308	248	488	240	504

Section III.

Sheep V. 35.

Single bleeding of 600 ml. on Day 0.

Day	0	5	43
Haemomyelogram			
Haemocytoblasts			
Myeloblasts	0.6	1.1	0.7
Promyelocytes	0.0	0.0	0.2
N. Myelos.	9.7	3.1	5.4
N. M/myelos.	11.5	5.7	12.8
N. Bands	13.3	5.8	17.3
N. Polymorphs.	17.1	13.0	21.9
E. Myelos.	5.7	1.5	1.8
E. M/myelos.	9.7	4.7	2.7
E. Polymorphs.	3.6	1.4	3.5
Basophils	2.3	1.2	1.2
Pro-erythroblasts	0.5	4.1	1.0
Early Norms.	2.1	5.5	2.1
Interm. Norms.	22.2	44.4	22.0
Late Norms.	0.9	7.9	6.5
Plasma cells	0.3	0.3	0.4
Reticulum cells	0.2	0.3	0.2
Lymphocytes	0.2	0.2	0.1
Monocytes	0.1	0.0	0.0
M/E Ratio	1.86:1	0.61:1	2.13:1
Mitosis	0.8	1.4	0.3
Cellularity	III.	V.	IV.
Maturation Curves			
Granuloblasts			
N. Myelos.	18.8	11.2	9.4
N. M/myelos.	22.3	20.6	22.3
N. Bands	25.8	21.0	30.1
N. Polymorphs.	33.1	47.1	38.2
E. Myelos.	30.0	19.7	22.5
E. M/myelos.	51.1	61.8	33.8
E. Polymorphs.	18.9	18.4	43.7
Erythroblasts			
Pro-erythroblasts	1.9	6.6	3.2
Early Norms.	8.2	8.9	6.6
Interm. Norms.	86.4	71.7	69.6
Late Norms.	3.5	12.8	20.6

Sheep E. 63

Day	0	1	2	3	4	5	6	7
Blood withdrawn (ml.)	400	325	500	580	420	500	0	150
P.C.V. %	39.0	34.5	31.0	29.0	25.0	19.5	18.0	17.5
Hb. gms/100 ml.	11.8	9.4	9.2	8.5	7.7	5.7	5.3	5.2
R.B.C. 10 /cu.mm.	9.9	8.3	7.5	7.5	5.5	6.9	4.9	4.4
M.C.V. cu.	40.0	41.4	43.0	38.0	45.0	35.0	36.0	40.0
M.C.H.C. %	30.0	27.0	28.5	29.0	31.0	29.5	29.5	30.0
Reticulocytes %	0.00	0.00	0.00	0.00	0.65	0.60	0.75	0.51
Polychromasia %	0.00	0.00	0.00	0.00	0.03	0.21	0.52	0.36
Punctate Basophilia %	0.00	0.00	0.00	0.00	0.03	0.28	0.12	1.71
S.G. Blood	1.1526	1.0476	1.0444	1.0446	1.0382	1.0344	1.0328	1.0325
" Plasma	1.0255	1.0240	1.0226	1.0246	1.0204	1.0195	1.0196	1.0216
W.B.C. 10 /cu.mm.	7.0	8.0	7.8	6.8	6.3	6.2	6.9	5.1
D.L.C. /cu.mm.								
Neut. Bands	0	0	0	0	0	31	0	0
" Polymorphs.	1,155	1,560	1,014	578	1,764	1,209	1,794	1,377
Eosinophils	655	520	468	306	189	403	104	306
Basophils	70	40	78	170	32	124	0	0
Lymphocytes	4,655	5,560	6,045	5,508	4,221	4,185	4,865	3,213
Monocytes	455	320	195	238	95	248	138	204

Day	8	9	12	13	18	20	69
Blood withdrawn %	0	0	0	0	0	0	0
P.C.V. %	17.5	17.5	23.5	23.0	25.5	32.0	38.5
Hb. gms/100 ml.	4.5	4.8	6.0	6.4	7.6	9.0	11.5
R.B.C. 10 /cu.mm.	4.8	4.6	6.1	5.9	7.0	9.2	12.5
M.C.V. cu.	37.0	37.5	39.0	39.0	43.0	45.0	31.0
M.C.H.C. %	26.5	27.5	25.5	28.0	30.0	28.0	30.0
Reticulocytes %	0.25	0.30	0.00	0.00	0.00	0.00	0.00
Polychromasia %	0.85	0.31	0.04	0.02	0.00	0.00	0.00
Punctate Basophilia %	2.75	1.86	0.63	0.01	0.01	0.00	0.00
W.B.C. 10 /cu.mm.	5.8	4.6	5.2	5.3	4.7	5.5	5.9
S.G. Blood	1.0326	1.0326	1.0376	1.0382	1.0396	1.0461	1.0530
" Plasma	1.0208	1.0206	1.0224	1.0226	1.0228	1.0232	1.0255
D.L.C. /cu.mm.							
Neut. Polymorphs.	1,566	1,334	1,014	504	1,175	1,403	1,564
Eosinophils	116	184	104	80	94	83	561
Basophils	0	92	0	0	0	27	30
Lymphocytes	3,828	2,668	3,666	4,611	3,149	3,300	3,186
Monocytes	290	322	416	106	282	688	561

Sheep E. 63.

Day	0	5	20	69
Haemomyelogram				
Haemocytoblasts	0.0	0.0	0.0	0.0
Myeloblasts	0.2	0.6	1.0	0.6
Promyelocytes	0.0	0.0	0.2	0.0
N. Myelos.	4.8	1.5	1.8	4.5
N. M/myelos.	8.2	1.7	4.1	7.7
N. Bands	16.3	2.1	5.2	16.1
N. Polymorphs.	30.0	11.1	6.6	17.7
E. Myelos.	2.7	1.5	1.0	1.8
E. M/myelos.	4.2	0.7	0.3	2.1
E. Polymorphs.	6.8	4.4	1.4	10.2
Basophils	2.2	0.5	0.2	1.5
Pro-erythroblasts	0.2	3.4	3.1	1.3
Early Norms.	1.1	15.7	6.8	3.0
Interm. Norms.	18.6	47.1	54.6	34.5
Late Norms.	3.5	9.2	12.1	8.4
Plasma cells	0.4	0.1	0.2	0.1
Reticulum cells	0.0	0.4	1.4	0.1
Lymphocytes	0.4	0.0	0.0	0.2
Monocytes	0.4	0.0	0.0	0.2
M/E Ratio	3.40:1	0.32:1	0.28:1	1.11:1
Mitosis	0.4	1.4	1.1	0.5
Cellularity	IV.	V.	IV.	III.
Maturation Curves				
Granuloblasts				
N. Myelos.	8.1	9.1	10.2	12.5
N. M/myelos.	13.8	10.4	23.2	21.4
N. Bands	27.5	12.8	29.3	16.9
N. Polymorphs;	50.6	67.7	37.3	49.2
E. Myelos.	19.7	22.7	37.0	12.8
E. M/myelos.	30.7	10.6	11.1	14.9
E. Polymorphs.	49.6	67.7	51.9	72.3
Erythroblasts				
Pro-erythroblasts	0.0	4.5	4.0	2.8
Early Norms.	4.7	20.8	8.9	6.4
Interm. Norms.	79.5	62.5	71.3	73.0
Late Norms.	14.9	12.2	15.8	17.8

Sheep 50

Day	0	1	2	3	4	5	6	7
Blood withdrawn (ml.)	780	780	0	250	250	250	0	250
P.C.V. %	47.5	39.0	31.0	28.0	27.0	25.0	25.0	25.0
Hb. gms/100 ml.	12.9	10.9	8.4	8.3	7.7	7.1	7.1	6.3
R.B.C. 10^6/cu. mm.	13.3	10.4	8.8	8.1	7.7	7.0	6.6	6.6
M.C.V. cu. μ	35.5	38.0	36.0	34.5	35.5	36.0	38.0	38.0
M.C.H.C. %	27.5	28.0	27.0	29.5	28.5	28.5	28.5	25.5
Reticulocytes %	0.00	0.00	0.00	0.00	0.80	0.64	1.14	0.55
Polychromasia %	0.00	0.00	0.00	0.02	0.06	0.18	0.58	0.14
Punctate Basophilia %	0.00	0.00	0.00	0.00	0.80	0.75	2.02	0.54
S.G. Blood	1.0561	1.0511	1.0455	1.0443	1.0426	1.0401	1.0400	1.0401
" Plasma	1.0287	1.0266	1.0251	1.0250	1.0249	1.0256	1.0248	1.0246
W.B.C. 10^3/cu.mm.	11.6	11.0	9.5	8.9	8.5	10.3	7.6	8.5
D.L.C./cu.mm.								
Neut. Polymorphs.	1,624	660	950	1,558	1,318	1,030	456	1,063
Eosinophils	1,276	1,265	1,188	979	553	824	532	468
Basophils	58	0	0	45	85	52	0	43
Lymphocytes	1,178	8,580	7,030	5,830	6,333	7,725	6,232	6,545
Monocytes	464	495	333	490	213	670	380	383

