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PROEFSCHRIFT

TER VERKRIJGING VAN DEN GRAAD VAN

DOCTOR IN DE VEEARTSENIJKUNDE

AAN DE RIJKS-UNIVERSITEIT TE UTRECHT OP GEZAG VAN DEN RECTOR MAGNIFICUS **JHR. DR. B. C. DE SAVORNIN LOHMAN** HOOGLEERAAR IN DE FACULTEIT DER RECHTSGELEERDHEID VOLGENS BESLUIT VAN DEN SENAAT DER UNIVERSITEIT TEGEN DE BEDENKINGEN VAN DE FACULTEIT DER VEEARTSENIJKUNDE TE VERDEDIGEN OP **DONDERDAG 4 JUNI 1931** DES NAMIDDAGS TE 4 UUR DOOR

P. J. J. FOURIE, M.R.C.V.S.

SUB-DIREKTEUR VAN VEEARTSENY DIENS
ONDERSTEPOORT

THE GOVERNMENT PRINTER, PRETORIA
1931

Aan de nagedachtenis van mijn Ouders.
Aan mijn Vrouw en Kinderen.

The Haematology and Pathology of Haemonchosis in Sheep.

BY

P. J. J. FOURIE, M.R.C.V.S.,
Sub-Director of Veterinary Services, Onderstepoort.

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INTRODUCTION.

In the literature available, no reference to a complete and systematic study of the haematology of sheep infected with wireworm (*Haemonchus contortus*) has been found. That anaemia develops as a result of the action of the gastro-intestinal worms, is well known; and, under field conditions, pale mucous membranes and the most extreme degrees of badly staining and watery blood, are features which are commonly associated with the effects of internal parasites. How the anaemia is brought about, and what part individual species of parasites play in its production, are points that have not been properly elucidated.

The main object of this study is the pathogenesis of the anaemia. In such an investigation it is necessary to produce in worm-free sheep, under properly controlled experimental conditions, a progressive and preferably fatal anaemia, by the administration of pure faeces cultures of the parasites. The subject matter of this work can conveniently be sub-divided into:

(1) General Survey and Plan of Work.
(2) Technique.
(3) Details of Experiments:
    (i) Haematology and Pathology of Haemonchosis in Sheep.
        A. Fatal cases of Haemonchosis.
        B. Recovery cases and those in which no effects were observed.
        C. Controls.
    (ii) Pathogenesis of the Anaemia in Haemonchosis of Sheep.
(4) Some unusual lesions of Haemonchosis.
(5) Summary.

GENERAL SURVEY AND PLAN OF WORK.

Weekly observations on the blood were recorded, before infection and throughout the experiment, until the animal died as a result of the parasitic infestation. In order that reliable conclusions can be drawn as to the effects produced in animals by particular parasites, it is necessary (1) that the animal host at the time of the commencement of the experiment be free from infestation, and (2) at the time of the completion of the experiment no other parasites than the one under investigation be recovered at post-mortem examination. These are the ideal conditions that must be aimed at, but it must be confessed that only rarely are they completely attained in practice, notwithstanding that the most elaborate precautions are strictly maintained.

Veglia (1928) describes in detail the method employed by him for rearing and maintaining worm-free lambs at this Institute. He removes the lambs from their mothers soon after birth, hand-rearing them in specially constructed cages with raised floors. In this way it is possible to control more or less successfully the worm infection of particular animals, but it was impossible to prevent this entirely, although such infestation with parasites other than those with which the experimental animals were infected was present only to a small and negligible extent.

Sixteen lambs reared in this way by Veglia were used in the first series of these experiments. Later on, when no more such lambs were available, young sheep, entirely free from worm infestation, were bought from certain farms in the Karroo. The complete absence of worm infestation was determined by making post-mortem examinations of some animals that were born and reared under the same conditions as others used for these experiments and was confirmed before these animals were actually drafted into an experiment by careful faeces cultures. This work was courteously directed by my colleagues, Drs. Veglia and Mönnig.

Lambs or young sheep were selected for these experiments because of the generally accepted view that young animals are more susceptible than are older animals to the effects of parasites. Hadwen (1925), referring chiefly to Ascariasis in horses, maintains that this is due to an immunity or tolerance acquired or developed by older animals as a result of infection with parasites. Young animals, not having had an opportunity to develop this power of tolerance, suffer severely when they become infected. This immunity and tolerance is due to anti-substances and eosinophiles, both of which function in neutralizing the cast-off products of the worms. He believes that the eosinophiles secrete in addition to these agents which neutralize effete porducts of worms, a substance which paralyses or kills the worms themselves. He states that the percentage of eosinophiles in the circulation may be low, or even altogether absent in severe cases of parasitism. There is an apparent leucopenia as far as the eosinophiles are concerned, but this is due to their withdrawal from the circulation and their concentration around the parasites in the tissues. Similarly, an eosinophilia may mean that the parasites have been overcome. In such cases the excess of eosonophiles has again returned to the circulation (from which they gradually disappear).

The use of young lambs for these experiments has the additional advantage that they are less likely to be seriously infested with any parasite other than the one under investigation.

It is naturally essential that pure cultures of the parasites should be used for infecting experimental animals. One may have the good fortune to obtain a pure culture from a sheep that happens to harbour the pure infestation of the parasite that it is intended to use. In cases of mixed infestation, undesirable parasites can be destroyed by specific drugs, which must not be parasiticidal for the parasites which are to be cutivated or a pure culture can be obtained by keeping a sample of mixed faeces culture until the unwanted larvae have died out. Mönnig (1930) shows that eggs of *Trichostrongylus* spp. containing complete embryos are more resistant to desiccation than such eggs of *Haemonchus contortus*, *Oesophatgostomum columbianum* and *Strongyloides papillosus*. In the same paper this author discusses a successful method of producing a pure infestation of Oesophagostomes in sheep by administering the adult nodular worms in saline per rectum.

A further method consists in infecting worm-free sheep with adult males and females of the parasite. In some cases eggs are passed in the faeces in due course, and in this way a pure culture can be obtained with which further clean animals can be infected, and rich cultures from these can be used for gross infection of experimental animals.

Recently Stoll (1929) described an ideal method for the natural infection of animals on the pasture. Natural infection of the pasture is introduced by means of lambs which have been given larvae developed from eggs obtained from a single female wireworm. If it is possible to prevent gross infestation of the pasture by other parasites, one should with this method be able to study the haematology of haemonchosis under natural conditions. This is very desirable, as none of the animals artificially infected with wireworm and kept under controlled experimental conditions in the stable developed any oedematous swellings, e.g. "bottle jaw," which are characteristic clinical features of verminosis.

The experimental animals were infected by injecting a saline suspension of larvae, obtained from faeces cultures, into the mouth by means of a hypodermic syringe without the needle.

Pure faeces cultures of the parasites having been obtained on the one hand and clean animals to infect on the other, it was thought that no great difficulty would be experienced in producing typical cases for haematological study. Accordingly a fairly ambitious programme was drawn up for the comparative study of the blood of a number of animals infected at the same time with pure cultures of parasites commonly encountered in the gastro-intestinal tract of sheep under natural conditions in South Africa. These are (1) *Haemonchus contortus*, (2) *Oesophagostomum columbianum*, (3) Various species of *Trichostrongylus*, (4) *Strongyloides papillosus*.

When mixed cultures of these parasites were used, no difficulty was experienced in artificially producing a fatal panverminosis, but

the production of a progressive disease in sheep by infecting these with pure cultures of these parasites was found to be altogether a different matter. This was especially the case when the sheep were stabled and properly fed on a well balanced ration. There are quite a number of factors which may, to a greater or lesser degree, be responsible for this, viz.:—

(1) Under field conditions the worm infestation is usually a mixed one, and it seems that the animal is less able to withstand the onslaught of an attack at several different portions of the gastro-intestinal tract;

(2) Where the grazing is poor, particularly in quality, the parasites thrive exceedingly at the expense of the host;

(3) Exercise seems to be detrimental. This is especially evident in those areas where no jackal-proof fencing has been carried out, for it becomes necessary, in order to protect the sheep against the ravages of this vermin, to drive them to the shelter of kraals every night. This increases the amount of daily exercise of these animals, with the result that the resistance to the parasites is decreased, enabling them to bring about deleterious effects more rapidly, especially in those sheep in which the anaemia is already present;

(4) The method of infection is probably also of importance. Veglia (1918) believes that the larvae, in the case of natural infection in haemonchosis, pass into the rumen of sheep with the solid food, whereas when 10 c.c. of saline suspension of larvae are injected into the mouth, the larvae may pass straight into the abomasum with this liquid and are in these circumstances liable to pass into the intestine without having had time to undergo those changes which enable them to remain in the abomasum.

It was only after many disappointments and with a great deal of difficulty that a sufficient number of typical, fatal cases of pure haemonchosis were artificially produced for haematological study. The work on the haematology and pathology of Oesophagostomiasis and Trichostrongylosis, will be discussed in later papers. In connection with Strongyloides papillosus, no haematological observations will be recorded (numerous observations were actually made) as in not a single instance was a progressive disease produced, in spite of the fact that many of the animals infected passed faeces from which strong colonies were obtained by faeces cultures.

Throughout these experiments, all the animals, including the controls, were kept under identical conditions of housing, feeding and watering. No special attempt was made to draw the blood at definite intervals before or after watering. It was thought that the most uniform results would be obtained if the amount of water that the animals consumed was allowed to be regulated by the natural development of thirst. In order to achieve this, a plentiful supply of water was made always available. On this account it is believed that no undue concentration of the blood could have occurred at any time as a result of diminished ingestion of water.

TECHNIQUE.

The technique developed by Neser (1923) and exhaustively discussed by him in his work on "The Blood of Equines," is with few modifications applicable to haematological work in sheep. The methods of examination employed are exactly those advocated by Neser, except where such modifications were found to be necessary, and details of the latter only will be discussed. For the other details Neser's work should be consulted.

Collection of Blood.

It is obvious that a direct examination of the whole blood would give the most accurate absolute results concerning the morphology and other attributes of both the erythrocytes and the leucocytes, but where a systematic examination of the blood of a number of sheep had to be completed in one day, it was clearly impossible to make determinations on the whole blood.

Rossdale (1923) compares the haematocrit results obtained when working with whole blood and citrated blood. Solid crystals were used, as well as definite proportions of citrate solutions, for which a correction was made afterwards. In a number of cases investigated, he found the greatest deviation from the mean volume index to be 4 per cent. in the case of whole blood, 11 per cent. in the case of citrate crystals, and 14 per cent. where citrate solution was employed. He concludes that the addition to the blood of citrate or oxalate affects the haematocrit readings by reducing the percentage volume of red cells, unless that particular proportion of citrate to blood be chanced upon which happens to be isotonic with the particular blood under investigation. Rossdale gives a comprehensive review of anticoagulants used by various workers, and he quotes in each case the haematocrit values obtained by these authors when centrifuging at certain definite speeds samples of blood of which the red counts, and in some cases average measurements of the red cells had been determined.

The anticoagulant selected was the one used by Neser for horse blood. This consisted of a 7½ per cent. citrate solution used in the proportion of .1 c.c. citrate to 1 c.c. of blood. With this method quite uniform relative results were obtained. Bryuchonenko and Steppuhn (1927) claim that Bayer 205, when injected intravenously, will prevent coagulation of the blood for some hours. This would be an ideal method for haematological work, provided the drug has no specific effect on any of the elements of the blood. The chief advantages of this method of collecting the blood would be: (1) If coagulation is prevented for some hours, there is ample time for a complete systematic study of several samples. (2) No measured amount of blood need be drawn. Errors in haematological studies are frequently due to the inaccurate measuring of the blood when it is being withdrawn from the vein. (3) No changes in isotonicity can possibly occur, so that values dependent on the size of the red cells will be more reliable. Hirudin as an anticoagulant was not available.

When an anticoagulant is used, the possibility of error is less the larger the amount of blood that is drawn. Where, however,

anaemia is studied, particularly in a small animal like the sheep, it is advisable that only small amounts of blood be collected, so that the factor of bleeding cannot be blamed for the production of the anaemia; consequently small bottles with narrow necks, measuring approximately 6 c.c. were utilized for collecting the blood.

Methods of Examination of the Blood.

The examination of the blood included the study of (1) a study of the erythrocytes—(*a*) the red counts, (*b*) the red precipitate or the percentage volume of the red cells, (*c*) the percentage of haemoglobin, (*d*) the size of the red cells, (*e*) the viscosity. (2) The leucocytes—(*a*) the total white counts, and (*b*) the differential counts.

(1) (*a*) *Counting of the Red Cells.*

This was done in the usual way, using a Burker Turk counting chamber.

(1) (*b*) *The Determination of the Percentage Volume of Red Cells (Red Precipitate).*

This was obtained by centrifuging the citrated blood in an electric centrifuge running at 1,500 revolutions per minute, for 60 minutes. The centrifuge tubes were calibrated as advocated by Neser (1923). In the horse this author obtained constant centrifuge readings in 10 mintes, irrespective of whether the centrifuge was running at medium or high speed. At a low speed (250 revolutions per minute), the same reading was obtained in 15 minutes. Neser concluded that within certain limits of speed the volume of the red corpuscles in equine blood is in no way influenced by the speed.

The blood of the sheep as compared with that of the horse, takes a much longer time to precipitate completely, as will be seen from the following experiment.

A young healthy Merino sheep, No. 24197, was bled daily from the jugular vein, 200-300 c.c. of blood being removed at a time, until a severe degree of anaemia was produced. Before bleeding, this animal had a red count of 15 million per c.mm. of blood, a percentage volume of 35, and 102 per cent. of haemoglobin. As a result of the bleeding, the red cell counts, the percentage of haemoglobin and the red precipitate rapidly decreased. When the sheep had developed what could be regarded as a mild degree of anaemia, with a red count of 4.9 millions per c.mm., a haemoglobin of 45 per cent. and a red precipitate of 15, the precipitate during centrifugalization was read every 10 minutes and the blood from another but normal sheep, No. 24188, was treated in the same way, at the same time, and in the same centrifuge running at 1,500 revolutions per minute. The results obtained were:—

Sheep No. 24197 (mild anaemia).					Sheep No. 24188 (normal).
ppt.	after	10	minutes	20	50
,,	,,	20	,,	18	34
,,	,,	30	,,	16	31
,,	,,	40	,,	15.5	30
,,	,,	50	,,	15	29.5
,,	,,	60	,,	15	29
,,	,,	70	,,	15	29
,,	,,	80	,,	15	29

Two days later, when a more severe degree of anaemia had developed, with a red count of 2.7 million per c.mm., thep recipitate was again read at intervals of 10 minutes, together with that of the same normal sheep. The results obtained were :—

Sheep No. 24197 (severe anaemia).					Sheep No. 24188 (normal).
ppt.	after	10	minutes	10	45
,,	,,	20	,,	9.5	38
,,	,,	30	,,	9	35
,,	,,	40	,,	9	34
,,	,,	50	,,	9	32
,,	,,	60	,,	9	31
,,	,,	70	,,	9	30
,,	,,	80	,,	9	30
,,	,,	90	,,	9	30
,,	,,	100	,,	9	30

From the above it can be concluded that in normal sheep constant red cell percentage volume readings are obtained in 60-70 minutes, when citrated blood is precipitated in an electric centrifuge running at 1,500 revolutions per minute. In the case of anaemic blood, constant readings are obtained very much earlier. In very severe degrees of anaemia, constant readings are obtained in 20-30 minutes time and in less severe degrees of anaemia in 40-50 minutes. In this work, therefore, the precipitate was read as a routine procedure after 60 minutes with the centrifuge running at 1,500 revolutions per minute.