Day	8	9	10	11	12	14	31	41
Blood withdrawn (ml.)	250	250	0	250	0	0	0	0
P.C.V. %	25.0	26.5	21.0	18.0	20.5	24.0	36.5	40.0
Hb. gms/100 ml.	6.4	6.7	5.6	4.9	5.6	7.0	9.9	12.3
R.B.C. 10^6/cu.mm.	6.0	6.3	4.0	5.1	6.1	7.3	11.3	14.2
M.C.V. cu. μ	41.5	42.0	41.5	35.0	33.5	33.0	32.0	28.5
M.C.H.C. %	25.5	25.5	27.0	27.0	27.0	29.0	27.0	30.5
Reticulocytes %	0.46	0.55	0.40	0.22	0.37	0.07	0.00	0.00
Polychromasia %	0.08	0.18	0.00	0.03	0.05	0.00	0.00	0.00
Punctate Basophilia %	0.51	1.28	0.91	0.64	1.56	0.22	0.00	0.00
S.G. Blood	1.0536	1.0399	1.0401	1.0371	1.0356	1.0371	1.0405	1.0506
" Plasma	1.0266	1.0236	1.0237	1.0235	1.0236	1.0244	1.0248	1.0266
W.B.C. 10^3/cu.mm.	10.5	9.4	7.6	8.4	8.8	8.5	10.6	12.1
D.L.C./cu.mm.								
Neut. Polymorphs.	1,628	1,269	1,064	1,176	1,716	340	742	3,146
Eosinophils.	945	658	456	588	440	425	424	1,392
Basophils.	0	0	76	42	0	0	0	0
Lymphocytes	7,508	7,003	5,662	6,174	6,292	7,225	9,116	6,655
Monocytes	420	470	342	420	352	510	318	908

Sheep 50.

Day	0	3	7	31	41
Haemomyelogram					
Haemocytoblasts	0.0	0.3	0.2	0.0	0.0
Myeloblasts	0.9	1.0	0.7	0.6	0.5
Promyelocytes	0.0	0.0	0.0	0.1	0.0
N. Myelos.	3.7	3.7	3.3	5.2	4.0
N. M/myelos.	4.9	4.3	3.0	4.6	5.1
N. Bands	7.9	7.7	2.0	6.5	10.0
N. Polymorphs.	19.6	18.8	7.5	19.3	26.8
E. Myelos.	6.2	5.8	1.8	3.6	5.1
E. M/myelos.	8.3	8.7	2.6	4.5	8.0
E. Polymorphs.	4.4	4.6	3.3	2.2	2.1
Basophils	0.6	0.4	0.5	1.6	1.0
Pro-erythroblasts	1.1	6.1	4.3	2.0	1.6
Early Norms.	3.2	9.4	20.8	11.3	5.4
Interm. Norms.	28.9	21.6	43.2	27.6	27.9
Late Norms.	0.6	5.5	5.7	6.5	2.0
Plasma cells	0.2	0.6	0.1	1.3	0.2
Reticulum cells	1.0	1.5	0.8	2.8	0.5
Lymphocytes	0.1	0.0	0.0	0.1	0.0
Monocytes	0.0	0.0	0.0	0.2	0.0
M/E Ratio	1.34:1	1.29:1	0.34:1	1.01:1	1.69:1
Mitosis	0.6	0.5	1.9	1.6	0.1
Cellularity	IV.	V.	V.	V.	IV.
Maturation Curves					
Granuloblasts					
N. Myelos.	10.2	10.7	20.9	14.6	8.7
N. M/myelos.	13.6	12.5	18.9	12.9	11.1
N. Bands	21.9	22.3	12.7	18.3	21.8
N. Polymorphs.	54.3	54.5	47.5	54.2	58.4
E. Myelos.	32.8	30.4	23.4	34.9	33.6
E. M/myelos.	43.9	45.5	33.7	43.7	52.6
E. Polymorphs.	23.3	24.1	42.9	21.4	13.8
Erythroblasts					
Pro-erythroblasts	2.6	14.3	5.8	4.2	4.3
Early Norms.	7.6	22.1	28.1	23.8	14.6
Interm. Norms.	68.5	50.7	58.4	58.2	75.6
Late Norms.	21.3	12.9	7.7	13.7	5.4

Sheep E. 98

Results on	1st day	7th day	14th day	21st day	28th day	35th day	42nd day
Haemomyelogram							
Haemocytoblasts	0.1	0.0	0.0	0.0	0.0	0.0	0.0
Myeloblasts	0.6	0.7	0.5	0.2	0.7	0.7	0.8
Promyelocytes	0.0	0.2	0.0	0.1	0.0	0.0	0.1
N. Myelos.	3.2	1.4	2.5	3.4	5.2	2.8	2.4
N. M/myelos.	3.5	3.6	4.9	8.1	10.6	5.1	5.5
N. Bands	4.9	2.7	4.1	1.9	8.0	5.9	3.5
N. Polymorphs.	16.3	15.5	16.1	9.3	17.7	16.2	12.0
E. Myelos.	4.6	3.7	5.4	5.7	8.0	7.0	3.6
E. M/myelos.	2.3	3.0	5.2	7.2	8.8	8.4	7.3
E. Polymorphs.	6.9	4.6	3.0	4.9	7.4	7.5	6.1
Basophils	0.4	0.1	0.7	0.1	0.4	0.5	0.1
Pro-erythroblasts	0.1	1.3	1.1	0.6	1.7	1.4	1.4
Early Norms.	2.9	5.0	2.2	4.1	1.6	5.4	1.5
Interm. Norms.	45.6	50.0	50.7	52.6	26.2	32.6	46.2
Late Norms.	6.2	7.9	2.6	1.0	2.3	6.1	8.6
Plasma cells	0.1	0.2	0.1	0.2	0.1	0.1	0.1
Reticulum cells	0.3	0.2	0.5	0.5	0.5	0.3	0.4
Lymphocytes	0.4	0.5	0.3	0.1	0.8	0.0	0.3
Monocytes	0.0	0.0	0.1	0.0	0.0	0.0	0.1
M/E Ratio	0.79:1	0.54:1	0.75:1	0.70:1	2.1:1	1.2:1	0.72:1
Mitosis	0.3	0.6	0.1	0.5	0.4	0.5	0,2
T. M. C.	256,600	121,800	143,800	51,000	39,200	129,000	101,200
Cellularity	IV.	III.	IV.	IV.	I.	II.	IV.
Peripheral Blood							
P.C.V. %	38.5	37.0	40.0	35.0	38.0	38.0	37.25
Hb. gms/100 ml.	11.2	10.9	11.6	9.9	12.9	11.5	10.2
R.B.C. 10 /cu.mm.	12.4	11.6	13.7	10.6	13.4	12.7	12.5
M.C.V. cu.	31.0	32.0	29.0	33.6	28.5	30.0	30.0
M.C.H.C. %	29.0	29.5	29.0	28.5	34.0	30.3	27.4
W.B.C. 10 "cu.mm.	11.8	17.7	16.4	12.3	17.8	17.5	13.2
D.L.C./cu.mm.							
Neut. bands			123	123			
" Polymorphs	1,534	973	1886	4,305	3,471	4,025	1,122
Eosinophils	2,006	2,478	1,394	1,415	1,956	4,463	1,848
Basophils	59	0	0	0	89	0	66
Lymphocytes	8,142	13,275	12,628	6,150	11,748	8,663	9,042
Monocytes	59	973	492	308	534	350	1,122
Weight (in lbs).	108½	107	108¾	104¾	107	107½	108½

Maturation Curves.

Sheep E. 98.

	1st day	7th day	14th day	21st day	28th day	35th day	42nd day
	%	%	%	%	%	%	%
Granuloblasts							
N. Myelos.	11.5	6.2	9.0	15.0	12.5	9.3	10.3
N. M/myelos.	12.5	15.9	17.8	35.6	25.5	17.0	23.5
N. Bands	17.6	9.3	14.9	8.4	19.3	19.7	15.0
N. Polymorphs.	58.4	88.6	58.3	41.0	42.7	54.0	51.2
E. Myelos.	31.1	32.7	39.7	32.0	33.1	30.6	21.2
E. M/myelos.	22.3	26.5	38.2	40.5	36.4	36.7	42.9
E. Polymorphs.	46.6	40.8	22.1	27.5	30.5	32.7	35.9
Erythroblasts.							
Pro-erythroblasts	1.3	2.0	1.9	1.0	5.3	3.1	2.4
Early Norms.	5.2	7.8	3.9	7.0	5.0	11.9	2.6
Interm. Norms.	82.3	78.8	89.6	90.2	82.4	71.6	80.0
Late Norms.	11.2	12.3	4.6	1.7	7.2	13.4	14.9

Sheep E. 99.

Results on	1st day	7th day	14th day	21st day	28th day	35th day	42nd day
Haemomyelogram							
Haemocytoblasts	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Myeloblasts	0.9	0.2	0.4	0.5	0.2	0.6	0.8
Promyelocytes	0.0	0.1	0.1	0.1	0.0	0.0	0.0
N. Myelos.	2.7	3.1	4.1	5.9	5.8	6.4	9.4
N. M/myelos.	3.8	3.6	8.7	12.9	11.1	6.0	15.9
N. Bands	5.7	2.9	9.0	6.2	7.2	9.0	3.1
N. Polymorphs.	13.6	10.0	13.9	18.3	24.7	23.8	9.1
E. Myelos.	3.2	2.1	5.6	5.7	4.5	10.6	4.9
E. M/Myelos.	2.2	2.4	6.9	5.8	3.8	10.3	7.7
E. Polymorphs.	0.7	0.6	1.3	2.1	2.0	2.7	3.9
Basophils	0.3	0.6	2.1	0.1	1.0	1.3	1.1
Proerythroblasts	2.5	0.7	1.8	0.4	0.9	0.3	2.5
Early Norms.	7.4	1.3	1.4	1.7	2.4	2.9	2.5
Interm. Norms.	51.5	63.5	42.5	36.5	28.2	23.4	33.3
Late Norms.	4.8	8.7	1.0	2.6	6.8	2.0	5.0
Plasma cells	0.0	0.0	0.3	0.2	0.3	0.0	0.0
Reticulum cells	0.7	0.1	0.3	0.8	0.5	0.6	0.3
Lymphocytes	0.0	0.0	0.1	0.1	0.5	0.1	0.3
Monocytes	0.0	0.0	0.0	0.0	0.0	0.0	0.2
M/E Ratio	0.50:1	0.37:1	1.13:1	1.4:1	1.6:1	2.5:1	1.3:1
Mitosis	0.2	0.2	0.2	0.1	0.0	0.6	0.6
T. M. C.	266,000	clotted	127,400	117,400	36,800	9,800	47,800
Cellularity	IV.	IV.	IV.	IV.	II.	III.	II.
Peripheral Blood							
P.C.V. %	35.0	36.25	39.5	38.5	42.0	37.75	39.25
Hb. gms/100 ml.	10.4	10.2	11.5	11.1	13.6	11.1	11.9
R.B.C. 10^6/cu.mm.	11.5	12.9	14.2	12.5	15.2	13.7	13.6
M.C.V. cu. μ	30.5	28.0	2810	31.0	27.6	27.6	28.9
M.C.H.C. %	29.5	28.0	29.0	29.0	32.4	29.4	30.3
W.B.C. 10^3/cu.mm.	9.3	9.4	8.0	9.7	9.5	7,5	10.2
D.L.C./cu.mm.							
Neut. bands			40	49	48	75	-
" Polymorphs	3,116	2,021	1,280	2,571	2,803	1,613	1,275
Eosinophils	279	376	320	291	238	900	816
Basophils	140	0	80	97	95	0	102
Lymphocytes	5,022	6,721	6,040	6,402	6,128	4,725	7,701
Monocytes	744	282	240	6,291	6,190	4,188	306
Weight (in lbs.)	91¾	92¼	91	90	92	91	92½

Maturation Curves.

Sheep E. 99.

	1st day	7th day	14th day	21st day	28th day	35th day	42nd day
	%	%	%	%	%	%	%
Granuloblasts							
N. Myelos.	10.5	15.8	11.5	13.6	11.9	14.2	25.1
N. M/myelos.	14.7	18.4	24.4	29.8	22.7	13.3	42.4
N. Bands	22.1	14.8	25.2	14.3	14.8	19.9	8.3
N. Polymorphs.	52.7	51.0	38.9	42.3	50.6	52.6	24.2
E. Myelos.	52.5	41.2	39.2	41.9	43.7	44.9	29.7
E. M/Myelos.	36.1	47.1	48.3	42.6	36.9	43.6	46.7
E. Polymorphs.	11.4	11.7	12.5	15.4	19.4	11.4	23.6
Erythroblasts							
Pro-erythroblasts	3.8	0.9	3.9	1.0	2.3	1.1	5.8
Early Norms.	11.2	1.8	3.0	4.1	6.3	10.1	5.8
Interm. Norms.	71.8	85.6	91.0	88.6	73.6	81.8	76.9
Late Norms.	7.2	11.7	2.1	6.3	17.8	7.0	11.5

Sheep E. 100.

Results on	1st day	7th day	14th day	21st day	28th day	35th day	42nd day
Haemomyelogram.							%
Haemocytoblasts	0.0	0.0	0.0	0.0	0.0	0.0	0.1
Myeloblasts	0.7	0.5	0.4	0.1	0.7	0.6	0.9
Promyelocytes	0.0	0.1	0.0	0.0	0.0	0.0	0.1
N. Myelos.	3.1	3.2	4.0	3.8	2.4	6.4	4.5
N. M/myelos.	4.9	4.7	7.5	9.3	7.1	6.0	10.1
N. Bands	3.6	5.8	7.5	2.3	6.3	9.0	5.6
N. Polymorphs.	12.9	13.8	15.6	2.3	13.1	23.8	12.0
E. Myelos.	4.9	2.6	1.7	2.0	4.7	10.6	2.4
E. M/myelos.	5.0	4.4	4.3	2.4	3.4	10.3	6.2
E. Polymorphs.	3.0	2.9	4.2	1.2	3.0	2.7	3.7
Basophils	0.7	0.7	0.6	0.7	1.2	1.3	0.9
Proerythroblasts	1.7	1.7	0.6	1.0	0.9	0.3	1.3
Early Norms.	7.8	2.4	2.2	3.8	3.5	2.9	3.1
Interm. Norms.	43.3	47.7	40.5	65.0	41.9	23.4	43.1
Late Norms.	7.2	8.4	9.3	5.6	6.9	2.0	4.9
Plasma cells	0.0	0.5	0.3	0.0	0.7	0.0	0.1
Reticulum cells	0.7	0.5	0.4	0.3	1.7	0.6	0.2
Lymphocytes	0.3	0.1	0.6	0.2	0.4	0.1	0.7
Monocytes	0.0	0.0	0.3	0.0	0.1	0.0	0.1
M/E Ratio	0.66:1	0.64:1	0.87:1	0.32:1	0.83:1	2.5:1	0.89:1
Mitosis	0.4	0.2	0.2	0.5	0.1	0.6	0.4
T. M. C.	210,200	121,000	36,800	153,800	144,800		
Cellularity	IV.	IV.	III.	IV.	III.	II.	I.
Peripheral Blood							
P.C.V. %	31.75	31.75	35.5	40.5	39.0	35.5	39.25
Hb.gms/100 ml.	8.4	9.7	9.9	11.8	12.0	11.2	11.8
R.B.C. 10^6/cu.mm.	10.4	10.1	12.1	14.6	12.1	12.6	13.6
M.C.V. Cu. μ	30.5	31.5	29.0	28.0	32.2	28.2	28.9
M.C.H.C. %	26.5	30.5	28.0	29.0	30.8	31.5	30.1
W.B.C. 10^3/cu.mm.	9.4	10.9	11.7	10.0	13.7	12.3	11.0
D.L.C. /cu.mm.							
Neut. bands	-	-	-	100	-	-	-
" Polymorphs.	3384	1799	2106	3000	3768	3321	1155
Eosinophils	1175	1417	1697	400	2055	1353	605
Basophils	0	109	0	50	0	123	0
Lymphocytes	4794	7358	7254	6150	7535	7319	8690
Monocytes	47	218	644	300	343	185	550
Weight (in lbs.)	109 5/8	110 1/4	109 1/2	109 1/4	103 1/2	105 1/2	106

Maturation Curves.

Sheep E.100.

	1st day	7th day	14th day	21st day	28th day	35th day	42nd day
	%	%	%	%	%	%	%
Granuloblasts.							
N. Myelos.	12.6	11.6	11.6	21.5	7.8	14.2	14.0
N. M/myelos.	20.0	17.1	21.7	52.5	29.4	13.3	31.4
N. Bands.	14.7	21.1	21.7	13.0	20.4	19.9	17.3
N. Polymorphs.	52.7	50.2	45.0	13.0	42.4	52.6	37.3
E. Myelos.	38.0	26.0	16.7	35.7	42.3	44.9	19.5
E. M/myelos.	38.7	45.0	42.2	42.9	30.6	43.6	50.4
E. Polymorphs.	23.3	29.0	41.1	21.4	27.0	11.4	30.1
Erythroblasts							
Pro-erythroblasts	2.8	2.8	1.1	1.3	1.7	1.0	2.5
Early Norms.	13.0	4.0	4.2	5.0	6.6	10.1	5.9
Interm. Norms.	72.2	79.2	77.0	86.2	78.7	81.8	82.2
Late Norms.	12.0	14.0	17.7	7.4	13.0	7.0	9.4