(1) (*c*) *The Determination of the Percentage of Haemoglobin.*

This was determined calorimetrically in a Dubosq calorimeter, a Newcomer disc being used as the standard. The application of this disc to haemoglobin determinations was discussed by Newcomer in 1919. Daylight illumination was used as it was found difficult to match the colours with the artificial light available. Occasionally, when observations were made on cloudy days, it was found difficult to obtain consistent readings. Throughout this work an average of 10 calometric readings was taken, typical examples of which are :—

Sheep No. 11806—normal animal—dilution 1 in 200—8.2, 8.3, 8.2, 8.3, 8.2, 8.2, 8.2, 8.2, 8.2, 8.4—giving an average reading of 8.2. When this is read against the chart supplied by the manufacturers, the value of 92 per cent. haemoglobin is obtained.

Sheep No. 14197—showing anaemia—dilution 1 in 200 17.6, 17.7, 17.7, 17.8, 18.0, 17.9, 18.0, 17.6, 17.6, 17.9 giving an average of 17.8, which is equivalent to 42 per cent. of haemoglobin.

The Newcomer disc was standardized against Van Slyke's gasometric method, and the chart supplied by the manufacturers of the disc in the Williamson standard was converted into the Haldane standard. Newcomer (1923) described " a new optical instrument for the determination of haemoglobin." This should be a very convenient apparatus, as one is able to read off directly on the instrument the percentage of haemoglobin.

The diluting fluid is N/10 hydrochloric acid which converts the blood into acid haematin. The chart supplied with the disc is only

prepared for reading dilutions of 1 in 200 and 1 in 500. In this work the dilution of 1 in 200 was employed.

Newcomer (1923) points out it takes some time before the full colour depth of the acid haematin is developed. After the flasks have stood for half an hour, the readings are correct to approximately 1 per cent. Readings were never made within an hour after making the dilutions, but some readings were not made until some hours after dilution. If it is necessary to determine the haemoglobin content almost immediately after making the dilutons, the readings should be corrected in accordance with Newcomer's (1919 and 1923) equation.

Terrill (1922) discusses (1) a method for making concentrated stock solutions of acid haematin. When these are kept in the dark at room temperature, or in a refrigerator, the colour fades slowly. He does not mention to what extent fading occurs if the stock solution be exposed to light. (2) A method for the preparation of acid haematin protein powder, from which standard solutions are prepared. (3) A method of preparing acid haematin films on cover glasses by means of a mixture of solutions of acid haematin and of gelatin. This author in the same paper shows that turbidity in the acid haematin solutions can be got rid of, by means of laking the blood with distilled water, before adding it to the hydrochloric acid solution. He maintains that the whole blood diluted with N/10 hydrochloric acid gives reading 6-12 per cent. higher than those obtained for the same blood first laked by adding it to distilled water. This error should not occur in the figures obtained in this investigation as the disc was standardized against Van Slyke, by obtaining the average of a number of calorimetric readings by different individuals on an acid haematin prepared without previous laking.

There is one important point to bear in mind when making the dilutions, viz., to shake the citrated blood very thoroughly before it is drawn off with the pipette for making the dilutions. If this is not done, sedimentation may occur and proportionately more red corpuscles are drawn into the pipette, which naturally is conveniently rested against the bottom of the bottle during manipulations. A considerable source of error in all the determinations may be introduced if this point is neglected. It is also important to avoid the formation of bubbles in the pipette, as well as in the flasks in which the acid haematin dilutions are made. Such bubbles are especially liable to form in the pipette on top of blood withdrawn for the first time from a bottle which just previously has been vigorously shaken. When this is the case the blood should again be blown into the bottle, whilst holding the pipette slightly obliquely and resting it against the bottom of the bottle. No bubbles will be present when the blood is sucked up the second time. In order to avoid bubbles in the diluting flask, the blood should be run down the side of the flask and should not be vigorously squirted into the N/10 hydrochloric acid solution. It was found convenient to fill the flask to within 10-20 cc. of the 200 cc. mark and then to run in the citrated blood as described. If the flask is now tilted or shaken, bubbles will develop, and it will be difficult to fill the flask to the mark. The flask should only be tilted after further N/10 hydrochloric acid has been carefully

added to the 200 cc. mark. Flasks with long necks having the 200 cc. mark on the neck, are the most convenient. The 1 cc. pipettes used were either standard or previously checked against a standard pipette.

(1) (*d*) *Size of the Red Cells.*

The size of the red cells was determined by direct microscopic measurement with an "Okular Schraubenmikrometer," having a 6 compensating ocular. When this apparatus is used with a 2 mm. apochromatic lens, the figures obtained must be multiplied by .14 in order to convert them into values of microns. It is realized that the true average size of red cells in stained smears will not be obtained by measuring even 400 or more red cells in numerous fields, and as this is most exacting work, requiring a great deal of time, only relatively few cells could be measured, mainly with the object of obtaining corroborative evidence as to whether any increase at all in the size of the cells had occurred, as was suggested by changes in the colour index and in the volume index. Pyper (1929) describes a diffraction method whereby the true average size of the red cells in suitably prepared unstained smears can be simply and quickly determined spectroscopically. If a sufficient number of smaller and larger cells are present in such smears, they will also produce measurable spectra, consequently this author can by this method calculate the diameters of the smallest, the average, and the largest cells. The method was unfortunately not available for application in this study.

(1) (*e*) *The Viscosity.*

Hess' viscosimeter was used. Schridde and Naegeli (1921) give a detailed description of this apparatus. Unless the determinations are made with dexterity, the values obtained can be very unreliable. In the tables submitted, the temperatures at which determinations were made are stated, but no corrections for these were made. It is noteworthy that in some cases in which there was observed an apparent agglutination of red cells in the counting chamber, the viscosity was abnormally high for the particular blood, if it is considered that more or less normal values were obtained for the red counts, haemoglobin and percentage volume of red cells.

2. *Leucocytes.*

The total white cell counts were made by the usual methods and the differential counts were made according to Neser's (1923) modification of Ehrlich's method.

DETAILS OF EXPERIMENT.

As already stated it was at first intended to make a comparative haematological study of sheep with pure infestations of *Haemonchus contortus*, various species of *Trichostrongylus*, *Oesophagostomum columbianum* and *Strongyloides papillosus*.

Seventy sheep were used in these experiments. Thirty-eight were infected with *Haemonchus contortus*, eleven with *Trichostrongylus*, seven with *Oesophagostomum columbianum*, three with

Strongyloides papillosus and eleven were kept as controls. In this work details in connection with the haematology and pathology of haemonchosis only will be presented.

THE HAEMATOLOGY AND PATHOLOGY OF HAEMONCHOSIS.

Sheep infected with a pure or practically pure culture of *Haemonchus contortus* can be conveniently classified in terms of the effects observed as: (A) those sheep in which a progressive and fatal disease was produced and (B) those in which an anaemia developed from which the animals soon recovered, without medicinal or any other treatment—these can be designated as recovery cases and those in which no effects were observed. (C) Controls.

(A) Changes Observed in the Blood of Sheep in Fatal Cases of Haemonchosis.

The outstanding feature of these cases is a progressive anaemia. This is conveniently illustrated in the accompanying tables and graphs, where the changes in the red cells are recorded in so far as are concerned (1) the number of red cells, (2) the percentage of haemoglobin, (3) the percentage volume of red cells (precipitate), and in some cases, (4) the viscosity.

The total number of leucocytes is also charted, together with differential counts, where the latter could conveniently be dealt with in this way.

Of the thirty-eight sheep infected artificially with *Haemonchus contortus*, nine developed a progressive anaemia, with a fatal termination. There were six so called recovery cases and the remaining twenty-three either resisted infestation or did not show any effects of infection. On the nine cases in which a progressive disease was produced, the haematological observations of five will be presented in detail.

Haematology and Pathology of Sheep No. 16027.

Table 1 and graph 1 contain the haematological records of Sheep No. 16027. Infection was commenced on the 27.4.27, at the rate of approximately 1,000 larvae per day, until 52,000 larvae had been given. Infection was, therefore, completed towards 16.6.27.

Haematology of Sheep No. 16027.

During the first fourteen (14) days after infection there is an increase in the number of red cells, with a corresponding increase in the percentage of haemoglobin and in the percentage volume of red cells. Such increases during the initial stages of the infection period have been observed in quite a number of cases, and are interpreted as probably due to increased activity of the blood-forming organs. The destruction and removal of red cells during the early stages of *Haemonchus contortus* infection serve as the stimulus for this increased activity. After that there is a progressive decrease in (1) the number of red cells, (2) the percentage of haemoglobin, and (3) the percentage volume of red cells, except for a slight increase which

TABLE 1.

SHEEP No. 10627.

Method of treatment: Infection commenced 27.4.27, *giving* 1,000 *larvae of* Haemonchus contortus *per day until* 52,000 *larvae had been administered.*

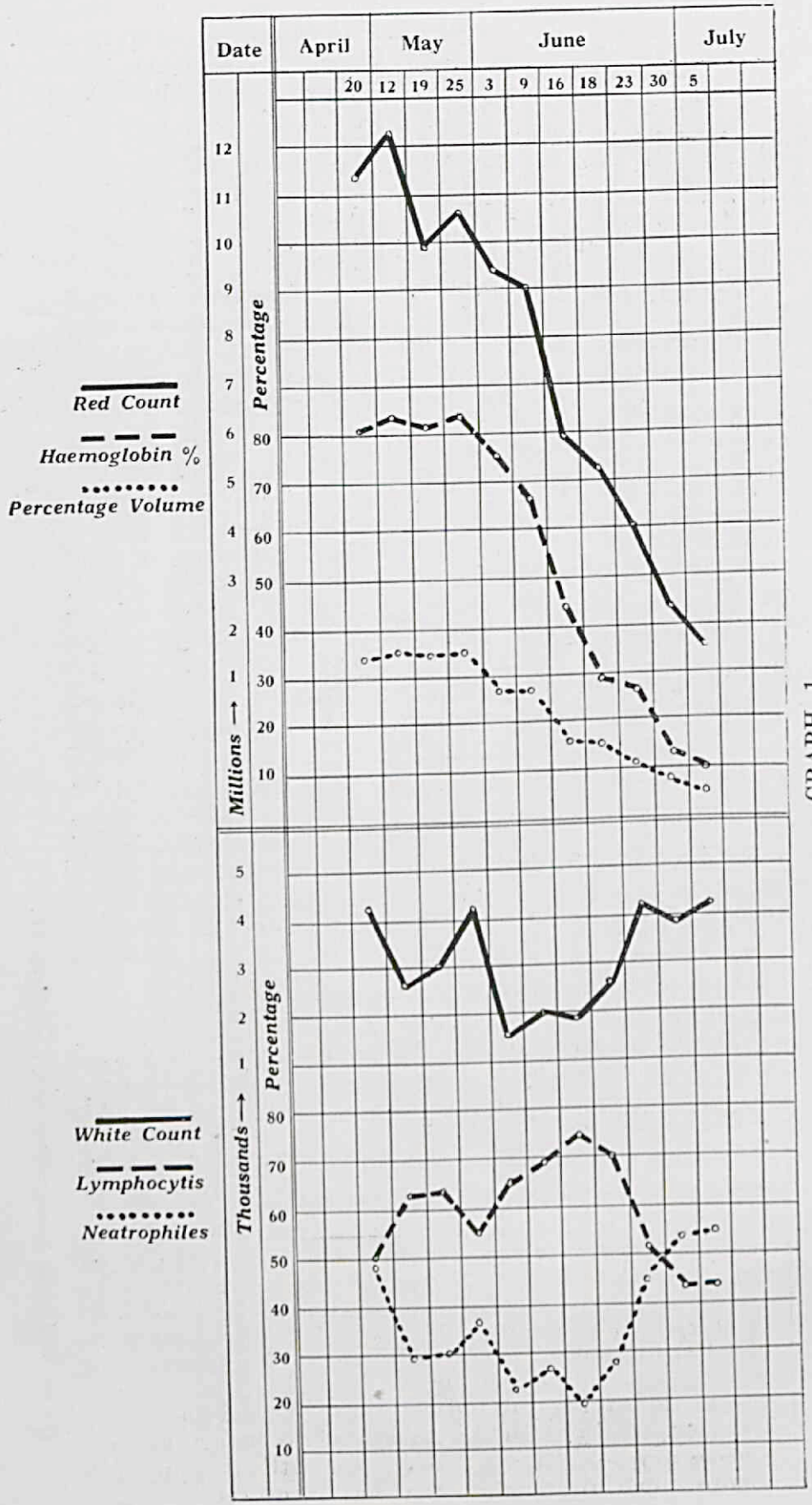
Date.	Source.	R.C.	R.P.	Hglb. %	Serum.	W.C.	L.	M.	N.	E.	B.	V.C.I.	C.I.	V.I.	Remarks.
20/4/27	Jugular	11·4	34	82*	Clear	4,200	50	1	49	0	0	1·00	0·94	0·95	Red cells, normal.
12/5/27	,,	12·2	35	84	,,	2,700	63	4	29	3	1	0·99	0·91	0·92	Red cells, normal.
19/5/27	,,	9·9	34	81*	,,	3,000	64	5	30	1	0	0·99	1·07	0·83	Red cells, normal.
25/5/27	,,	10·6	35	83	,,	4,200	56	2	37	3	2	0·99	1·04	1·06	Red cells, normal.
3/6/27	,,	9·4	27	76	,,	1,600	66	4	22	8	0	1·17	1·30	0·92	Red cells, normal.
9/6/27	,,	9·0	27	67	,,	2,000	69	1	27	3	0	1·03	0·99	0·96	Red cells, normal.
16/6/27	,,	5·9	16	43	,,	1,900	75	5	19	1	0	1·12	0·97	0·87	Anisocytosis with occasional punctate basophilia.
18/6/27	,,	5·3	15	29	,,	2,600	70	2	28	0	0	0·80	0·72	0·90	Anisocytosis with occasional punctate basophilia.
23/6/27	,,	4·0	11	27	,,	4,200	51	4	45	0	0	1·02	0·90	0·88	Polychromasia-anisocytosis punctate basophilia.
30/6/27	,,	2·3	8	13	,,	3,900	43	2	53	2	0	0·68	0·76	1·11	Marked anisocytosis, poikilocytosis, polychromasia, punctate basophilia.
5/7/27	,,	1·6	5±	10	,,	4,200	43	3	54	0	0	0·84	0·83	1·00	Marked anisocytosis, poikilocytosis, polychromasia, punctate basophilia. Excepting polychromasia less marked. Jolly bodies present. DIED.

* On these two days haemoglobin was not actually measured but was calculated.

Although infection only occurred on 27/4/27, the first four determinations, i.e., up to and including those on 25/5/27, are regarded as normal.

Abbreviations: R.C. = Red count. R.P. = Red precipitate. Hglb. = Haemoglobin. W.C. = White count. L. = Lymphocyte. M. = Monocyte. N. = Neutrophiles. E. = Eosinophiles. B. = Basophilia. V.C.I. = Volume colour index. C.I. = Colour index. V.I. = Volume index.

For method of calculating colour indices, see Table IV.



GRAPH 1.

SHEEP No. 16027.

Infected *Haemonchus contortus*
(52,000 larvae).

Killed by pure infection of *Haemonchus contortus* (Wireworm).

occurs during the period from the 19th until the 25th of May. From the 9th to the 16th of June the decrease of 3 million red cells per c.mm. of blood, of 24 per cent. in the haemoglobin, and of 11 per cent. in the percentage volume is particularly striking. During this time no clinical evidence of icterus or haemoglobinuria was present. The animal was destroyed on the 5th of July, on which day the red count was 1.6 million per c.cm. of blood, the haemoglobin 10.5 per cent. and the precipitate approximately 5 per cent.

During the course of the disease the only clinical symptoms observed were (1) pale mucous membranes, particularly the conjunctivat, the buccal and the anae (just before death the pallor was of an extreme degree), (2) accelerated respirations, and (3) frequent pulse, with pounding heart action, especially in the later stages. The accelerated respirations and frequent heart action were particularly noticeable on exertion. There was no clinical evidence of icterus or oedema.