Sheep B. 198

Results on	1st day	7th day	14th day	21st day	28th day	35th day	42nd day
Haemomyelogram							
Haemocytoblasts	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Myeloblasts	0.3	1.1	0.5	0.5	0.5	0.8	1.0
Promyelocytes	0.0	0.2	0.2	0.1	0.1	0.2	0.0
N. Myelos.	2.8	4.1	4.6	6.9	3.3	8.1	5.5
N. M/myelos.	5.1	4.9	7.2	11.9	8.3	17.5	8.4
N. Bands.	5.8	7.2	7.4	8.5	5.4	7.0	6.7
N. Polymorphs.	10.4	11.7	18.4	19.2	22.0	15.9	13.5
E. Myelos.	3.5	4.1	4.2	5.3	4.9	4.8	1.9
E. M/myelos.	3.6	5.5	4.0	8.0	4.1	5.3	4.8
E. Polymorphs.	2.6	2.0	0.9	1.1	4.5	1.4	2.6
Basophils	0.2	0.4	0.9	1.1	0.4	0.6	1.0
Pro-erythroblasts	2.3	2.1	1.8	0.4	1.8	2.2	1.7
Early Norms.	2.9	7.1	4.3	1.1	2.5	2.4	6.5
Interm. Norms.	53.3	41.1	40.6	27.5	34.3	26.0	42.3
Late Norms.	6.9	6.8	3.5	7.5	6.3	6.5	2.6
Plasma cells	0.0	0.1	0.3	0.0	0.1	0.2	0.0
Reticulum cells	0.3	1.2	0.5	0.7	0.7	0.3	0.2
Lymphocytes	0.0	0.3	0.4	0.1	0.8	0.8	1.4
Monocytes	0.0	0.1	0.3	0.1	0.0	0.0	0.0
M/E Ratio	0.52:1	0.80:1	0.96:1	1.7:1	1.2:1	1.7:1	0.86:1
Mitosis	0.3	0.6	0.0	0.2	0.5	0.5	1.7
T. M. C.	195,200	51,800	30,600	45,000	55,000	22,000	22,200
Cellularity	IV.	II.	II.	III.	III.	I	I
Peripheral Blood							
P.C.V. %	36.5	40.0	43.5	46.0	41.0	42.25	41.5
Hb. gms/100 ml.	9.1	11.2	12.0	13.2	10.6	12.7	12.5
R.B.C. 10^6/cu.mm.	11.4	12.5	13.2	13.6	13.1	31.3	25.9
M.C.V. cu. μ	32.0	32.0	33.0	34.0	31.3	34.6	32.4
M.C.H.C. %	25.0	28.0	27.5	28.5	25.9	30.1	30.1
W.B.C. 10^3/cu.mm.	9.8	9.4	8.8	8.8	8.7	8.6	7.9
D.L.C. /cu.mm.							
Neut. bands		47			44		
" Polymorphs.	2,352	3,572	1,892	1,716	2,436	1,548	1,817
Eosinophils	637	705	528	440	435	172	316
Basophils	196	94	44	88	0	86	0
Lymphocytes	6,125	4,700	6,248	6,424	5,655	6,450	5,570
Monocytes	490	282	88	132	131	344	198
Weight (in lbs.)	101	96	97	91¼	91¼	89½	85

Maturation Curves.

Sheep B. 198

	1st day	7th day	14th day	21st day	28th day	35th day	42nd day
	%	%	%	%	%	%	%
Granuloblasts							
N. Myelos.	11,6	14.7	13.2	14.8	8.5	16.7	16.1
N. M/myelos.	21.2	17.6	19.2	25.6	21.3	36.1	24.6
N. Bands.	24.1	25.8	19.7	18.3	13.8	14.4	19.6
N. Polymorphs.	43.1	41.9	48.9	41.3	56.4	32.8	39.6
E. Myelos.	36.1	35.3	46.2	36.8	36.3	41.7	20.4
E. M/myelos.	37.1	47.4	44.0	55.6	30.4	46.1	51.6
E. Polymorphs.	26.8	17.2	9.8	7.6	33.3	12.2	28.0
Erythroblasts							
Pro-erythroblasts	3.5	3.7	3.6	1.1	4.0	5.9	3.2
Early Norms.	4.4	12.4	8.6	3.0	5.6	6.5	12.3
Interm. Norms.	81.5	72.0	80.8	75.3	76.4	70.1	79.6
Late Norms.	10.6	11.9	7.0	20.5	14.0	17.5	4.9

Sheep B. 199.

Results at	1st day	7th day	14th day	21st day	28th day	35th day	42nd day
Haemomyelogram							
Haemocytoblasts	0.0	0.0	0.0	0.0	0.0	0.1	0.1
Myeloblasts	0.1	0.7	0.6	0.3	0.7	0.2	0.2
Promyelocytes	0.0	0.0	0.2	0.0	0.1	0.0	0.0
N. Myelos.	3.5	3.1	4.2	7.5	4.0	3.9	3.5
N. M/Myelos.	4.5	5.8	6.8	10.2	5.7	6.6	8.0
N. Bands.	4.2	3.1	7.1	6.8	6.6	6.6	3.4
N. Polymorphs.	9.9	19.1	25.5	19.1	15.3	15.4	20.3
E. Myelos.	4.0	3.5	4.3	7.8	6.8	5.6	4.6
E. M/myelos.	2.8	3.0	5.8	6.5	6.0	5.0	7.3
E. Polymorphs.	4.0	5.0	4.3	1.3	7.0	4.5	6.3
Basophils	0.7	0.8	0.8	0.0	0.4	0.4	0.2
Pro-erythroblasts	1.3	0.3	0.6	0.4	0.9	1.2	0.6
Early Norms.	7.9	4.5	3.4	2.3	1.5	5.5	2.5
Interm. Norms.	46.7	46.7	32.1	35.1	36.9	37.2	37.2
Late Norms.	9.9	4.1	3.8	2.4	7.1	7.6	4.6
Plasma cells	0.0	0.2	0.1	0.2	0.2	0.0	0.1
Reticulum cells	0.4	0.1	0.3	0.0	0.6	0.5	0.4
Lymphocytes	0.1	0.0	0.1	0.0	0.2	0.0	0.7
Monocytes	0.0	0.0	0.0	0.0	0.0	0.0	0.0
M/E Ratio	0.51:1	0.79:1	1.49:1	1.5:1	1.1:1	0.9:1	1.2:1
Mitosis	3.6	0.3	0.3	0.2	0.1	0.4	0.2
T. M. C.	106,200	25,000	24,800	46,000	59,000	31,600	clotted
Cellularity	IV.	III.	III.	III.	III.	III	II.
Peripheral Blood							
P.C.V. %	38.25	40.0	37.75	38.5	36.0	40.0	34.5
Hb. Gms/100 ml.	10.6	10.8	10.5	10.8	10.8	12.5	10.1
R.B.C. 10 /cu.mm.	13.2	14.1	13.1	13.0	13.1	12.9	10.6
M.C.V. cu.	29.0	28.5	29.0	29.5	27.5	31.0	32.5
M.C.H.C. %	28.0	27.0	28.0	28.0	30.6	31.3	29.3
W.B.C. 10 /cu.mm.	9.3	9.1	11.1	8.6	9.8	10.0	9.7
D.L.C./cu.mm.							
Neut. bands	46	46			49		
" Polymorphs	4,557	3,458	2,442	2,279	2,254	2,650	2,959
Eosinophils	837	683	1,332	1,118	980	1,400	1,795
Basophils	93	91	111	0	49	0	194
Lymphocytes	3,395	4,550	6,882	5,031	6,076	5,600	4,317
Monocytes	419	273	333	172	392	350	437
Weight (in lbs.)	111¾	108	106¾	102¾	102½	101½	101

Maturation Curves.

Sheep B. 199.

	1st day	7th day	14th day	21st day	28th day	35th day	42nd day
	%	%	%	%	%	%	%
Granuloblasts							
N. Myelos.	15.8	10.0	9.6	17.2	12.7	12.2	9.9
N. M/myelos.	20.4	18.6	15.6	23.4	18.0	20.7	22.7
N. Bands.	19.0	10.0	16.3	15.6	20.9	18.8	9.7
N. Polymorphs.	44.8	61.4	58.5	43.8	48.4	48.3	57.7
E. Myelos.	37.0	30.4	29.9	50.0	34.3	37.1	25.3
E. M/myelos.	25.9	26.1	40.2	41.7	30.3	33.1	40.1
E. Polymorphs.	37.0	43.5	29.9	8.3	35.4	29.8	34.6
Erythroblasts.							
Pro-erythroblasts	2.0	0.5	1.5	1.0	1.9	2.3	1.3
Early Norms.	12.0	8.1	8.5	5.7	3.2	10.7	5.6
Interm. Norms.	71.0	84.0	80.5	87.3	79.5	72.2	82.9
Late Norms.	15.0	7.4	9.5	6.0	15.3	14.8	10.2