Changes in the Red Cells.—In referring to Table No. 1 it will be seen that no morphological changes occurred in the red cells until 16.6.27. On that day punctate basophilia was for the first time observed. During the further course of the disease, and as the anaemia became progressively worse, the morphological changes became more pronounced. On 30.6.27 and 5.7.27, when the anaemia was of an extreme degree, there was well marked anisocytosis, and there were poikilocytosis, polychromasia, punctate basophilia and Jolly bodies in quite a number of red cells, but in this particular case no normoblasts were identified.

Changes in Size of the Red Cells.—As already stated, Pyper's (1929) apparatus for determining quickly and simply the average size of the red cells in smears was not available. To measure say 400 cells with a micrometer requires a great deal of time and even if this is done the true average size of the red cells in smears will not be obtained with any degree of accuracy. It is, however, important for the interpretation of changes in colour and volume indices, to know if changes in size have occurred. In this particular sheep measurements were made, of a small number of cells from smears made (1) before infection, (2) when a marked decrease in the oxygen carrying capacity of the blood was taking place, and (3) towards the end of the disease. In all three cases 15 cells were measured in several fields. The results are:—

Date.	*Smallest cell.*	*Largest cell.*	*Average.*
(1) 20.4.27	4.17μ	5.2μ	4.6μ
(2) 16.6.27	3.9μ	5.3μ	4.6μ
(3) 30.6.27	4.2μ	7.1μ	5.7μ

The relationship of the haemoglobin content to the number of red cells, i.e., the colour index, the relationship of the haemoglobin to the precipitate, i.e. the volume colour index, and the relationship of the precipitate to the number of red cells, i.e. the volume index, will be fully discussed elsewhere in this paper. This being the case, it will only be necessary to indicate briefly and without going into any details the more important changes that have occurred.

The Colour Index.—There is a tendency towards a decrease of this index, probably due to a relative decrease in the haemoglobin content of the cells and to an increase in the size of the cells.

The Volume Colour Index.—There is at first a tendency towards an increase of this index (up to 16.6.27) probably due to a decrease in the size of the red cells. Towards the end of the disease the index is definitely decreased. This would indicate a relative deficiency in haemoglobin and an increase in the size of the red cells.

Volume Index.—There is at first a tendency towards a decrease of this index, indicating a decrease in the size of the red cells, but towards the end of the disease the volume index increases. This confirms the conclusion previously arrived at, that an increase in the size of the red cells had occurred. As previously stated, too few cells were measured in order that reliable conclusions as to the true average size of the red cells could be drawn; nevertheless, the measurements made suggest that the cells on 16.6.27, for instance, are on the whole smaller than the cells on 20.4.27. There is no doubt at all that on 30.6.27 the cells were definitely larger than before infection and during the earlier stages of the disease.

The cells stained less intensely in the later stages of the disease and many had a vacuolated appearance. Although unstained central areas are sometimes encountered in cells of apparently normal sheep, these staining reactions would tend to confirm the conclusion that a relative deficiency of haemoglobin was present.

Changes in the Leucocytes.—No changes which can be regarded as pathogonomic for haemonchosis were observed. Soon after the red cells begin to decrease in number a decrease in the total number of leucocytes also occurs, but during the later sages of the disease the total number of leucocytes again increases. If the differential counts be referred to, it will be observed that this increase affects mainly the neutrophiles. The neutrophile counts are at their lowest when the first evidence of morphological changes in the red cells is noticed, Later on, when these morphological changes in the red cells are more pronounced, the neutrophiles increase in number. It is possible that this may be associated with greater activity of the blood-forming organs. No marked changes were observed in connection with the number of eosinophiles, only on one day (3.6.27), during the early stages of the disease, was there what one could describe as an eosinophilia.

Pathology of Sheep No. 16027.

On 5.7.27 the animal was killed *in extremis* for post mortem examination.

Macroscopic Examination.—Signalment: Merino wether in good condition, having only temporory teeth.

The mucous membranes of the natural openings were very pale and almost dead white in colour. The *blood* was extremely watery, barely staining the fingers, and was of almost an amber colour. A good deal of fat was present in the fat depôts. No abnormal fluid was present in the abdominal cavity. The *lymphatic glands* on section were pale and moist. The pericardial sac contained about

20 c.c. of a clear colourless fluid. The *liver* was slightly enlarged and firm on palpation. On section a watery fluid escaped from the cut surface, which was of a mottled appearance, the central portion of the lobule being brown and opaque and the periphery almost transparent and grey in colour. The heart fat was normal in amount and appearance. The *heart* itself appeared quite normal, except that the myocardium was of a greyish colour and opaque, suggesting degenerative changes *of a fatty nature*. The *lungs* were extremely pale, but excepting for a certain amount of emphysema, were not altered in any way. The *kidney* had a fair amount of pericapsular fat, the capsule stripped easily, the cortex was of a yellowish brown colour and the medulla and boundary zones were extremely pale. The *spleen* was decreased in size and measured 7.5 by 5 by 1cm. The capsule was markedly wrinkled, the trabeculae were distinct, but the Malpighian bodies were indistinct. The other organs, such as the *adrenals*, *thyroid*, etc., were all systematically examined, but apart from the fact that they were paler in colour than normal, no abnormalities were observed macroscopically. The contents as well as the mucous membranes of the *rumen*, *recticulum* and *omasum* were normal. In the *abornasum* numerous wire-worms were present. The mucous membrane was pale and the contents were liquid and of a dark brownish colour. In the *small intestine* the ingesta were liquid and of a dark chocolate colour and a few *Strongyloides papillosus* were present. The faeces in the large intestine were of a dark colour. Very few adults of the *Oesophagostomum columbianum* were identified, but no nodules were present. No changes were recognized in the bone-marrow.

Anatomical Pathological Diagnosis.—Very marked anaemia, paleness of all the organs and of the visible and other mucous membranes, throughout the body; degenerative changes of the liver, myocardium and kidneys; emphysema of the lung; numerous wire-worms; very few nodular worms; few *Strongyloides papillosus;* complete absence of icterus and haemoglobinuria.

Aetiological Diagnosis.—Killed in extremis (practically pure infection of *Haemonchus contortus*).

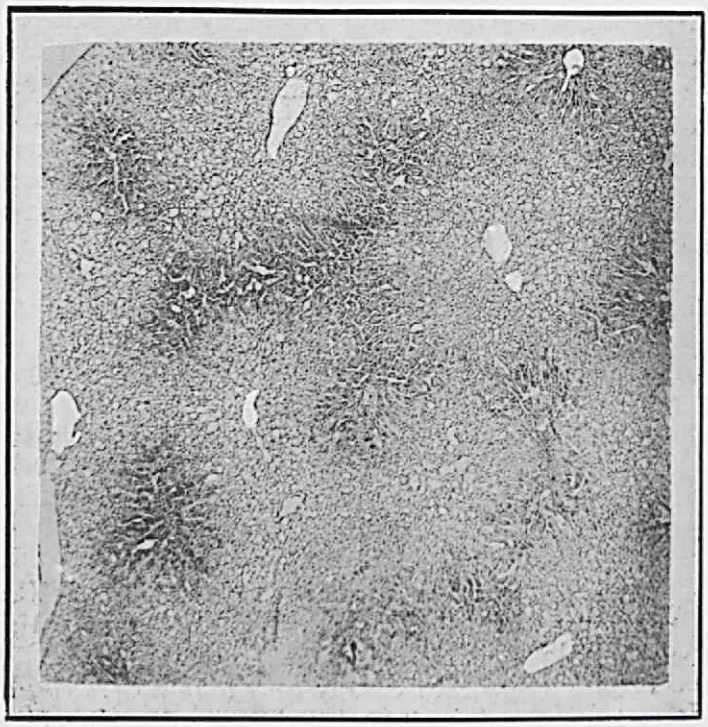
Microscopic Examination: *Kidneys.*—There were slight degenerative changes of a fatty nature. Some of the cells, especially of the convoluted tubules, stained a faded orange with Sudan III, and with a 4 mm. objective were seen to be distended with fine radiating crystals, which stained similarly. There was no evidence of any iron-containing pigment in the kidney. No other lesions were present.

Spleen and Lung.—No changes were seen microscopically, except that the lymphoid follicles in the spleen were not well defined, and emphysema of lungs was present. With specific staining for iron, very little iron-containing pigment could be identified in the spleen and none at all could be recognized in the lungs.

Liver.—When the haemalum-eosin stained sections were examined with the naked eye by holding them up against the light, a distinct mottled appearance was observed. This was due to some portions staining slightly or not at all and other staining more evenly and more intensely (see Figs. 1 and 2.) On microscopic examination the slightly stained or even unstained areas were seen to be

SHEEP No. 16027: SPEC. No. 7045.

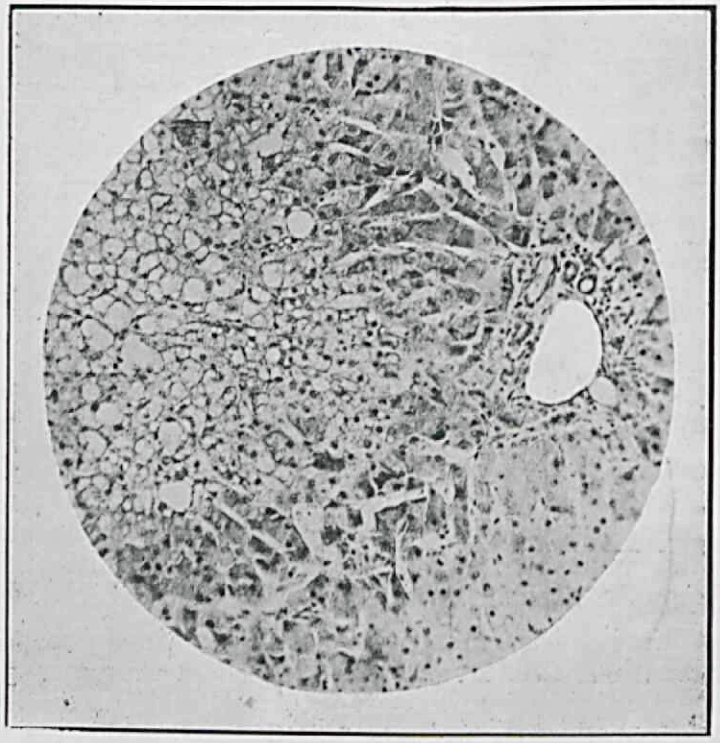
FIG. 1.



Liver. ×33.

Lesions around Central Veins.

FIG. 2.

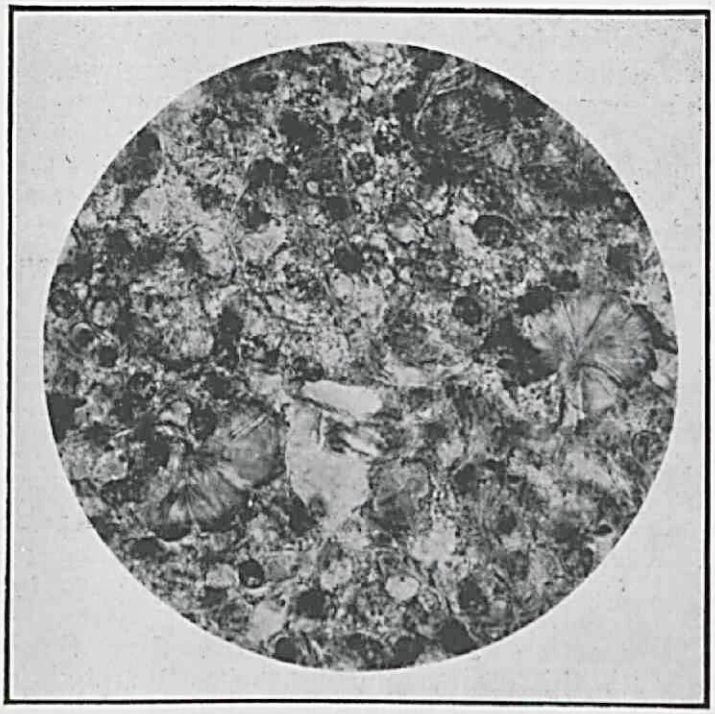


Liver. ×110.

Lesions around Central Veins.

SHEEP No. 16027: SPEC. No. 7045.

FIG. 3.

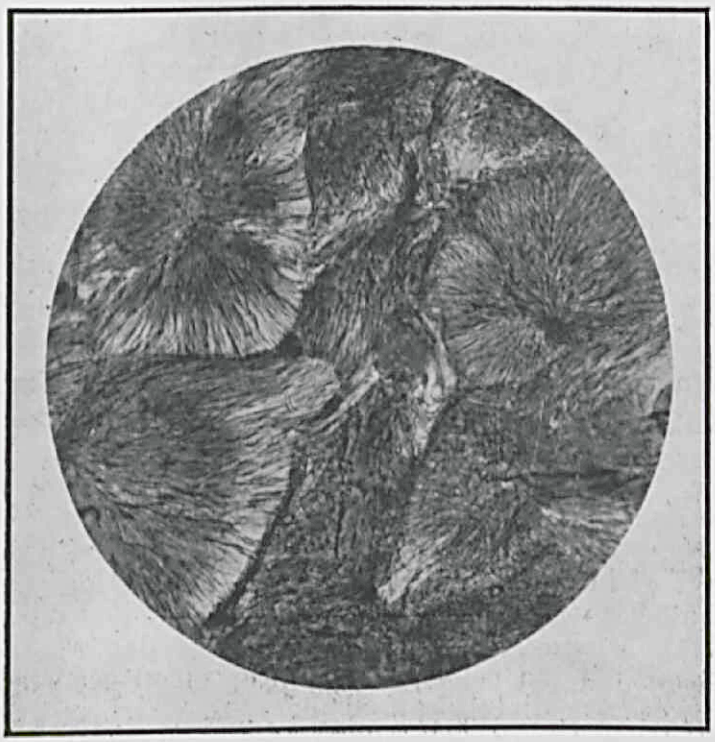


Liver. ×450.

Crystals in Liver Cells.

SPEC. No. 11038.

FIG. 4.



×225.

Fat Necrosis in Sheep.

those situated around the central veins. Here the cells contained large vacuoles, which did not stain at all, but the peripheries of the cells were well defined as slender pink staining outlines. Many cells were devoid of a nucleus and in others the nucleus was situated against the periphery of the cytoplasm, although cells having a more centrally situated nucleus were also present. Towards the periphery of the lobule, i.e. around Glisson's capsule, the cells were intact and normal in appearance. The extent to which the cells with these vacuoles occurred, varied. The greater proportion of some lobules were so affected, with the result that there remained only a narrow peripheral portion of the lobule, with more or less normally staining liver cells.

This vacuolated appearance of the cells was also seen to be present in van Gieson and Giemsa stained sections, but with Sudan III the contents of these cells stained in most cases a faded orange colour. A large number of cells was distended with fine radiating crystals (see Fig. 3) somewhat like those seen in fat necrosis (see Fig. 4). These crystals also stained a faded orange colour with Sudan III. It was only here and there that cells containing true fat were encountered. The presence of a very little true fat was confirmed when sections stained with Nile Blue Sulphate were examined. With this stain, some of the cells around the central veins stained blue and others remained unstained. The crystals previously referred to were for the most part unstained, although some did stain a light blue. According to Mallory and Wright (1922), such colour reactions are obtained with soaps, fatty acids and lipochromes. The interpretation of these lesions will be fully discussed elsewhere. No demonstrable iron containing pigment was present in the liver.

These peculiarly staining crystals were found in epithelial cells of nearly all the organs including the lung, thyroid, intestine and adrenals. In the case of the latter organs only the cells containing these crystals stained with Sudan III. No crystals were, however, demonstrable in the myocardium.

Haematology and Pathology of Sheep No. 11896.