Sheep's Number	50	68	54	56	63	67	51
Date of Sampling	14/12/49	14/12/49	11/1/50	11/1/50	25/1/50	25/1/50	8/2/50
Haemmyelogram							
Haemocytoblasts	0.0	0.1	0.0	0.0	0.2	0.1	0.1
Myeloblasts	0.2	0.3	0.2	0.0	1.1	0.4	0.5
Promyelocytes	0.0	0.6	0.3	0.2	0.6	0.1	0.3
N. Myelos.	6.7	2.7	4.0	5.9	4.9	3.2	5.7
N. M/myelos.	26.4	23.6	16.5	26.0	21.3	16.4	19.5
N. Polymorphs.	12.4	5.6	2.4	6.7	2.3	1.4	5.4
E. Myelos.	3.1	3.3	5.6	4.6	4.2	3.7	4.8
E. M/myelos.	16.8	13.5	8.6	8.5	15.0	14.5	12.2
E. Polymorphs.	4.7	3.2	3.9	2.6	0.8	3.0	1.8
Basophils	1.7	1.1	0.6	2.3	1.0	0.3	1.1
Pro-erythroblasts	0.2	0.9	0.8	0.5	1.1	1.1	1.1
Early Norms.	0.8	4.9	1.5	2.5	2.7	2.9	3.4
Interm. Norms.	15.4	29.6	37.3	29.5	27.2	37.1	30.0
Late Norms.	6.6	6.8	15.0	7.1	15.1	12.4	8.7
Plasma cells	0.1	0.0	0.3	0.3	0.3	0.4	0.7
Reticulum cells	1.1	1.2	1.4	0.9	0.8	1.1	1.6
Lymphocytes	3.0	2.3	1.5	2.2	1.2	1.2	2.6
Monocytes	0.8	0.3	0.1	0.2	0.2	0.7	0.5
M/E Ratio	3.13:1	1.28:1	0.77:1	1.43:1	1.11:1	0.81:1	1.19:1
Mitosis	0.1	0.7	0.2	0.5	1.6	0.0	1.3
Cellularity	I	III	II	III	III	II	III
Peripheral Blood							
P.C.V. %	36.0	35.0	39.0	33.0	37.0	38.5	35.5
Hb. gms/100 ml.	10.1	10.1	10.5	10.2	10.2	10.8	10.9
R.B.C. 10^6/cu.mm.	11.4	0.2	11.3	11.2	10.2	11.2	10.7
M.C.V. cu. μ	31.5	38.0	35.0	29.5	37.0	34.0	33.0
M.C.H.C. μ	28.0	28.5	27.0	31.0	27.5	28.0	31.0
Frag. I.H.	-	-	0.68	0.68	0.70	0.68	0.70
" O.H.	-	-	0.52	0.50	0.50	0.48	0.52
S.G. Blood	-	-	-	-	1.0526	1.0540	1.0521
" Plasma	-	-	1.0272	1.0251	1.0292	1.0286	1.0272
W.B.C. 10^3/cu.mm.	11.1	8.0	5.3	7.4	7.4	7.0	5.3
D.L.C./cu.mm.							
Neut. bands	55	20	122	74	111	105	50
" Polymorphs	2,675	1,660	2,475	3,175	1,665	2,250	1,700
Eosinophils	700	540	320	260	407	350	210
Basophils	0	0	0	37	0	35	0
Lymphocytes	6,700	5,300	2,125	3,500	4,514	3,950	3,100
Monocytes	955	500	265	370	703	350	170

Continued/

Maturation Curves.

Sheep's Number	50	68	54	56	63	67	51
	%	%	%	%	%	%	%
Granuloblasts							
N. Myelos.	14.7	8.5	17.5	15.2	17.2	15.2	18.6
N. M/myelos.	58.0	74.0	72.0	67.4	74.7	78.1	63.7
N. Polymorphs.	27.3	17.5	10.5	17.4	8.1	6.7	17.6
E. Myelos.	12.6	16.5	30.9	29.3	21.0	17.5	25.5
E. M/Myelos.	68.3	67.5	47.5	54.1	75.0	68.4	64.9
E. Polymorphs.	19.1	16.0	21.5	16.6	4.0	14.1	9.6
Erythroblasts							
Pro-erythroblasts	0.9	2.1	1.5	1.3	2.4	2.1	2.5
Early Norms.	3.5	11.6	2.7	6.3	5.9	5.4	7.9
Interm. Norms.	67.0	70.2	68.3	74.5	59.0	69.3	69.4
Late Norms.	28.6	16.1	27.5	17.9	32.7	23.2	20.2

Continued/

Sheep's Number	52	57	58	53	65	55	69
Date of Sampling	8/2/50	22/2/50	22/2/50	8/3/50	8/3/50	22/3/50	22/3/50
Haemomyelogram							
Haemocytoblasts	0.0	0.0	0.0		0.0	0.0	0.1
Myeloblasts	1.4	0.1	0.7		1.0	0.7	0.6
Promyelocytes	0.0	0.1	0.0		0.2	0.1	0.1
N. Myelos.	1.5	4.3	5.7		8.1	3.6	5.0
N. M/myelos.	18.0	28.5	21.3		15.5	13.8	11.5
N. Polymorphs.	3.6	6.2	6.9		5.3	2.1	3.2
E. Myelos.	5.4	6.2	5.5		5.2	1.5	4.4
E. M/myelos.	8.2	8.6	11.5		7.8	2.0	4.8
E. Polymorphs.	5.1	3.8	3.0		0.8	0.4	1.4
Basophils	1.2	1.0	0.8		1.0	1.0	1.7
Pro-erythroblasts	0.9	0.9	0.9		1.4	1.0	1.3
Early Norms.	3.6	3.4	3.2		4.9	2.8	5.0
Interm. Norms.	33.6	27.9	31.9		34.5	52.2	43.0
Late Norms.	14.2	4.9	5.8		9.1	13.3	12.5
Plasma cells	0.3	1.0	0.0		0.2	0.5	0.9
Reticulum cells	1.3	1.8	2.0		1.9	2.7	3.1
Lymphocytes	1.5	1.1	0.6		3.1	1.8	1.2
Monocytes	0.2	0.2	0.2		0.0	0.5	0.2
M/E Ratio	0.85:1	1.68:1	1.32:1		0.90:1	0.37:1	0.53:1
Mitosis	1.0	0.9	0.7		0.7	0.7	1.5
Cellularity	III	I	IV	I	II	IV	III
Peripheral Blood							
P.C.V. %	37.5	32.0	35.0	34.0	31.0	26.0	26.0
Hb. gms/100 ml.	11.6	9.7	10.8	9.7	9.1	7.7	8.1
R.B.C. 10^6/cu.mm.	12.1	8.4	10.3	9.5	8.9	7.2	7.7
M.C.V. cu. μ	31.0	38.5	34.0	30.5	30.5	34.5	36.0
M.C.H.C. %	31.0	30.0	30.5	28.5	29.0	29.5	29.5
Frag. I.H.	0.68	0.68	0.70	0.64	0.64	0.70	0.66
" C.H.	0.46	0.48	0.54	0.52	0.48	0.52	0.50
S.G. Blood	1.0536	1.0497	1.0518	1.0488	1.0486	1.0471	1.0456
" Plasma	1.0275	1.0290	1.0301	1.0272	1.0280	1.0285	1.0261
W.B.C. 10^3/cu.mm.	8.4	8.8	10.4	8.3	10.6	9.0	10.0
D.C.L./cu.mm.							
Neut. bands	80	270	160	0	110	0	100
" Polymorphs	3,230	2.650	4,450	3,400	4,650	2,800	7,500
Eosinophils	210	750	740	80	220	0	100
Basophils	0	40	0	170	0	0	0
Lymphocytes	4.400	4,280	4,600	4,250	5,300	5,680	2,000
Monocytes	460	850	420	420	320	540	300

Continued/

Maturation Curves.

Sheep's Number	52	57	58	65	55	69
	%	%	%	%	%	%
Granuloblasts						
N. Myelos.	6.5	11.0	16.8	28.0	18.5	25.4
N. M/myelos.	77.9	73.1	62.8	53.6	70.8	58.4
N. Polymorphs.	15.6	15.9	20.4	18.3	10.7	16.2
E. Myelos.	28.9	33.3	27.5	37.7	38.5	41.5
E. M/Myelos.	43.9	46.2	57.5	56.5	51.3	45.3
E. Polymorphs.	27.2	20.4	15.0	5.8	10.2	13.2
Erythroblasts						
Pro-erythroblasts	1.7	2.4	2.2	2.8	1.4	2.1
Early Norms.	6.9	9.2	7.6	9.8	4.0	8.1
Interm. Norms.	64.2	75.2	76.3	69.2	75.3	69.6
Late Norms.	27.2	13.2	13.9	18.2	19.2	20.2