Table 2 and graph 2 contain the haematological records of Sheep No. 11896. Infection was commenced on 16.11,25 at the rate of 200 larvae every second day until 600 larvae had been given. Thereafter 5,000 larvae were given every second or third day until approximately 50,600 larvae had been given. The last 5,000 larvae were given on 23.12.25.

Haematology of Sheep No. 11896.

Changes in the red cells.—Very soon after infection the number of red cells begins to decrease. This is also reflected as a decrease in the percentage of haemoglobin and the red precipitate. By 20.11.25 600 larvae had been administered (200 on 16/11, 200 on 18/11 and 200 on 20/11). During the following 5 days a remarkable drop of more than 3 million red cells per c.mm. of blood occurred and the question which immediately arises here is whether this marked decrease of the number of red cells is or is not entirely due to the action of the parasites. This is very difficult to prove, and one

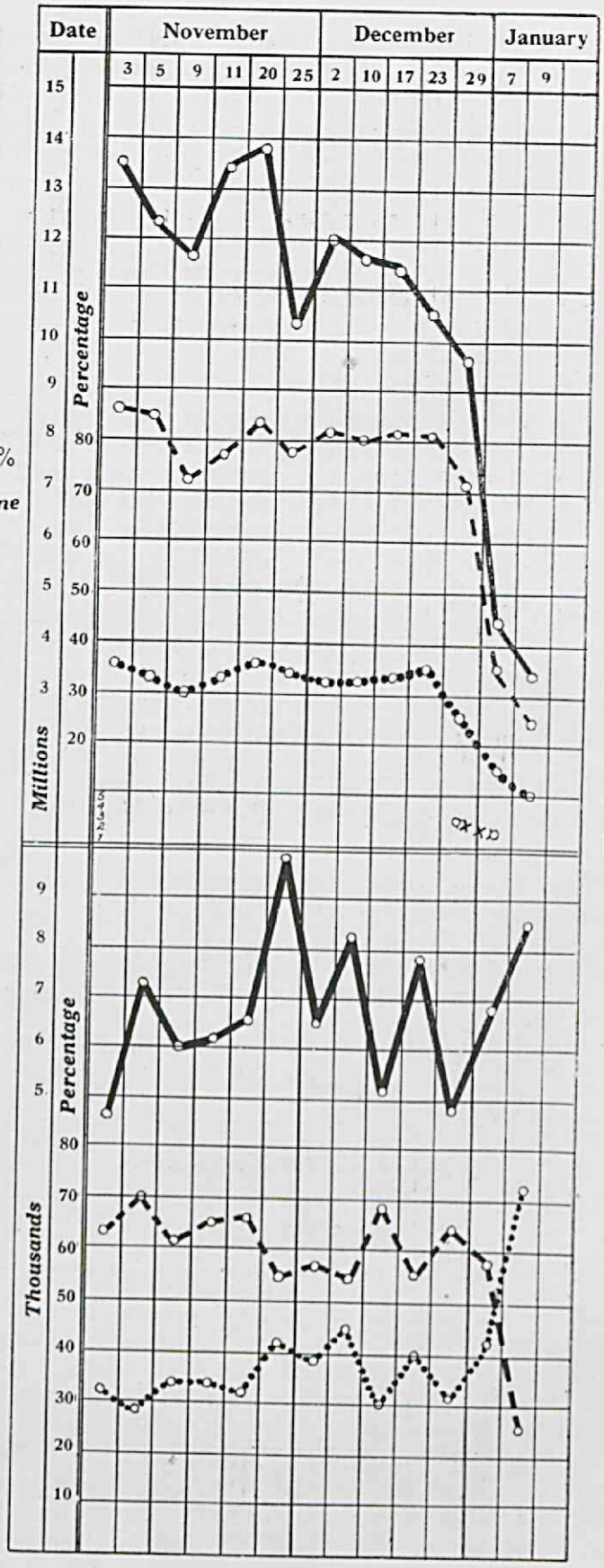
TABLE 2.

SHEEP No. 11896.

Method of treatment: Infection commenced 16.11.25 *with* Haemonchus contortus *and continued every second or third day until* 50,600 *larvae had been given. Infection completed by* 23.12.25.

Date.	Source.	R.C.	R.P.	Hglb. %	Serum.	W.C.	L.	M.	N.	E.	B.	V.C.I.	C.I.	V.I.	Remarks.
3/11/25	Jugular	13·5	36	86	Clear	4,600	63	5	31	1	0	1·00	0·98	0·99	Red cells, normal.
5/11/25	,,	12·3	34	84	,,	7,200	70	0	29	1	0	1·03	1·06	1·03	Red cells, normal.
9/11/25	,,	11·7	30	72	,,	6,000	61	3	34	2	0	1·00	0·96	0·95	Red cells, normal.
11/11/25	,,	13·4	33	78	,,	6,100	65	0	34	1	0	0·99	0·91	0·91	Red cells, normal.
20/11/25	,,	13·9	36	83	,,	6,500	66	1	32	1	0	0·96	0·93	0·96	Red cells, norma.
25/11/25	,,	10·3	34	78	,,	9,800	54	1	42	2	1	0·96	1·18	1·22	Red cells, normal.
2/12/25	,,	12·0	33	81	,,	6,400	57	2	39	1	1	1·03	1·05	1·02	Red cells, normal
10/12/25	,,	11·7	33	80	,,	8,200	55	0	45	0	0	1·05	1·10	1·04	Red cells, normal.
17/12/25	,,	11·4	33	81	,,	5,100	69	0	30	1	0	1·03	1·11	1·04	Red cells, normal.
23/12/25	,,	10·5	35	81	,,	7,200	56	2	40	2	0	0·97	1·20	1·23	Red cells, normal.
29/12/25	,,	9·6	26	70	,,	4,800	64	2	31	3	0	1·13	1·13	1·00	Red cells, normal.
7/1/26	,,	4·4	15	34	,,	6,200	58	0	42	0	0	0·95	1·20	1·26	Anysocytosis. Suggestion of polychromasia occ. Jolly bodies.
9/1/26	,,	3·3	10	23	,,	8,400	26	2	72	0	0	0·96	1·08	1·12	Anysocytosis. Suggestion of polychromasia occ. Jolly bodies.

Although infection was commenced on 16/11/25, the first seven determinations, i.e., up tc and including 2/12/25, are regarded as normal.
For abbreviations see Table I.
For method of calculating colour indices, see Table IV.



GRAPH 2.

SHEEP No. 11896.

Infected *Haemonchus contortus*
(50,600 larvae).

Killed by pure infection of *Haemonchus contortus* (Wireworm).

hesitates to draw that conclusion, particularly in view of the fact that infection was commenced only 9 days previously. According to Veglia (1915) the larvae that were administered on 16.11.25 would be on 25.11.25, just on the point of undergoing their 4th ecdysis before entering on the adult stage. In the same paper Veglia describes blood coagula in the mucous membrane of the abomasum, 3 days after infection. In the first parasitic stage of *Haemonchus* larvae, the mouth parts are not armed with a lancet (which only develops during the 2nd parasitic stage and apparently only comes into use in the adult stage), but the larvae are already able to produce in the 2nd parasitic stage, i.e. the 4th stage larvae the blood coagula described by Veglia. It seems, therefore possible, that these young forms can produce changes in the blood, but it is doubtful, if this is likely to occur to the extent of decreasing the red counts by 3 million per c.mm. of blood in 5 days (from 20.11.25-25.11.25). That the effects produced by them must be regarded as a definite contributory cause of this is without question, but a number of other factors may also be partly responsible for this big decrease in the red counts. If the blood volume had become increased such low counts might have been obtained. On the other hand, the blood might have been unduly concentrated on 11.11.25 and on 20.11.25. This could have occurred under certain abnormally dry conditions and high temperatures of the atmosphere, or if the animal incidentally had a diarrhoea or polyuria. No observations to obtain information concerning these several factors were made at the time. If there was overconcentration of the blood, the true counts with a normal blood volume may really have been somewhere in the neighbourhood of 12 million cells (instead of 13.9 million, as actually recorded on 20.11.25). This decrease would then have been correspondingly less.

From 2.12.25 there is a continuous and progressive decrease in the number of red cells every week. During the second week after the administration of the last dose of 5,000 larvae, there is practically a collapse of the red count, and this decrease by more than 5 million red cells per c.mm. of blood during 9 days time, must be regarded as phenomenal. It does seem rather paradoxical that the anaemia develops progressively more rapidly when the animal is no longer being infected. It may of course be that just about this time the limits of compensation were being reached, and that, if infection had been continued, a similar or even greater collapse would have occurred. Did this not occur with continuous administration of infective larvae, then it would appear that the method of infection employed was wrong. Instead of giving large doses at frequent intervals, a single large dose, or smaller doses at greater intervals, should perhaps have been given. When large doses are given at short intervals disturbances in the abomasum may be brought about, as a result of which a large proportion of the infection may become lost. This may actually be responsible for the so-called recovery cases.

It will be observed that the progressive decrease in the number of red cells which occurred from 2.12.25 to 23.12.25, is not reflected in the haemoglobin and percentage volume curves. The haemoglobin remains more or less stationary, but the precipitate is even somewhat increased on 23.12.25. If one could at this time have obtained reliable data of the true average size of the red cells, a satisfactory

explanation of these results would probably have been obtained. Measurements that were made indicate that there was an increase in the size of the cells, but it is realized, as previously stated, that too few cells were measured in order that reliable conclusions could be drawn in this connection. It is nevertheless of interest to present the figures that were obtained. These comprise measurements that were made during the pre-infection period, and at various intervals during the course of the disease, 15 cells being measured in various fields in each case. The results are tabulated below:—

Sheep No.	*Date.*	*Minimum.*	*Maximum.*	*Average.*
11896	9.11.25	4.1μ	5.0μ	4.3μ
,,	23.12.25	3.8μ	4.8μ	4.5μ
,,	7.1.26	4.0μ	5.2μ	4.9μ
,,	9.1.26	3.9μ	5.4μ	4.6μ

Changes in colour and volume indices.—On referring to Table 2, it will be seen that there is an increase in the volume index and in the colour index, but there is a decrease in the colour volume index. These changes in the indices can be caused by an increase in the size of the red cells.

On 7.1.26 and 9.1.26 morphological changes, such as anisocytosis, a suggestion of polychromania and occasional Jolly bodies were recorded, but no other evidence of anaemia was found on microscopic examination.

Changes in the Leucocytes.—A leucocytosis was present on 25.11.25, i.e. about 9 days after infection was commenced, and a neutrophile leucocytosis was recorded towards the end of the disease.

Pathology of Sheep No. 11896.

(1) *Macroscopic Examination.*—This merino ewe lamb died on 11.1.26. In presenting the results of the post-mortem examination, it is not considered necessary to give a detailed description of all the organs examined. Thus reference need only be made to organs in which changes were recognized.

The blood was coagulated. There was marked anaemia with paleness of all the organs and of the visible and other mucous membranes. There were degenerative changes in the heart and the liver and slight oedema of the lungs. The abomasal contents were of a yellowish chocolate colour. Wireworms were exceedingly numerous. A few nodules of *Oesophagostomum columbianum* were present in the small intestine, but there were no adult nodular worms. The spleen showed slight atrophy, measuring 10 by 6.5 by 1.5 cm. No other parasites were present.

(2) *Anatomical Pathological Diagnosis.*—This was similar to that of sheep 16027, except that no parasites other than *Haemonchus contortus* were recorded.

(3) *Aetiological Diagnosis.*—Anaemia due to a pure infection of *Haemonchus contortus.*

(4) *Microscopic Examination.*—The lesions in all the organs were similar to those described for Sheep No. 16027, except in the case of the liver. In this organ the orderly arrangement of the cells around

the central veins was disturbed. The nuclei of these cells were pycnotic, and in some cases absent altogether. The cytoplasm of the cells stained more intensively with haemalumeosin than that of normal cells. These changes in the cytoplasm and the nuclei of the cells immediately around the central veins, were interpreted as early stages of necrosis. Only occasional cells some of which contained crystals similar to those previously described stained with Sudan III. The staining reactions were otherwise similar to those described for Sheep No. 16027.

Haematology and Pathology of Sheep No. 11921.

Table 3 and graph 3 contain the haematological records of Sheep No. 11921. Infection with *Haemonchus contortus* larvae was commenced on 16.11.25, and continued every 2nd or 3rd day until 20,600 larvae had been administered. In the beginning each dose consisted of 200 larvae. The last of 3 such doses was given on 20.11.25. From 2.12.25 each dose consisted of 2,000 larvae. On 23.12.25 infection was completed.

Haematology of Sheep No. 11921.

Changes in the red cells.—During the pre-infection period, there is a certain amount of what is interpreted as normal variations in the red counts and other determinations that were made. During the infection period, there is even greater fluctuation in the counts, etc., but during this time there is a tendency towards an increase in the red counts, accompanied by a corresponding increase in the amount of haemoglobin and in the percentage volume of red cells. One is very much tempted to explain this as being due to increased activity on the part of the blood-forming organs, as a direct result of the action of the parasites. No demonstrable evidence of such activity could, however, be found when making careful microscopic examinations of stained smears during this time.

It is again somewhat significant that, within 14 days after infection was completed, the progressive decrease in the number of red cells commenced, and again the remarkable decrease of 5 million red cells per c.mm. of blood was registered within a period of 8 days. The percentage of haemoglobin is the lowest, and the percentage volume of red cells least, on the 3rd and 5th of February, 1926. On these dates, however, there is a slight increase in the red counts. There are quite a number of factors that may be responsible for this: (1) Error in counting; this is possible, and it is generally admitted that one of the most frequent sources of error in haematological work is in connection with the counting of the red cells. (2) Poikilocytosis and anisocytosis was of an extreme degree on these dates; microcytes were very numerous; macrocytes were less frequent. The anisocytosis is well shown in Fig. 6 (from smears made on 21.1.26), and should be compared with the normal cells of more or less uniform size, as seen in Fig. 5 (from smears made on 3.12.25). Fig. 6 is a microphotograph of cells from smears made on 21.1.26, when the red counts fell from 10.3 million of the previous week to 5.5 million per c.mm. of blood. The values obtained when measuring 15 cells from smears made on 3.12.25 are: 34.5, 33.5, 33.5, 31.0, 32.0, 34.5, 33.5, 31.5, 34.0, 31.5, 32.5, 36.0, 31.0, 32.5, 33.0, when the " Okular Schraubenmikrometer," having a 6 comp. ocular, with a 2 mm. apochromatic objective

TABLE 3.

SHEEP No. 11921.

Method of treatment: Infection with Haemonchus contortus *commenced on* 16.11.25 *and continued every second or third day until* 20,600 *larvae had been given.*

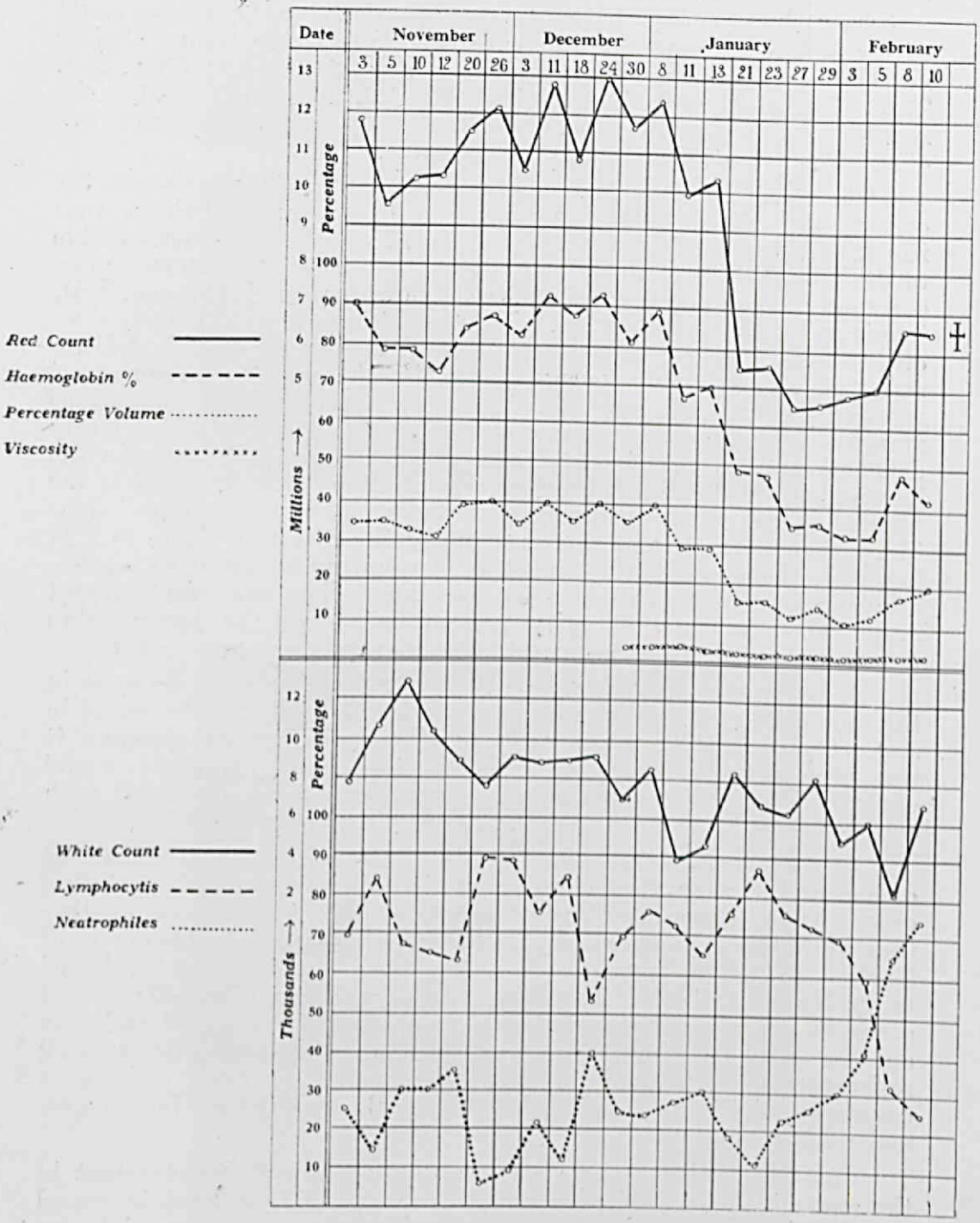
Date.	Source.	R.C.	R.P.	Hglb. %	Viscosity.	W.C.	L.	M.	N.	E.	B.	V.C.I.	C.I.	V.I.	Remarks.
3/11/25	Jugular	11·8	35	90	—	7,800	69	5	26	0	0	1·11	1·01	—	—
5/11/25	,,	9·6	35	78	—	10,500	84	0	14	1	1	0·96	0·92	—	—
10/11/25	,,	10·2	33	78	—	12,700	67	3	30	0	0	1·02	0·98	—	—
12/11/25	,,	10·3	31	72	—	10,300	65	4	30	1	0	1·01	1·08	—	—
20/11/25	,,	11·6	39	84	—	9,000	63	1	36	0	0	0·93	0·96	—	—
26/11/25	,,	12·1	40	87	—	7,800	89	5	6	0	0	0·90	0·95	—	—
3/12/25	,,	10·5	34	83	—	9,100	88	3	9	0	0	1·05	1·04	—	—
11/12/25	,,	12·8	40	92	—	8,900	75	3	21	0	1	0·99	0·96	—	Cells normal.
18/12/25	,,	10·8	35	87	—	9,100	84	2	12	1	1	1·08	1·07	—	Cells normal.
24/12/25	,,	13·0	40	93	—	9,300	53	6	40	1	0	1·01	0·95	—	Cells norma.
30/12/25	,,	11·7	35	81	3·5	7,000	69	4	24	3	0	1·00	0·92	—	Cells normal.
8/1/26	,,	12·4	40	89	3·6	8,300	76	1	23	0	0	0·91	0·95	—	Cells normal.
11/1/26	,,	9·9	29	67	3·9	3,900	72	1	27	0	0	1·00	0·90	—	Cells normal.
13/1/26	,,	10·3	29	70	3·0	4,500	66	3	30	0	1	1·05	0·90	—	Cells normal.
21/1/26	,,	5·5	16	48	2·3	8,400	76	4	18	1	1	1·29	1·16	—	Anisocytosis well marked.
23/1/26	,,	5·6	16	47	2·2	6,900	87	2	11	0	0	1·27	1·11	—	Anisocytosis well marked.
27/1/26	,,	4·5	12	35	2·0	6,300	76	0	23	1	0	1·26	1·03	—	Anisocytosis and poikilocytosis.
29/1/26	,,	4·6	14	36	2·0	8,200	72	1	26	0	1	1·11	1·04	—	Anisocytosis and poikilocytosis.
3/2/26	,,	4·8	11	33	1·6	5,000	69	1	30	0	0	1·29	0·91	—	Anisocytosis and poikilocytosis, occasional Jolly bodies.
5/2/26	,,	5·0	12	33	1·6	6,000	59	0	41	0	0	1·19	0·87	—	Anisocytosis and poikilocytosis.
8/2/26	,,	6·6	17	48	1·9	[illegible],000	32	3	65	0	0	1·05	0·82	—	Anisocytosis and poikilocytosis.
10/2/26	,,	6·5	20	42	1·9	7,000	25	1	74	0	0	0·91	0·86	—	Anisocytosis and poikilocytosis.

Viscosity from 30/12/25–10/2/26, was determined at the following temperatures: 30, 31, 31, 30, 28, 26, 30, 28, 28, 30, 30, 30.
An average of the first five determinations is regarded as normal for this sheep for calculating the colour and volume colour indices.
For abbreviations, see Table 1.
For method of calculating colour indices, see Table 4.

GRAPH 3.

SHEEP No 11921.

Infected Haemonchus contortus
(20600 larvae)



is used, the above results must be multiplied by .14, in order to convert them into values of microns. All the meaurable cells in a field were measured as they were encountered. The values are fairly uniform. The smallest cell measured 4.3μ, the largest 5μ and the average was 4.6μ.

On 21.1.26, when without measuring any cells anisocytosis could with ease be diagnosed microscopically from stained smears, similar measurements were made. The values are: 28.0, 41.5, 30.5, 28.5, 27.0, 37.0, 31.0, 28.0, 40.0, 30.0, 40.0, 41.0, 33.5, 31.0, 36.0. The smallest cell measured 3.8μ, the largest 6.8μ and the average was 4.5μ. The variation in size is considerable.

The changes in the shape of the cells (poikilocytosis) towards the end of the disease were of a most varied nature. This is well illustrated in the microphotographs Figs. 7 and 8. It is not possible to describe all the various shapes seen, but those poikilocytes more commonly encounteerd were of the shape of a pear, half-moon, bell, tennis racket, or a comma, etc. Many cells stained less intensively than others and in numerous cells are central clear areas which did not stain at all, the so-called " glasskörper " and " pessarformen," referred to by Schilling (1922). If one can draw any conclusions from the morphological study of stained smears, these cells are clearly deficient in haemoglobin, and this would explain a decrease in the haemoglobin content when no corresponding decrease occurred in the number of red cells. In other words one would expect a lower colour index than normal, and this is actually the case (see Table 3). If however, the possibility of an error in counting be admitted, such a minus colour index will also be calculated when the counts reveal more cells than are actually present but when the haemoglobin determinations have been correct.

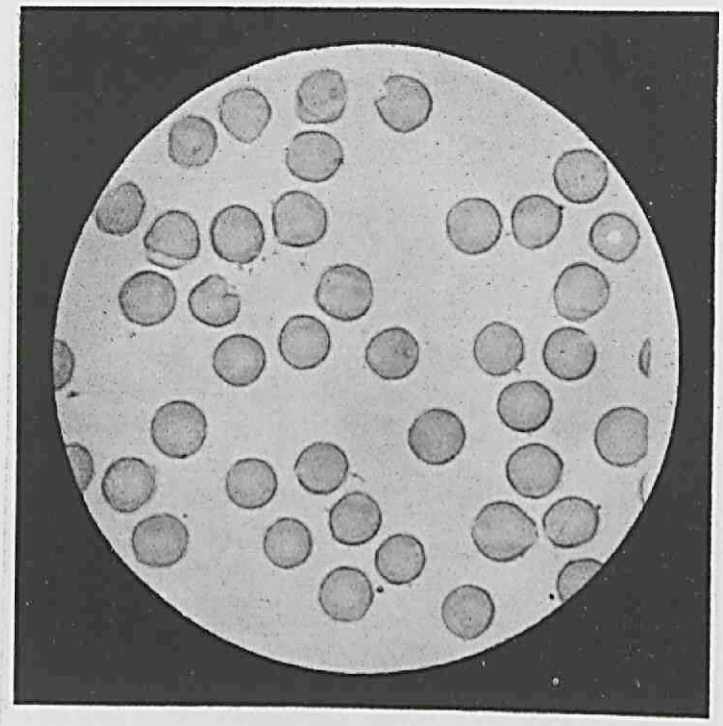
A decrease in the precipitate without a corresponding decrease in the number of red cells can occur when the red cells are decreased in size. On account of the extreme poikilocytosis that was present, it was useless to measure the cells, as the figures obtained would obviously have been unreliable. It can nevertheless be stated with confidence that a large number of microcytes were present.

In these smears there were not infrequently very small cells, but owing to the very marked anisocytosis and poikilocytosis it is not quite clear if these are also to be regarded as microcytes or whether they are perhaps haemoglobin fragments, the so-called schistocytes of Ehrlich, quoted by Krumbhaar (1928), or the " schizocyten " referred to by Naegeli (1912). According to this author, these cells do not occur in the bonemarrow, but only in the peripheral blood and may develop with disturbances in isotonicity, chiefly when cells of small resistance are produced in the bonemarrow. If such cells were counted as individual red cells, this would tend to produce abnormally high red counts.

Just before death there was an apparent slight improvement in the anaemia. Morphologically there was no evidence that increased regenerative changes in the haemapoetic organs was responsible for this. This was probably due to an increased concentration of the blood as a result of diarrhoea, serous atrophy of the fat, hydropericardium and disturbances in the appetite and the amount of water

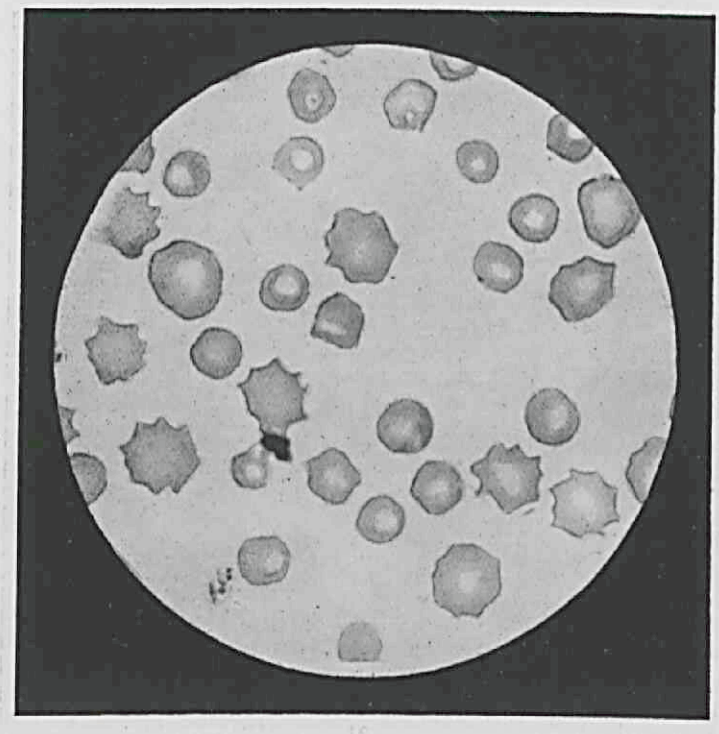
SHEEP No. 11921.

FIG. 5.



3.12.25. ×1300.

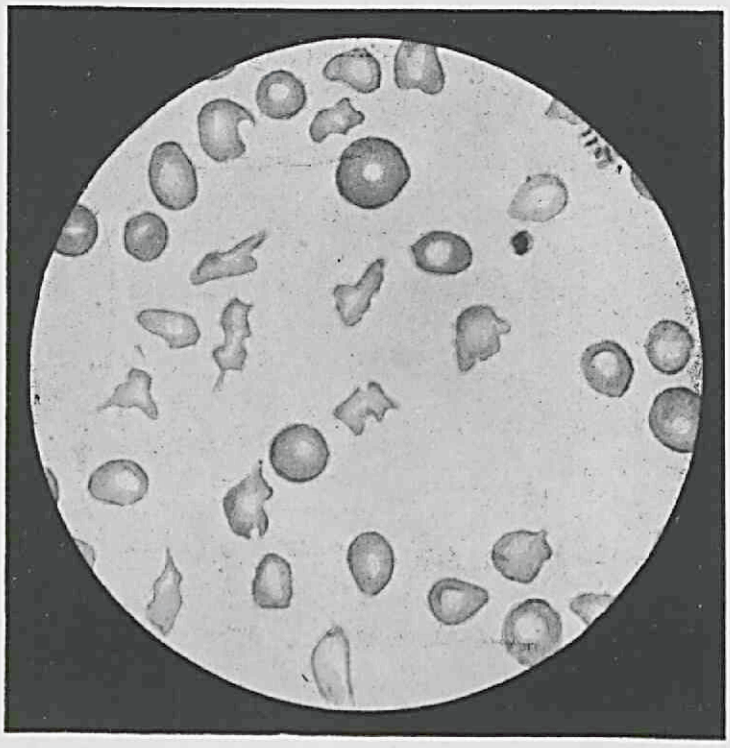
FIG. 6.
Normal.



21.1.26. ×1300

Anisocytosis.

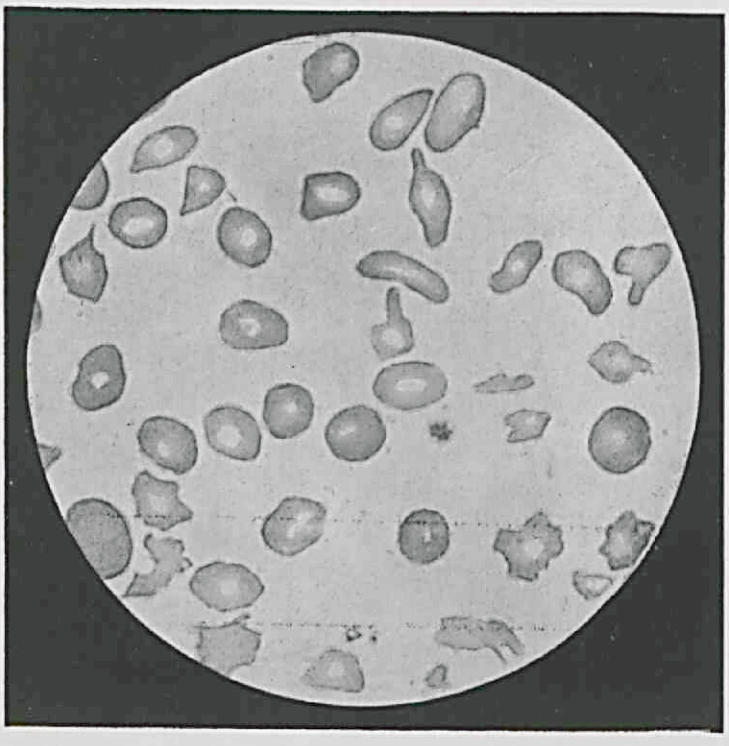
SHEEP No. 11921.
FIG. 7.