Continued/

Sheep's Number	60	62	61	64	70	74	66
Date of Sampling	5/4/50	5/4/50	19/4/50	19/4/50	3/5/50	3/5/50	17/5/50
Haemomyelogram							
Haemocytoblasts	0.0	0.0	0.0	0.0	0.0	0.1	0.1
Myeloblasts	0.6	0.6	0.5	0.6	0.9	0.5	0.5
Promyelocytes	0.0	0.0	0.0	0.0	0.2	0.1	0.0
N. Myelos.	2.1	5.9	5.5	4.3	3.3	2.7	2.3
N. M/myelos.	17.5	15.8	13.5	13.1	24.2	18.9	7.9
N. Polymorphs.	23.4	4.0	2.0	2.6	6.6	3.3	0.9
E. Myelos.	1.8	2.7	2.7	2.8	4.2	5.0	3.9
E. M/myelos.	3.8	3.5	2.9	6.1	12.1	8.8	4.5
E. Polymorphs.	7.0	1.9	0.9	1.2	3.9	2.1	1.6
Basophils	0.6	0.6	1.3	2.2	1.1	3.6	0.9
Pro-erythroblasts	0.1	1.2	0.4	0.5	0.6	0.7	0.6
Early Norms.	0.4	5.7	3.7	5.5	3.5	4.1	9.7
Interm. Norms.	33.4	45.7	52.0	41.4	26.8	34.5	56.3
Late Norms.	8.5	10.3	12.5	15.9	7.6	12.5	9.1
Plasma cells	0.5	0.2	0.3	0.6	0.7	0.5	0.1
Reticulum cells	0.2	0.7	1.2	1.2	2.0	1.5	0.9
Lymphocytes	0.0	1.0	0.5	1.6	2.1	1.0	0.7
Monocytes	0.1	0.2	0.1	0.4	0.2	0.1	0.0
M/E Ratio	1.34:1	0.56:1	0.43:1	0.52:1	1.47:1	0.87:1	0.30:1
Mitosis	0.8	1.3	1.1	0.3	0.3	0.0	0.9
Cellularity	I	IV	IV	IV	IV	IV	IV
Peripheral Blood							
P.C.V. %	23.0	25.0	32.0	29.5	29.0	30.5	26.0
Hb. gms/100 ml.	6.9	7.4	9.8	8.7	8.7	9.5	7.3
R.B.C. 10^6/cu.mm.	7.0	7.5	8.5	8.5	7.6	9.0	7.6
M.C.V. cu. μ	33.0	33.5	38.0	35.0	38.0	33.5	35.0
M.C.H.C. %	30.0	30.0	30.5	29.5	30.0	31.5	28.0
Frag. I.H.	0.68	0.66	0.66	0.52	0.70	0.70	-
" C.H.	0.50	0.48	0.48	0.48	0.34	0.34	-
S.G. Blood	1.0415	1.0441	1.0490	1.0466	1.0491	1.0487	1.0464
" Plasma	1.0265	1.0275	1.0281	1.0276	1.0300	1.0276	1.0281
W.B.C. 10^3/cu.mm.	10.8	8.6	8.0	11.4	7.7	6.6	7.9
D.L.C./cu.mm.							
Neut.bands	0	40	80	50	0	230	60
" Polymorphs.	4,980	3,320	3,600	6,850	1,440	3,400	2,000
Eosinophils	220	260	280	170	1,080	40	630
Basophils	110	0	120	110	0	0	120
Lymphocytes	5,100	4,530	3,800	3,880	4,650	2,650	4,830
Monocytes	430	450	120	330	570	230	280

Continued/

Maturation Curves.

Sheep's Number	60	62	61	64	70	74	66
	%	%	%	%	%	%	%
Granuloblasts							
N. Myelos.	4.9	22.9	26.2	21.5	9.7	10.8	20.7
N. M/myelos.	40.7	61.5	64.3	65.5	70.9	75.9	71.2
N. Polymorphs.	54.4	15.6	9.5	13.0	19.4	13.3	8.1
E. Myelos.	14.3	33.3	41.5	27.7	20.8	31.4	39.0
E. M/Myelos.	30.1	43.2	44.6	60.4	59.9	55.3	45.0
E. Polymorphs.	55.6	23.5	13.8	11.9	19,3	13.2	16.0
Erythroblasts							
Pro-erythroblasts	0.2	1.9	0.6	0.8	1.6	1.4	0.8
Early Norms.	0.9	9.1	5.4	8.7	9.1	7.9	12.8
Interm. Norms.	78.8	72,7	75.8	65.5	69.6	66.6	74.4
Late Norms.	20.0	16.3	18.2	25.0	19.7	24.1	12.0

Continued/

Sheep's Number	75	71	72	59	73	76	77
Date of Sampling	17/5/50	31/5/50	31/5/50	14/6/50	14/6/50	28/6/50	28/6/50
Haemomyelogram							
Haemocytoblasts	0.0	0.1	0.1	0.1	0.0	0.0	0.0
Myeloblasts	0.7	0.9	0.7	0.5	1.1	0.8	0.7
Promyelocytes	0.0	0.2	0.1	0.1	0.1	0.0	0.0
N. Myelos.	2.8	4.7	3.1	2.5	2.7	4.0	3.8
N. M/myelos.	15.7	23.6	10.2	11.6	19.4	18.7	10.6
N. Polymorphs.	4.0	2.3	2.3	7.4	8.1	15.4	13.8
E. Myelos.	5.3	5.3	5.1	2.2	4.8	2.5	5.0
E. M/myelos.	8.6	8.6	11.3	4.8	13.2	2.6	4.5
E. Polymorphs.	1.5	3.8	2.1	4.9	3.8	7.4	7.2
Basophils	1.8	1.3	0.5	0.6	0.2	0.9	0.3
Pro-erythroblasts	0.9	1.7	1.5	0.6	0.6	1.0	1.3
Early Norms.	2.2	2.3	2.9	2.8	3.1	8.0	5.3
Interm. Norms.	40.0	25.9	38.1	45.6	32.2	32.7	39.4
Late Norms.	10.9	15.0	17.1	12.1	7.0	4.0	5.2
Plasma cells	1.3	0.3	0.2	0.3	0.7	0.5	1.0
Reticulum cells	2.4	2.8	1.9	0.9	2.2	0.9	1.8
Lymphocytes	1.9	0.7	2.4	2.7	0.8	0.2	0.1
Monocytes	0.0	0.5	0.4	0.3	0.0	0.2	0.0
M/E Ratio	0.75:1	1.13:1	0.59:1	0.57:1	1.24:1	1.14:1	0.90:1
Mitosis	0.9	0.4	0.5	0.3	0.7	0.7	0.2
Cellularity	IV	IV	IV	IV	IV	IV	IV
Peripheral Blood							
P.C.V. %	32.0	31.0	30.5	35.5	29.0	30.5	28.0
Hb. gms/100 ml.	9.1	8.7	9.1	10.8	8.7	9.0	8.3
R.B.C. 10^6/cu.mm.	9.5	8.6	9.2	10.7	8.8	9.1	8.5
M.C.V. cu. μ	34.0	36.5	33.5	33.0	33.0	33.5	33.0
M.C.H.C. %	28.5	28.0	29.5	30.0	30.0	29.0	29.5
Frag. I.H.	-	-	-	0.76	0.76	-	-
" C.H.	-	-	-	0.54	0.56	-	-
S.G. Blood	1.0506	-	-	1.0550	1.0518	1.0486	1.0471
" Plasma	1.0301	-	-	1.0336	1.0353	1.0281	1.0274
W.B.C. 10^3/cu.mm.	6.6	8.6	8.4	9.4	13.9	6.7	9.3
D.L.C. cu.mm.							
Neut. bands	100	2,800	2,480	3,150	4,730		
" Polymorphs	3,050						
Eosinophils	500	560	380	520	940		
Basophils	70	4,700	5,250	5,400	8,000		
Lymphocytes	2,580	560	300	330	210		
Monocytes	330						

Maturation Curves.

Sheep's Number	75	71	72	59	73	76	77
	%	%	%	%	%	%	%
Granuloblasts							
N. Myelos.	12.4	15.4	19.9	11.6	8.9	10.6	13.5
N. M/myelos.	69.8	77.1	65.4	54.0	64.2	49.1	37.6
N. Polymorphs.	17.7	7.5	14.7	34.4	26.8	40.4	48.9
E. Myelos.	34.4	29.9	27.5	18.5	22.0	20.0	30.8
E. M/myelos.	55.8	48.6	61.1	40.3	60.6	20.8	26.9
E. Polymorphs.	9.7	21.5	11.4	41.2	17.4	59.2	43.1
Erythroblasts							
Pro-erythroblasts	1.7	3.8	2.5	1.0	1.4	2.2	2.5
Early Norms.	4.1	5.1	4.9	4.6	7.2	17.4	10.4
Interm. Norms.	74.0	57.7	63.9	74.6	75.0	71.2	77.0
Late Norms.	20.2	33.4	28.7	19.8	16.3	9.2	10.1

Section VI.

Results of initial examination.