3.12.26. ×1300.

Anisocytosis and Poikilocytosis.

FIG. 8.

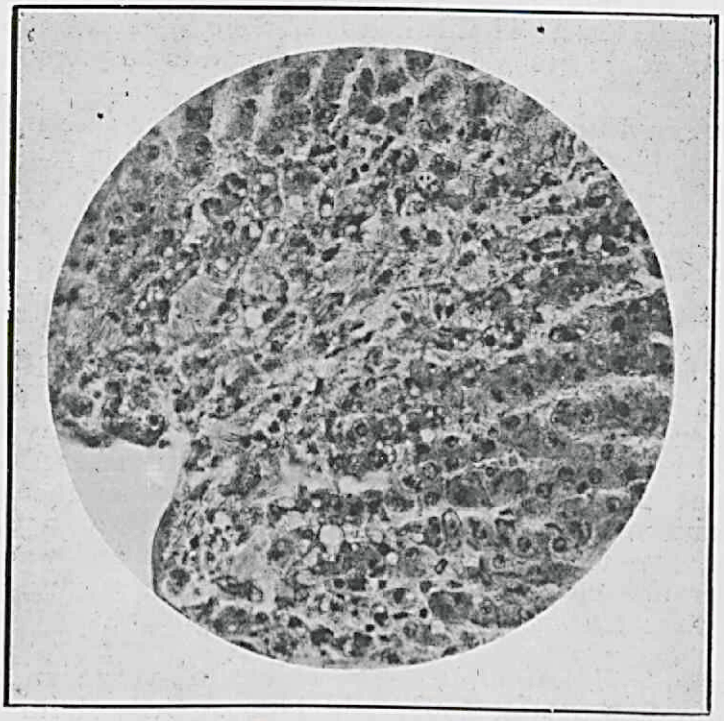


8.2.26. ×1300.

Anisocytosis and Poikilocytosis.

SHEEP No. 11921 : SPEC. No. 5748.

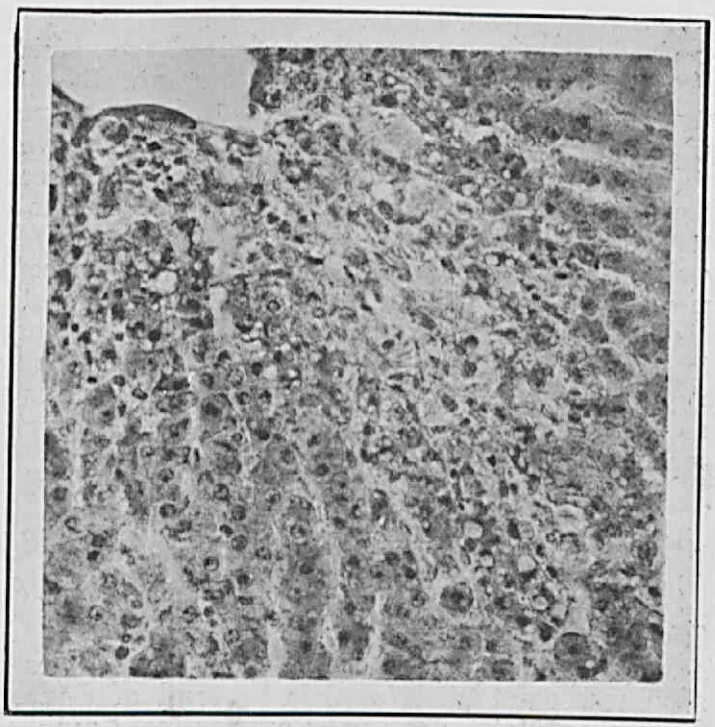
FIG. 9.



Liver. ×225.

Lesions around central veins with crystals in some of the cells.

FIG. 10.



Liver. ×225.

Lesions around central veins with crystals in some of the cells.

ingested. It is a pity that absolute blood volume determinations were not made at this time. If this had been done, a decrease in the total blood volume would probably have been found to be present.

Changes in Viscosity.—There is a decided decrease in the viscosity on 21.1.26, when a very marked decrease in the number of red cells occurred, but slight changes in the number of red cells were not reflected in the viscosity curve.

Pathology of Sheep No. 11921.

Macroscopic Examination.—This sheep died on 11.2.26. The post-mortem findings were somewhat different to those recorded in previous cases, in that the condition of this ewe lamb was poor, there was marked serous atrophy of the fat and a well marked hydropericardium was present. Except for these changes, the naked eye appearances were like those previously recorded, such as pale mucous membranes, absence of icterus, clear urine, pale and watery blood, and degenerative changes in the parenchymatous organs. No parasites other than *Haemonchus contortus* were present.

Microscopic Examination.—The lesions are very similar to those described for Sheep Nos. 16027 and 11896. Cells staining a faded orange colour with Sudan III were found in all the organs except the heart. As the lesions of the liver resemble those in Sheep Nos. 16027 and 11896 combined, it is necessary to give a little more detailed description of the microscopic appearance. There are in the haemolum-eosin stained sections numerous cells with vacuoles around the central veins. These cells stained a faded orange colour with Sudan III and the crystals previously described are present in many of them. Scattered almost irregularly throughout the substance of the liver are fairly large areas, up to 113μ in cross-measurement. In these are cells containing globules staining a lemon yellow colour with Sudan III. No crystals are present in the central cells of these lesions, but cells staining the characteristic faded orange colour and containing crystals are present towards the periphery. The lesions are usually situated in the immediate vicinity of the central veins, but sometimes they occupy variable positions in the substance of the lobule. It is not quite clear whether these are to be regarded as an intermediate or a further advanced stage in the development of the lesions. In quite a number of cases the nuclei of the cells around the central veins are pycnotic and these changes are interpreted as early stages of necrobiosis.

Haematology and Pathology of Sheep Nos. 22387 *and* 22388.

Tables 4 and 5 contain the haemotological records of Sheep No. 22387 and Sheep No. 22388 respectively. Infection with *Haemonchus contortus* larvae was commenced on 5.6.29. Small doses were given until infection was completed towards the end of June, 1929. In these two sheep exact details as to the approximate number of larvae administered are not available.

Table 4.

SHEEP No. 22387.

Method of treatment: Infected with Haemonchus contortus *larvae from* 5.6.29.

Date.	Source.	R.C.	R.P.	Hglb. %	Serum.	W.C.	L.	M.	N.	E.	B.	V.C.I.	C.I.	V.I.	Remarks.
29/4/29	Jugular	11·3	36	84	Clear	15,700	24	5	71	0	0	0·98	0·93	0·95	Cells normal in appearance.
10/5/29	,,	9·9	34	82	,,	4,700	45	3	52	0	0	1·01	1·04	1·03	Cells normal in appearance.
5/6/29	,,	9·4	32	79	,,	5,100	56	3	41	0	0	1·03	1·05	1·02	Cells normal in appearance.
10/6/29	,,	11·0	32	84	,,	6,500	52	1	45	2	0	1·10	0·96	0·87	Occasional very large cells.
17/6/29	,,	9·8	31	75	,,	5,600	59	2	39	0	0	1·01	0·96	1·00	Cells normal in appearance.
21/6/29	,,	7·9	25	65	,,	4,500	57	1	41	1	0	1·09	1·03	0·95	Cells normal in appearance.
26/6/29	,,	5·6	19	48	,,	5,500	54	1	41	3	1	1·06	1·11	1·01	No evidence of anaemia.
1/7/29	,,	4·0	15	36	,,	5,200	59	0	40	0	1	1·00	1·13	1·12	Anisocytosis. Slight polychromasia and occasional punctate basophilia.
6/7/29	,	2·8	15	25	,,	5,200	54	2	43	1	0	0·69	1·12	1·60	Anisocytosis; polychromasia and punctate basophilia very marked.
8/7/29	,,	2·6	19	24	,,	8,800	44	4	50	1	1	0·53	1·16	2·14	Anisocytosis, polychromasia and punctate basophilia very marked. DIED, 10/7/29.

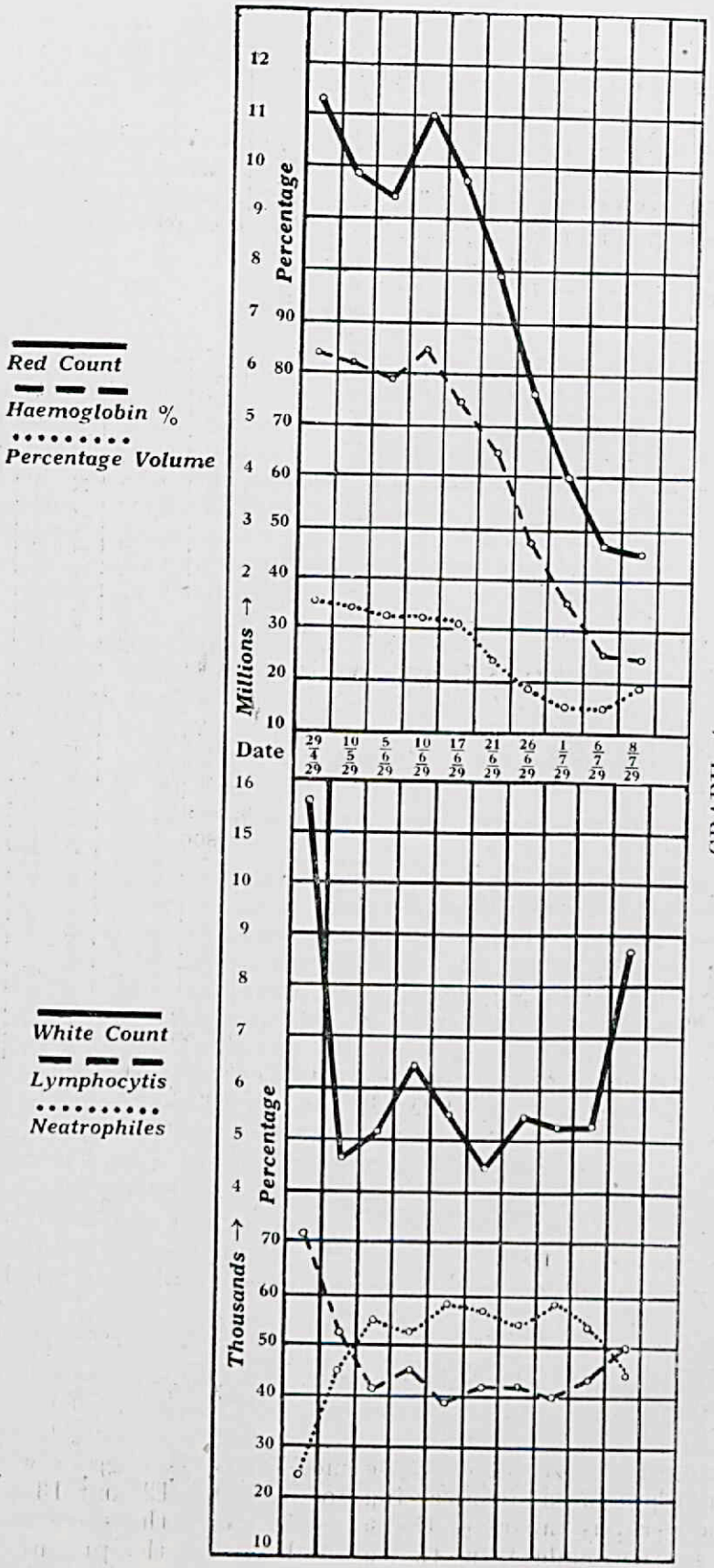
To determine the colour volume index calculate $\frac{100 \times \text{actual haemoglobin}}{\text{average normal haemoglobin (81)}} \times \frac{\text{average normal ppt. (34)}}{\text{actual ppt.} \times 100}$

To determine the colour index calculate $\frac{100 \times \text{actual haemoglobin}}{\text{average normal haemoglobin (81)}} \times \frac{\text{average normal R.C. (10·2)}}{\text{actual R.C.} \times 100}$

To determine the volume index calculate $\frac{100 \times \text{actual precipitate}}{\text{average normal precipitate (34)}} \times \frac{\text{average normal R.C. (10·2)}}{\text{actual red count} \times 100}$

Determinations on 29/4/29, 10/5/29, and 5/6/29, are regarded as normal for calculating the Volume and Colour indices.

For abbreviations, see Table I.



GRAPH 4.

SHEEP No. 22387.

Infected *Haemonchus contortus*
(20,600 larvae).

Haematology of Sheep No. 22387.

Changes in the Red Cells.—Within a week after infection there is a slight rise in the red counts and in the haemoglobin content. A corresponding increase in the percentage volume of red cells does not occur. This is possibly merely the normal variation that occurred in the number of red cells and is probably not due to increased activity on the part of the blood-forming organs. Thereafter a progressive decrease in the number of red cells and the total haemoglobin content takes place until 8.7.29, i.e. two days before death, when the red cells were 2.6 millions and the haemoglobin 24 per cent. A corresponding and progressive decrease also occurs in the percentage volume of red cells until 1.7.29; after this, instead of further decreasing, as might perhaps be expected, the precipitate remains constant until 6.7.29, and from this date until 8.7.29, i.e. in two days time, the precipitate actually increases from 15 per cent. on the two previous occasions to 19 per cent. The effects of these changes on the colour, the volume and the volume colour (saturation) indices, will be fully discussed subsequently.

Morphological Changes in the Red Cells.—Smears stained according to Pappenheim's May Grünwald-Giemsa method, were carefully examined microscopically. On 1.7.29 the first evidence of anaemia was observed. This consisted in changes such as anisocytosis, slight polychromasia and occasional punctate basophilia. Previous to this occasional very large red cells were observed, but to such no pathological significance was attributed. During the further course of the disease, these changes became progressively worse and, towards the end of the disease (6.7.29), the anisocytosis, polychromasia and punctate basophilia were very marked. In addiiton, poikilocytosis and not infrequent Jolly bodies were present, but the poikilocytosis was not of the extreme degree described for Sheep No. 11921.

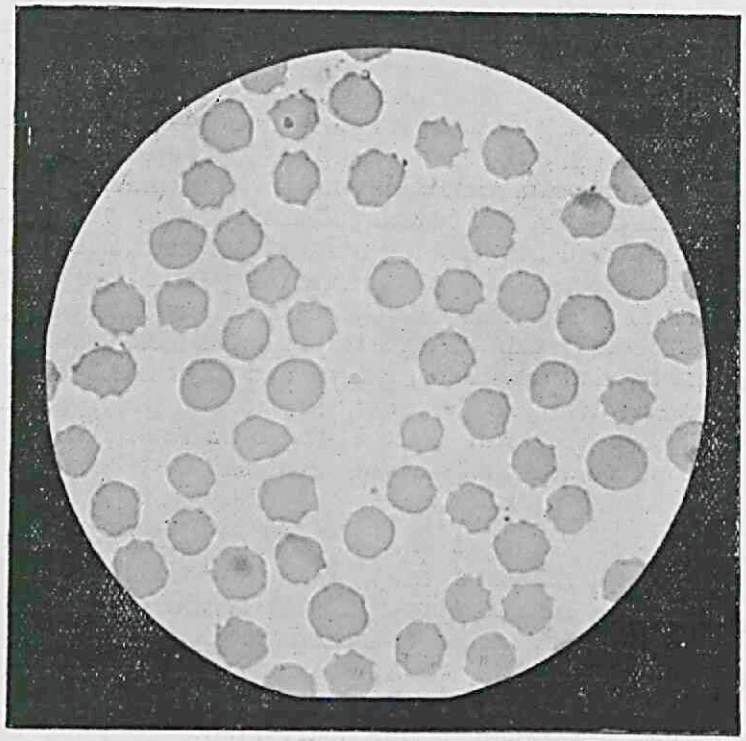
Many of the cells in the smears made during the later stages of the disease stained less intensely than normal, and in quite a large number there were central clear areas which did not stain at all. These morphological changes indicate that the cells are deficient in haemoglobin. On 8.7.29, two days before the animal died, similar changes were present, but these were, if anything, of a more severe degree.