Sheep's No.	51	52	P44	E63	J52	48	045
Haemomyelogram.							
Haemocytoblast	1.2	0.0	0.1	0.4	0.3	0.0	0.0
Myeloblast	4.2	0.7	2.6	0.4	0.3	0.5	0.5
Promyelocytes	1.2	0.1	0.0	0.2	0.0	0.0	0.0
N. Myelos.	17.2	8.5	6.0	5.6	4.4	6.5	6.4
N. M/myelos.	17.6	11.4	10.9	9.9	8.6	3.1	3.4
N. Bands	22.9	16.4	18.4	7.9	5.5	5.0	7.4
N. Polymorphs.	11.2	28.8	26.9	32.8	19.0	26.7	17.6
E. Myelos.	3.1	6.4	4.9	5.4	2.5	7.0	3.0
E. M/myelos.	2.6	6.0	6.9	8.9	9.9	8.6	3.4
E. Polymorphs.	5.5	4.9	4.6	15.2	[illegible]	[illegible]	2.9
Basophils	1.4	0.4	2.8	2.0	0.2	0.3	0.7
Proerythroblasts	0.0	0.2	0.2	0.0	1.0	2.0	1.0
Early Norms.	0.2	0.7	1.0	0.3	3.2	3.3	1.1
Interm. Norms.	1.9	6.9	8.2	4.5	32.0	20.4	38.8
Late Norms.	0.2	1.1	5.2	0.8	5.1	5.3	11.9
Plasma cells	5.5	2.2	0.4	1.1	0.2	0.3	0.1
Reticulum cells	2.6	5.3	0.4	3.6	1.2	4.0	1.6
Lymphocytes	2.6	0.0	0.4	0.8	0.2	0.0	0.2
Monocytes	0.0	0.0	0.1	0.0	0.0	0.0	0.0
M/E Ratio	37.3:1	9.27:1	5.75:1	15.2:1	1.38:1	2.09:1	0.85:1
Cellularity	IV	IV	IV	III	III	III	IV
Peripheral Blood							
P.C.V. %	19.0	24.0	23.5	33.5	36.0	31.0	36.0
Hb.gms/100 ml.	6.2	7.1	6.2	9.0	9.4	8.8	9.1
R.B.C. 10^6/cu.mm.	5.3	8.8	8.7	9.2	11.3	10.7	11.0
M.C.V. cu. μ	35.5	27.0	27.0	36.5	32.0	29.0	33.0
M.C.H.C. %	33.0	30.0	26.5	27.0	26.0	28.5	25.0
W.B.C. 10^3/cu.mm.	11.9	7.0	18.6	9.0	9.5	5.2	4.0
D.L.C. /cu.mm.							
Neut. Juv.	30						
Neut. Band	1131	70	186	90	143	26	0
" Polymorphs.	9223	3640	11532	5040	6460	2444	1600
Eosinophils	0	70	279	45	333	156	120
Basophils	0	0	0	90	0	0	0
Lymphocytes	1250	2870	5859	2610	2470	2236	2000
Monocytes	268	350	744	1125	95	338	280

Results of initial examination continued.

Sheep's No.	G2	32	X22	50	V35	60	D65
Haemomyelogram.							
Haemocytoblast	0.0	0.1	0.0	0.0	0.0	0.1	0.0
Myeloblast	0.3	0.6	0.1	0.4	0.1	0.4	1.0
Promyelocytes	0.0	0.0	0.3	0.0	0.0	0.0	0.1
N. Myelos.	7.2	3.4	4.5	2.9	6.1	4.4	6.7
N. M/myelos.	6.5	4.1	6.0	5.8	3.4	3.4	4.6
N. Bands	7.8	6.7	6.4	6.7	9.5	5.9	4.9
N. Polymorphs.	23.1	42.1	30.3	21.7	40.8	16.5	10.5
E. Myelos.	10.5	4.6	4.2	6.7	10.1	2.7	5.3
E. M/myelos.	5.9	5.3	3.2	8.2	8.1	2.1	8.4
E. Polymorphs.	0.8	2.4	1.8	3.6	3.1	2.2	8.7
Basophils	0.9	0.1	0.4	1.6	0.2	1.2	0.2
Proerythroblasts	0.4	0.1	0.4	0.6	0.2	0.4	2.0
Early Norms.	4.6	1.1	1.9	1.8	0.6	1.0	4.7
Interm. Norms.	2.3	20.6	33.2	24.9	5.1	40.9	30.8
Late Norms.	6.8	2.1	3.2	11.8	1.6	12.7	8.4
Plasma cells	0.0	0.0	0.2	0.4	1.1	0.9	1.3
Reticulum cells	2.7	6.5	3.8	2.5	9.8	4.8	2.2
Lymphocytes	0.1	0.2	0.1	0.2	0.2	0.4	0.2
Monocytes	0.1	0.0	0.0	0.2	0.0	0.0	0.0
M/E Ratio	1.80:1	2.89:1	1.48:1	1.47:1	10.8:1	0.71:1	1.12:1
Cellularity	IV	III	II	IV	III	II	IV
Peripheral Blood							
P.C.V. %	32.0	36.0	39.5	42.5	32.0	34.0	34.5
Hb.gms/100 ml.	8.1	9.8	10.4	10.8	8.4	9.2	9.8
R.B.C. 10^6/cu.mm.	9.8	11.4	12.0	11.9	10.0	12.5	9.0
M.C.V. cu μ	32.5	32.0	33.0	36.0	32.0	27.0	38.5
M.C.H.C. %	25.5	27.5	26.0	25.5	26.0	27.0	28.5
W.B.C. 10^3/ cu.mm.	3.5	6.9	5.8	8.5	4.8	7.4	17.2
D.L.C. / cu.mm.							
Neut. Band	0	100	0	0	48	0	172
Neut. Polymorphs.	1050	3590	1856	1660	1080	1776	13250
Eosinophils	385	278	87	469	24	444	86
Basophils	70	34	87	42	0	148	0
Lymphocytes	1890	2780	3596	5900	3336	4588	3350
Monocytes	105	138	174	469	360	444	465

Section VI.

Results of second examination. (Results of initial examination also included for 45

Sheep's No.	45 (1st)	45 (2nd)	E63	V35	P44
Haemomyelogram.					
Haemocytoblast	0.0		0.0	0.0	0.0
Myeloblast	0.5		0.2	0.6	0.4
Promyelocyte	0.0		0.0	0.0	0.0
N. Myelos.	6.3		4.8	9.7	6.1
N. M/myelos.	7.2		8.2	11.5	10.4
N. Bands	4.6		16.3	13.3	10.2
N. Polymorphs.	57.2		30.0	17.1	19.3
E. Myelos.	2.1		2.7	5.7	2.4
E. M/myelos.	3.4		4.2	9.7	5.0
E. Polymorphs.	4.6		6.8	3.6	2.8
Basophils	0.2		2.2	2.3	0.8
Procrythroblasts	0.0		0.2	0.5	1.6
Early Norms.	0.5		1.1	2.1	3.8
Interm. Norms.	3.6		18.6	22.2	22.0
Late Norms.	0.5		3.5	0.9	12.4
Plasma cells	0.0		0.4	0.3	0.4
Reticulum cells	9.3		0.4	0.2	2.0
Lymphocytes	0.0		0.4	0.2	0.2
Monocytes	0.0		0.0	0.1	0.2
M/E Ratio	18.7:1		3.40:1	2.86:1	1.36:1
Cellularity	IV	0	III	III	IV
Peripheral Blood.					
P.C.V. %	35.5	23.0	42.5	42.5	38.5
Hb.gms/100 ml.	9.8	6.4	13.0	12.0	11.3
R.B.C. 10^6/cu.mm.	10.8	6.5	12.1	11.8	12.3
M.C.V. cu. μ	29.0	35.0	35.0	36.0	31.0
M.C.H.C. %	26.0	28.0	30.5	28.5	29.5
W.B.C. 10^3/cu.mm.	5.1	3.3	7.0	6.5	5.9
D.L.C. /cu.mm.					
Neut. Bands	0	16	0	0	15
Neut. Polymorphs.	2525	1914	1155	260	3319
Eosinophils	0	0	665	265	192
Basophils	0	16	70	60	0
Lymphocytes	2295	1287	4655	5460	2183
Monocytes	281	66	455	455	192

Section VI.

Maturation Curves

Sheep's Number	48	G.2	J.52	0.45	X.22	60	51
	%	%	%	%	%	%	%
Granuloblasts							
N. Myelos	15.7	16.1	11.7	18.4	9.5	14.6	25.0
N. M/Myelos.	7.5	14.6	22.9	9.8	12.7	11.3	25.5
Neut. Band.	12.1	17.5	14.7	21.3	13.6	19.5	33.2
Neut. Polymorphs	64.6	51.8	50.7	50.5	64.2	54.6	16.3
E. Myelos.	31.0	61.0	13.1	32.2	45.6	38.6	27.7
E. M/Myelos.	38.0	34.3	51.8	36.6	34.8	30.0	23.2
E. Polymorphs.	31.0	4.7	35.1	31.2	19.6	31.4	
Erythroblasts							
Pro-erythroblasts	6.5	1.2	2.4	1.9	1.0	0.7	0.0
Early Norms.	10.6	13.4	7.7	2.1	4.9	1.8	10.0
Interm. Norms.	65.8	65.4	77.5	73.5	85.8	74.4	80.0
Late Norms.	17.1	19.9	12.3	22.5	8.3	23.1	10.0

SECTION VI.