Changes in Size of the Red Cells.—Measurements were made (20 cells) during the pre-infection period (29.4.29), and again (20 cells) when the disease was at its height (6.7.29), and a third time (40 cells) two days before the animal died (8.7.29). These results are tabulated below:—

Date.	*Smallest Cell.*	*Largest Cell.*	*Average.*
29.4.29	3.3μ	5.7μ	4.2μ
6.7.29	4.1μ	7.4μ	5.4μ
8.7.29	4.3μ	8.0μ	5.5μ

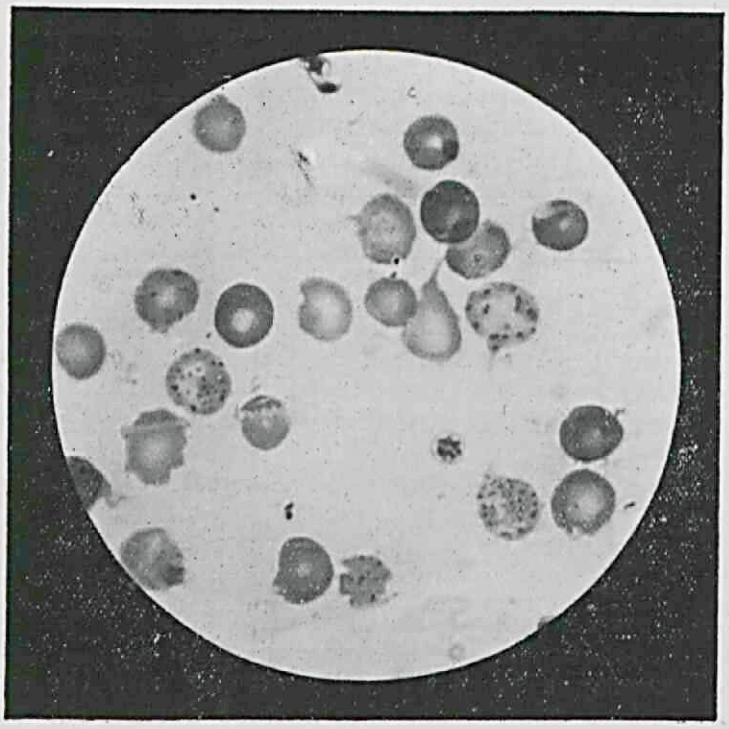
The changes in size which these measurements suggest will be much better appreciated by referring to Figs. 11, 12 and 13 which are microphotographs taken on the same day with the same magnification, of smears made from this animal during the pre-infection period (29.4.29) and later on when the anaemia was well established (6.7.29).

SHEEP No. 22387.
FATAL CASE OF HAEMONCHOSIS.
FIG. 11.



Bloodsmear made 29.4.29. ×1300.

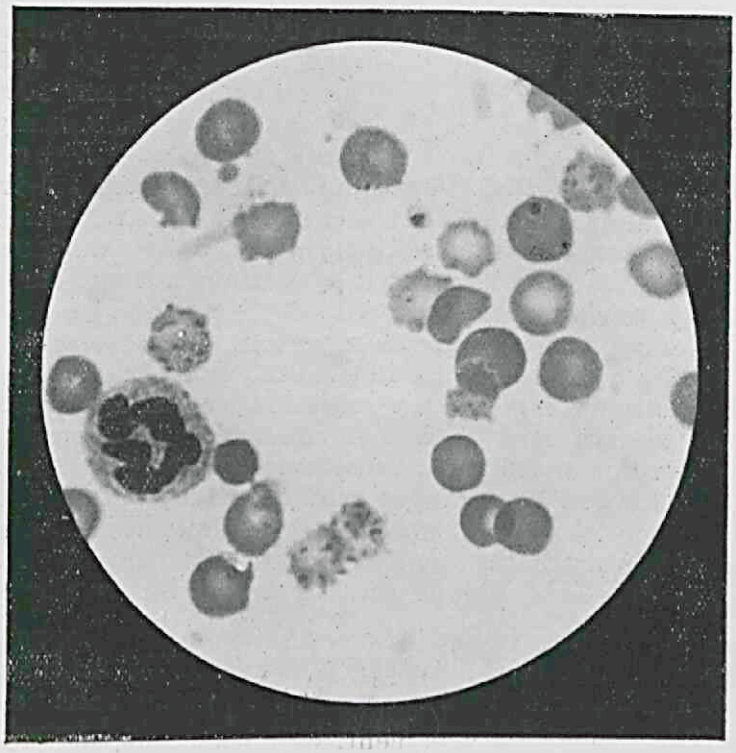
FIG. 12.



×1300.

Bloodsmear made 6.7.29, showing Anisocytosis, Punctate Basophilia and Jolly bodies.

SHEEP No. 22387.
FATAL CASE OF HAEMONCHOSIS.
FIG. 13.



×1300.
Bloodsmear made 6.7.29, showing Anisocytosis, Punctate Basophilia and Jolly bodies.

Changes in the Colour, Volume, and Volume Colour Indices.— The changes in these indices in this particular case are very interesting. In calculating the various indices in the human subject, it is usual to regard 5 million cells per c.mm. of blood as being equal to 100 per cent., the amount of haemoglobin contained in 5 million red cells per c.mm. of blood as representing 100 per cent., and the volume of packed red cells (precipitate) obtained by centrifuging a blood containing 5 million red cells per c.mm. at certain definite speeds, until such time as constant readings are obtained, as representing 100 per cent. There seems to be very little agreement as to what this last figure should actually be. Rossdale (1923) showed that anticoagulants materially affect the centrifuge readings. The values obtained by the various authors quoted by Rossdale are: Hedin (1889) 51.6, using Müller's fluid as anticoagulant and centrifuging for 5-7 minutes at a speed of 8,000 revolutions per minute; Daland (1891) 51.8, using 2.5 per cent. bichromate solution as anticoagulant and centrifuging for 3-6 minutes at a speed of 10,000 revolutions per minute, with red count of 5.07 million per c.mm.; Capps (1903) 51.5, using whole blood and centrifuging for 3 minutes at a speed of 10,000 revolutions per minute, with red counts (in males) of 5.06 million per c.mm.; Harvey (1919), average 46.5; Groen (1921), 51.4-55.1 (for males).

Rossdale (1923) himself found an average of 50, when centrifuging for 20 minutes, at a speed of 3,600 revolutions per minute. Haden (1925) used sodium oxalate at anticoagulant and centrifuged

the blood for 30 minutes at 2,500 revolutions per minute, as suggested by Hooper, Smith, Belt, and Whipple (1920). His figure is 46 for a blood containing 5 million cells. In calculating the various colour and volume indices, this author regards the average normal percentage volume of red cells at 46, which would represent 100 per cent. Whatever figure is agreed upon as a result of examining a large number of normal individuals as being the normal average for the human subject, that figure may differ considerably from the normal of any particular patient. When one is working with animals under experimentally controlled conditions, it is unnecessary to take as normal, the standards as laid down by various authors for particular species. It is much more accurate to lay down the normal for each individual during the pre-infection period. As there is a normal variation in the counts of each individual, one should make at least 5 determinations during the pre-infection period on different days. This was not always possible, and in some cases values obtained after infection was commenced were considered as normal. This was as a rule only done when no changes had occurred, which could be regarded as other than the normal variation. With this method, which can obviously be applied to the human subject on rare occasions only, more reliable colour and volume indices can be calculated.

When the colour index, the volume index (Capps), and the volume colour index (Neser 1921, Rossdale 1923) or the saturation index (Haden 1925) are calculated, when 5 million cells per c.mm. of blood are regarded as 100 per cent. and when the haemoglobin contained in such blood as well as the precipitate obtained from it under the conditions previously stated are regarded as 100 per cent., then in normal individuals these indices should always be 1, within admissable limits of technical error and within the limits of normal variation in an individual, that is, if normality for each individual is first of all established as advocated in this work. In this work it was not possible to investigate what variation, if any, may occur in sheep or in an individual sheep, dependent on changes in size and haemoglobin content of the red cells when technical errors have been eliminated by making in each case a number of check counts.

There are undoubtedly tremendous differences in the red counts of apparently normal sheep. Counts ranging between 10 and 14 million per c.mm. of blood are quite common, i.e. for well-grown lambs up to a year old. One would, however, expect that as the red counts become increased or decreased in normal sheep, the haemoglobin content and the percentage volume should show corresponding variations, so that the various colour and volume indices will remain unaltered, except where the cells may be increased or decreased in size and contain a proportionately increased or decreased amount of haemoglobin.

Blood containing a normal number of cells with microcytes predominating will give a relatively small precipitate and the volume index will be decreased, but on the other hand if macrocytes predominate blood with a normal number of cells will give a relatively large precipitate and the volume index will be increased. In normal individual sheep, such a deviation from the normal volume index (which is 1) may occur, if one uses the average obtained of a large

number of determinations on normal sheep as the normal for this species, but should not occur when the normality of the individual which is being examined has been determined and is being used.

The colour index is the relative ratio of the haemoglobin to the number of red cells; the volume index (Capps) is the relative ratio of the percentage volume of red cells (more conveniently termed the precipitate), to the number of red cells; the volume colour index (Neser and Rossdale) or the saturation index (Haden) is the relative ratio of the haemoglobin to the percentage volume of red cells. These can be simply calculated in the following way:—

1. Colour index =

$$\frac{100 \times \textit{actual haemoglobin}}{\textit{average normal haemoglobin}} \times \frac{\textit{average normal red count}}{\textit{actual red count} \times 100}$$

2. Volume index =

$$\frac{100 \times \textit{actual precipitate}}{\textit{average normal precipitate}} \times \frac{\textit{average normal red count}}{\textit{actual red count} \times 100}$$

3. Volume colour index of saturation index =

$$\frac{100 \times \textit{actual haemoglobin}}{\textit{average normal haemoglobin}} \times \frac{\textit{average normal precipitate}}{\textit{actual precipitate} \times 100}$$

Where the average normal red count, haemoglobin percentage and precipitate of an individual cannot be previously determined, the average normal for the species can be substituted.

The papers on the volume index by Capps (1903), Wroth (1907), and Larrabee (1911), quoted by Haden (1925), were unfortunately not available to me.

The term " volume colour " was used by Neser (1921) to express the ratio of the haemoglobin percentage to the percentage volume of red cells. Unfortunately Neser did not calculate the relative ratio, but made a direct calculation instead, simply by dividing the precipitate into the haemoglobin-percentage. His index in normal horses could, therefore, never be 1. Previous to this Herz (1893), quoted by Rossdale (1923) and Haden (1925), calculated this index in exactly the same way and called it the " specific haemoglobin content of the cell " (Specifischer Haemoglobin gehalt). In the case of a normal human blood having a haemoglobin of 100 per cent. and a red precipitate of 50, Herz's index would be 100/50.

Rossdale (1923) independently used the term volume colour index to express " the capacity of the cell volume for haemoglobin." Haden (1925) suggested for exactly the same thing the term saturation index. Both these latter workers give values which are expressed in terms of the normal. Consequently all these indices should be 1 in the case of normal adult individuals, and where they are either greater or less than 1, a deviation from the normal is indicated, particularly changes in the haemoglobin content of the cells and changes in the size of the cells.

On referring to Table IV, it will be seen that definite changes occurred in the colour index, the volume index, and the volume colour

index. Any changes occurring in these indices up to and including the 21.6.29 are probably due to technical errors, particularly in the counting of the red cells.

Changes in the Colour Index.—Towards the later stages of the disease there is a tendency to a slight increase in this index. Haden (1925) states that a colour index greater than 1.00 means only that the cells are larger than normal. Theoretically, a plus colour index would indicate either an increase in the haemoglobin content or a decrease in the number of red cells, if these are larger than normal; but in the latter case the larger and fewer cells must contain the same amount of haemoglobin as the greater number of smaller cells. There is, however, definite evidence, on morphological grounds, that the cells are to some extent deficient in haemoglobin; such deficiency would therefore tend to correct the tendency towards a plus colour index; in other words, one may actually be dealing with a pathological blood containing less, but larger cells than normal, and the colour index can remain 1.00, if there is a corresponding decrease in the amount of haemoglobin in the cells. There is very little doubt that this had occurred in this particular sheep (No. 22387). Notwithstanding, there is a slight increase in this index and one must conclude that the increase which occurred in the size of the cells was of such a degree, that it produced a plus colour index, even though the cells were deficient in haemoglobin.

Changes in the Volume Colour Index.—This index remained normal up to 1.7.29, but it was markedly decreased on 6.7.29 (5 days later), namely to .69, and a further decrease took place on 8.7.29 (2 days later) when it was .53. A decrease in the volume colour index can occur if there is a relative deficiency in the haemoglobin content of the cells, when the cells remain the same in size and number, or if the cells are decreased in number even though they may be increased in size. When, however, the larger but fewer cells are fully charged with haemoglobin, the volume colour index may remain unaltered. Theoretically, a decrease in the volume colour index can also occur if the precipitate is increased when the total haemoglobin remains unaltered. The precipitate can increase if there are more cells, which are at any rate not decreased in size, or if the cells normal in number are increased in size; but in both these cases the volume colour index will remain unchanged if the cells, whether they are normal or increased in size, are fully charged with haemoglobin. It is, therefore, obvious that when the volume colour index is decreased, the cells must be deficient in haemoglobin. As already stated, the presence of this deficiency was suggested by the morphological changes described and also by the changes in the colour index, although, admittedly, the plus colour index emphasizes rather more the increase in the size of the red cells.

Changes in the Volume Index.—Apart from slight changes, probably due to technical arrors, no changes occur in the volume index up to and including 26.6.29. On 10.6.29, however, there is a decided decrease in the volume index. This occurs within a week after infection was commenced. This was unfortunately not observed at the time and consequently the determinations could not be repeated

in order definitely to eliminate the possibility of a technical error during centrifuging. On 1.7.29 the volume index begins to increase and is decidedly increased on 6.7.29, namely to 1.6. It is even further increased 2 days later, i.e. on 8.7.29, when it is 2.14. This indicates very definitely that a marked increase in the size of the red cells has taken place. This was already suggested on general morphological grounds, and also by the measurements that were made, e.g., the average normal size of the red cells was 4.2μ during the pre-infection period, but towards the end of the disease (on 6.7.29 and 8.7.29) the average diameter of the red cells was 5.4μ and 5.5μ respectively. Slight differences in size, especially when uniform, may be difficult or impossible to appreciate microscopically, even by actually measuring the cells, but must nevertheless cause a well marked increase in the precipitate, with a consequent plus volume index. This is commonly encountered in early cases of pernicious anaemia in the human subject.

From a purely haemotological point of view, the outstanding features of this particular case are: marked morphological evidence of anaemia, e.g., anisocytosis, poikilocytosis, polychromasia, " glasskörper " and " pessarformen," punctate basophilia, and Jolly bodies. There is a slight increase in the colour index, a marked decrease in the volume colour or saturation index, and a marked increase in the volume index. These changes are interpreted as indicating that there is a relative deficiency in haemoglobin in the cells and that there is a marked increase in the size of the cells. Some of the macrocytes can appropriately be classified as megalocytes, but no megaloblasts, so characteristic of pernicious anaemia in the human subject, were recognized. Except for the absence of these last-named cells, the blood changes recorded in this particular animal, affected with a pure haemonchosis, correspond very closely with those described for pernicious anaemia in the human subject. Haden's (1925) average for 50 cases of pernicious anaemia is: Volume index 1.41, colour index 1.29, saturation index .92. His highest volume index is 1.77, with a saturation index of .53. This corresponds very closely with the indices of Sheep No. 22387, but the colour index in pernicious anaemia seem in general to be slightly higher.