Maturation Curves.

Sheep's Number	52	P.44	E. 63	V. 35	50	32	D. 65
	%	%	%	%	%	%	%
Granuloblasts							
N. Myelos	13.1	9.6	10.0	10.2	7.8	6.0	25.0
N. M/myelos.	17.5	17.5	17.6	5.7	15.6	7.3	17.2
Neut. Bands	25.2	29.6	14.0	15.9	18.1	11.9	18.3
Neut. Polymorphs.	44.2	43.3	58.4	68.2	58.5	74.8	39.5
E. Myelos.	37.0	29.9	18.3	47.4	36.2	37.4	23.5
E. M/myelos.	34.7	42.1	30.2	38.0	44.3	43.1	57.5
E. Polymorphs.	38.3	28.0	51.5	14.6	19.5	19.5	39.0
Erythroblasts							
Pro-erythroblasts	2.2	1.4	0.0	2.7	1.5	6.4	4.4
Early Norms.	7.9	6.8	8.6	8.0	4.6	9.4	10.2
Interm. Norms.	77.5	56.2	77.6	68.0	63.6	86.2	67.1
Late Norms.	12.4	35.6	13.7	21.3	30.2	8.7	18.3

SECTION VI.

Maturation Curves

For Second Examination.

Sheep's Number	45(1st)	P.44	E. 63	V. 35
Granuloblasts				
N. Myelos.	8.4	13.3	8.1	18.8
N. M/myelos.	9.5	22.6	13.8	22.3
Neut. Bands.	6.1	22.2	27.5	25.8
Neut, Polymorphs.	76.0	41.9	50.6	33.1
E. Myelos.	20.8	23.5	19.7	30.0
E. M/myelos.	33.7	49.0	30.7	51.1
E. Polymorphs.	45.5	27.5	51.5	18.9
Erythroblasts				
Pro-erythroblasts	0.0	4.0	0.9	2.0
Early Norms.	10.9	9.5	4.7	8.2
Interm. Norms.	78.2	55.3	79.5	86.4
Late Norms.	10.9	31.2	14.9	3.5

Section VII.

Sheep's Number	99/51	L.20	H.613	H.602	R.R.200	H.911
Haemomyelogram						
Haemocytoblast	0.1	0.2	0.0	0.0		
Myeloblast	1.4	0.3	0.8	0.7		
Promyelocyte	0.3	0.0	0.3	0.0		
N. Myelos.	11.1	0.7	7.4	11.0		
N. M/myelos. Jnr.	15.8	0.7	18.3	8.9		
N. " Band	17.0	3.7	12.9	16.1		
N. Polymorphs	35.1	8.2	12.8	38.8		
E. Myelos.	1.8	0.4	0.9	1.2		
E. M/Myelos.	2.3	1.1	1.8	2.3		
E. Polymorphs.	3.3	1.4	0.5	1.9		
Basophils	1.7	0.8	0.8	0.3		
Pro-erythroblasts	0.4	5.1	0.5	0.7		
Early Norms.	0.5	9.6	4.2	0.6		
Interm. Norms.	2.7	59.1	33.8	10.8		
Late Norms	1.4	7.0	2.6	4.5		
Reticulum Cells	3.1	1.6	1.7	0.4		
Plasma Cells	0.9	0.1	0.6	6.6		
Lymphocytes	0.1	0.0	0.1	0.2		
Monocytes	0.0	0.0	0.0	0.0		
M/E Ratio	15.0 : 1	0.21 : 1	1.37:1	4.6:1		
Cellularity	II.	V.	V.	V.	0.	0.
Peripheral Blood						
P.C.V. %	19.0	12.0	13.0	8.5	13.0	28.0
Hb. gms/100 ml.	5.5	3.5	2.8	2.0	3.9	8.3
R.B.C. 10 /cu.mm.	6.2	3.1	3.5	1.7	4.2	9.4
M.C.V. cu.	31.0	41.0	37.5	51.0	31.0	30.0
M.C.H.C. %	29.0	28.5	21.5	23.5	30.0	29.5
Punctate Basophilia %	0.3	0.12	0.2	3.1	0.0	0.0
W.B.C. 10 /cu.mm.	8,8	8.5	7.1	19.9	14.9	6.9
D.L.C. /cu.mm.						
Neut. Band.	88	00	71	2090	745	793
N. Polymorphs.	6424	2593	6035	15,234	12,814	4,865
Eosinophils	0	0	71	0	0	0
Basophils	0	0	0	0	0	0
Lymphocytes	1760	5865	639	2,388	1,043	1,139
Monocytes	528	35	284	100	198	104

Section VII.

Maturation Curves

Sheep's Number	99/51	L. 20	H. 613	H. 602
	%	%	%	%
Granuloblasts				
N. Myelos	14.1	5.2	14.4	15.8
N. M/myelos.	20.0	5.2	35.6	12.7
N. M/myelos Band	21.5	27.8	25.1	23.1
N. Polymorphs.	44.4	61.7	24.9	48.4
E. Myelos.	24.3	13.8	28.1	22.2
E. M/Myelos.	31.1	37.9	56.2	42.6
E. Polymorphs.	44.6	48.3	15.6	35.2
Erythroblasts				
Pro-erythroblasts	6.6	6.3	1.2	4.2
Early Norms	8.3	11.8	10.2	3.6
Interm. Norms.	61.7	73.1	82.2	65.1
Late Norms.	23.3	8.7	6.3	27.1

Section V.

Worm Burdens of sheep

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Sheep's No.	50	68	54	56	63	67	51	52
H.contortus	0	0	0	0	0	0	0	0
O.circumcincta	0	0	50	50	50	0	0	0
O.other spp.	0	0	0	0	0	0	0	0
T.axei	350	0	0	750	300	0	0	0
Larval stages	0	0	250	0	50	0	0	0
T.colubriformis	0	0	0	0	50	0	0	0
T.vitrinus	0	0	0	0	0	0	0	0
C.curticei	0	0	0	250	0	50	0	50
B.trigonocephalum	0	0	30	20	15	0	60	10
Nematodirus spp.	0	0	0	0	0	0	0	0
Larval stages	0	0	0	0	0	0	50	0
S.papillosus	50	0	0	0	0	0	50	0
O.venulosum	1	0	2	8	0	0	3	1
C.ovina	0	0	0	0	0	0	0	0
T.ovis	0	0	0	0	0	0	0	0

Section V.

Worm Burdens of sheep.

Sheep's No.	57	58	53	65	55	69	60
H.contortus	0	0	0	0	0	0	0
O.circumcincta	0	0	100	0	400	350	350
O.other spp.	0	0	100	0	150	50	0
T.axei	0	500	300	0	1,300	50	350
Larval stages	350	400	550	600	5,850	22,300	5,300
T.colubriformis	0	0	0	0	0	0	0
T.vitrinus	0	0	0	0	0	0	50
C.curticei	0	350	1,050	150	50	200	100
B.trigonocephalum	0	5	40	12	12	32	7
Nematodirus spp.	0	0	0	0	0	0	0
Larval stages	0	50	0	0	50	50	50
S.papillosus	50	0	0	0	0	50	50
O.venulosum	0	0	0	0	0	0	0
C.ovina	0	0	1	0	0	0	0
T.ovis	0	0	0	0	0	0	0

Sheep's No.	62	61	64	70	74	66	75
H.contortus	0	0	0	0	0	0	0
O.circumcincta	750	1,300	150	0	0	350	0
O.other spp.	150	150	0	0	0	0	0
T.axei	0	650	150	0	900	2,600	300
Larval stages	10,000	5,200	5,850	0	150	400	600
T.colubriformis	0	1,100	0	0	0	0	0
T.vitrinus	0	0	0	0	0	0	0
C.curticei	0	0	0	50	150	150	0
B.trigonocephalum	19	15	1	4	49	0	20
Nematodirus spp.	0	0	0	0	0	0	0
Larval stages	0	0	0	0	0	0	0
S.papillosus	0	0	0	0	0	0	0
O.venulosum	0	0	0	0	33	29	2
C.ovina	0	7	0	0	187	106	4
T.ovis	0	0	0	0	0	0	0

Sheep's No.	71	72	59	73	76	77
H.contortus.	24	0	0	0	0	0
O.circumcincta	8,500	350	0	0	150	50
O.other spp.	850	0	0	0	50	50
T.axei	550	200	50	900	200	400
Larval stages	4,200	150	50	0	50	100
T.colubriformis	0	0	0	0	0	0
T.vitrinus	600	0	0	0	0	0
C.curticei	0	300	50	0	1,550	450
B.trigonocephalum	9	30	10	8	40	0
Nematodirus spp.	200	0	0	0	0	0
Larval stages	0	0	0	0	0	100
S.papillosus	0	0	0	0	0	0
O.venulosum	0	0	0	0	0	0
C.ovina	0	2	0	0	1	0