Changes in the Leucocytes.—There is a remarkable leucocytosis with a neutrophilia on 29.4.29. This occurs during the pre-infection period and no explanation of the cause of this can be given. As occurs in other cases, there is a leucocytosis with a neutrophilia towards the end of the disease.

Pathology of Sheep No. 22387.

The animal which was a young sheep and had not yet shed any of its temporary or milk teeth, died on 10.7.29, when a post-mortem examination was made. The same general changes as described for previous cases were recorded. There was in addition a very interesting lesion which involved mainly the apex of the heart. There was thrombosis or embolism of one of the branches of a coronary artery, resulting in infarction of the myocardium in the region of the apex. A similar lesion was encountered in some of the other sheep with haemonchosis and this will be referred to again later on.

This animal must have died during the early part of the evening of 9.7.29, and was available for post-mortem examination only on the morning of 10.7.29, when a certain amount of autolysis and decomposition had unfortunately already occurred, particularly in the liver. In these circumstances it is not possible to make a definite statement in regard to specific lesions, but the outstanding features of the macroscopic and microscopic examinations are: absence of demonstrable iron pigment from all the organs, including even the spleen, which was markedly atrophied, being of small size and having a very much wrinkled capsule. There was general paleness of the visible and other mucous membranes, and of the tissues in general. There was no icterus. Microscopically the bonemarrow was found to contain the usual immature forms of granulocytes and erythrocytes, such as myeloblasts and myelocytes, etc. The presence of these cells was interpreted as indicating an actively functioning bonemarrow. In the fatty bonemarrow were seen scattered groups or islands of cells, many of which neutrophile and eosinophile myelocytes, and others were erythroblasts and normoblasts. This was interpreted as a compensatory myeloid hyperplasia involving the fatty bonemarrow. No parasites other than wireworms were found to be present.

Haematology of Sheep No. 22388.

Changes in the Red Cells.—On studying Table 5, with its corresponding graph, it will be observed that there is during the pre-infection period a peculiar decrease in the red counts and the absolute haemoglobin content of the cells, as well as in the precipitate. The cause of this is not definitely known, but may possibly be associated with the artificial conditions under which these animals were placed, when they were drafted into this experiment. As previously explained, these animals were obtained from a Karroo farm, where the conditions for parasitic life are generally unfavourable. On arrival at the Onderstepoort Laboratory, they were immediately placed in a loose box measuring 6.25 by 3 metres. This box was subdivided by 5 partitions into oblong compartments in each of which were placed 2 to 3 sheep. The sheep had a liberal supply of good food and abundant water, but obviously exercise was restricted to an absolute minimum. Neser (1923) showed that a remarkable increase in the number and in the percentage volume of red cells takes place with exercise or work in horses. No observations of this nature have so far been attempted on sheep under experimentally controlled conditions, but there is just a possibility that this may be the explanation of the marked decrease in the number of red cells that occurred in this sheep. Arguing by analogy, this should not be the case, as Neser has shown that in horses the reverse process, that is, decrease of the red cells with idleness and restricted exercise is slow. This may, however, not be the case with sheep. It is noteworthy that a similar decrease in the red cells of Sheep No. 22387 (see Table 4 and Graph 4) was recorded. This animal was bought from the same Karroo farm, arrived here at the same time and was further treated in exactly the same way as was Sheep No. 22388. This suggests that exercise will have the same interesting effects on the red cells of sheep as was shown to be the case in horses by Neser and in cattle by Canham (1930).

TABLE 5.

SHEEP No. 22388.

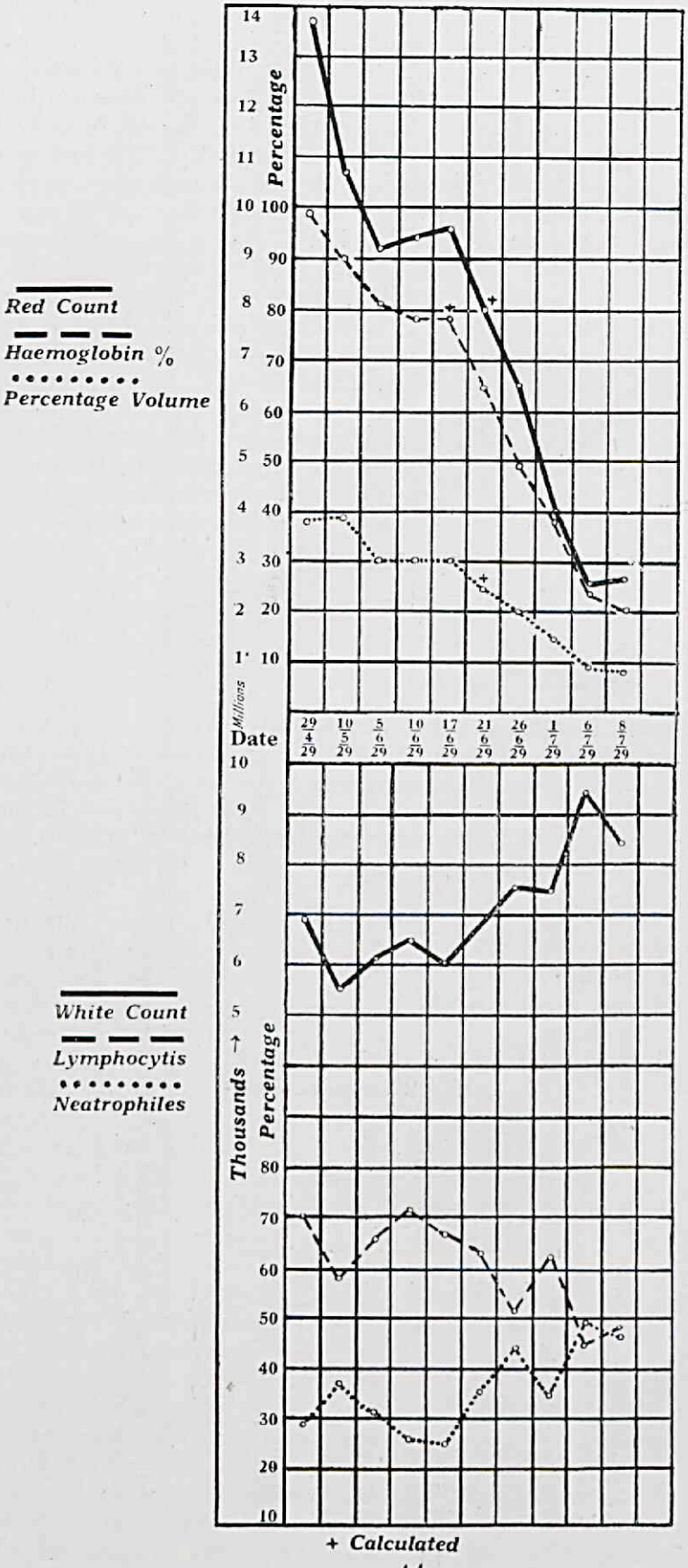
Method of treatment: Infected with Haemonchus contortus *larvae from* 5.6.29.

Date.	Source.	R.C.	R.P.	Hglb. %	Serum.	W.C.	L.	M.	N.	E.	B.	V.C.I.	C.I.	V.I.	Remarks.
29/4/29	Jugular	13·8	38	99	Clear	6,900	70	0	29	0	1	0·92	0·85	0·86	Cells normal.
10/5/29	,,	10·7	39	90	,,	5,500	58	3	37	1	1	0·88	1·00	1·14	Cells normal.
5/6/29	,,	9·2	30	81	,,	6,100	66	3	31	0	0	1·03	1·05	1·02	Occasional fairly large cells.
10/6/29	,,	9·4	30	78	,,	6,500	71	2	26	1	0	0·99	0·99	1·00	Cells normal.
17/6/29	,,	9·6	30	78	,,	6,000	67	3	25	4	1	0·99	0·92	0·98	Cells normal.
21/6/29	,,	8·0	24	65	,,	—	63	1	35	1	0	1·03	0·97	0·94	Cells normal.
26/6/29	,,	6·6	20	49	,,	7,600	51	3	44	2	0	0·93	0·88	0·95	Anisocytosis and punctate basophilia.
1/7/29	,,	4·0	14	39	,,	7,500	62	1	34	3	0	1·06	1·16	1·10	Anisocytosis and poikilocytosis.
6/7/29	,,	2·6	9	23	,,	9,400	45	2	49	3	1	0·97	1·05	1·08	Anisocytosis, poikilocytosis and punctate basophilia.
8/7/29	,,	2·7	8	20	,,	8,400	48	5	47	0	0	0·95	0·88	0·93	Anisocytosis, poikilocytosis and punctate basophilia. DIED 10/7/29.

The average of figures obtained on 10/5/29, 5/6/29, and 10/6/29, is regarded as normal for calculating the colour and volume indices. The figures obtained on 29/4/29 and 10/5/29, were rejected as being probably inaccurate.

For abbreviations, see Table 1.

For method of calculating colour indices, see Table 4.



GRAPH 5.

SHEEP No. 22388.

Infected *Haemonchus contortus*
(20,600 larvae).

If restricted exercise was responsible for the decrease in the number of red cells and the corresponding decrease in the haemoglobin content and the percentage volume of red cells, it is difficult to say whether the blood reached its normal level for conditions of complete inactivity on 5.6.29 (see Graph 5) or whether the downward trend of the curve was checked as from 5.6.29 when infection was commenced, as a direct result of the action of *Haemonchus* larvae in producing an initial stimulating effect on the blood-forming organs.

From 17.6.29 until 6.7.29, there is a progressive decrease in the number of red cells, together with a decrease in the amount of haemoglobin and in the pricipitate. The rate of decrease in the red cells varies from 1.5 to 2.5 millions per week. The course of the disease in this sheep as well as in Sheep No. 22387 can be regarded as acute or even peracute, as it is not often that sheep will die under artificial conditions of infection, from a pure *Haemonchus* infestation within 5 weeks after infection was commenced.

On 8.7.29 the red count and the precipitate remain practically the same, but there is a decrease in the haemoglobin content as compared with the figures obtained on 6.7.29.

Changes in the colour, volume, and volume colour indices.—The figures obtained during the pre-infection period, i.e. on 29.4.29 and 10.5.29, were regarded as abnormal, consequently they were rejected when calculating the average normal, red count, haemoglobin percentage, and precipitate. The average of the figures obtained on 5.6.29 (blood drawn on this date can also be regarded as having been collected during the pre-infection period) 10.6.29, and 17.6.29, was regarded as the average normal for this sheep.

Changes in the colour index.—No marked changes occur in this index. In the early stages of the anaemia there is a tendency towards a decrease in this index, which is calculated as .88 on 26.6.29. Within a week after this there is a plus colour index and towards the end of the disease the index again decreases. A decrease in the colour index is due either to a deficiency in haemoglobin, or, if the cells are fully charged with haemoglobin, to a decrease in the size of the red cells, which means that there must be a relatively greater number of these smaller cells in order to supply the normal amount of haemoglobin, that is, assuming that supersaturation of the cells with haemoglobin did not occur. It is difficult to measure directly a decrease in the haemoglobin content of the cells, but morphological evidence of anaemia, such as anisocytosis and punctate basophilia, was already present on 26.6.29. The other factor, namely the decrease in the size of the red cells, can be determined by direct measurement. Fifteen cells were measured on two occasions (10.5.29 and 5.6.29), when the animal's blood can be regarded as normal, 15 cells were measured during the early stages of the anaemia (26.6.29), and 40 cells were measured when the animal had developed a severe degree of anaemia. These results are tabulated below:—

Date.	*Smallest cell.*	*Largest cell.*	*Average.*
10.5.29	3.8μ	4.6μ	4.2μ
5.6.29	4.1μ	4.8μ	4.5μ
26.6.29	3.5μ	5.1μ	4.5μ
1.7.29	4.1μ	5.7μ	5.0μ

It must again be emphasized that too few cells were measured to regard the averages obtained as even approximately true for the smears of that particular date, but the measurements suggest that no decrease in size has occurred. According to these measurements no changes in the size of the red cells occur up to and including 26.6.29. The decreased colour index that is present on this date would, therefore, appear to be due to a relative deficiency in haemoglobin. On 1.7.29 there is definite evidence that the cells have increased in size and this is responsible for the plus colour index on that date. The more or less normal colour index on 6.7.29 is probably due to a relatively greater deficiency in haemoglobin, in spite of the increase in size which is probably still present, as the volume indices on 1.7.29 and 6.7.29 are practically the same. Owing to the severe degree of poikilocytosis, it was useless measuring the cells on the latter date, as too great a selection would have had to be practised in picking out the measurable cells. On 8.7.29 the colour index is again decreased and is practically the same as on 26.6.29.

Changes in the volume index.—One is probably not justified in interpreting the slight decrease in the volume index on 17.6.29, 21.6.29, and 26.6.29 as being of pathological significance. A plus volume index is present on 1.7.29 and 6.7.29, and this indicates that an increase in the size of the red cells has occurred on these dates. The measurements previously quoted tend to confirm this.

Changes in the volume colour index.—No marked changes occur in this index. There is a slight decrease on 26.6.29, when the colour index also is decreased and this tends to confirm the conclusion that a relative deficiency in haemoglobin was present on this date. Towards the end of the disease there is a slight tendency towards a decrease in this index.

Changes in the leucocytes.—During the pre-infection and post-infection periods, there is a good deal of fluctuation in the total white counts, but there is a tendency throughout the entire course of the disease towards a gradual rise in the total number of leucocytes. On referring to the differential counts, it will be seen that this increase in the number of leucocytes is due to an increase in the number of neutrophiles, and this commences at the same time, as the, so to speak, pathological decrease in the number of red cells occurs. This continuous increase in the number of neutrophiles is interrupted on 1.7.29, when these cells decrease from 44 per cent. to 34 per cent. It was not possible to determine whether this has any special pathological significance. On the other hand, the neutrophilia indicates a healthy activity on the part of the blood-forming organs, undoubtedly stimulated thereto as the result of the loss of red cells.

Pathology of Sheep No. 22388.

The animal died during the night of 9.7.29 and was available for post-mortem examination on 10.7.29. It is unnecesary to give a detailed description of all the organs, reference will only be made to outstanding features of this case: There was very marked paleness of the visible and other mucous membranes, as well as of all the tissues throughout the body. The blood was watery and stained

badly. The animal was in poor condition. There was very marked atrophy of the spleen, hydropericardium, oedema of the lungs, absence of icterus and haemoglobinuria, and what were interpreted macroscopically as degenerative changes in the parenchymatous organs. Numerous wireworms were present in the abomasum, the mucous membrane of which contained numerous dark red spots. The ingesta of the abomasum and of portions of the small intestine were of a chocolate colour and strongly positive for haemoglobin when tested chemically with guiaconic acid. No other parasites besides wireworms were found to be present.

Microscopic examination.—Liver: The cells around the central veins stain less intensely and contain a fair amount of true fat in the form of small droplets, otherwise no changes are present and the crystals described in other cases cannot be identified. Kidneys: Slight fatty changes are the only lesions identified. Bonemarrow: In the case of both the red and the yellow marrow the picture is the same as described for Sheep No. 22387.

(B) Recovery Cases.

Quite a number of cases were encountered in which the infective larvae that were administered developed to maturity and subsequently passed eggs (found in the faeces), without producing any noticeable effects on the hosts. The nature of this immunity or resistance, was not studied.

A number of other sheep developed varying degrees of anaemia and then recovered, with a gradual improvement of the anaemia. These can conveniently be referred to as recovery cases. Of quite a number of such cases encountered during the course of this investigation, the haematological details of three wil be presented. These are Sheep No. 15699 (Table 6 and Graph 6), Sheep No. 15680 (Table 7 and Graph 7) and Sheep No. 11828 (Table 8 and Graph 8).

Sheep No. 15699 was infected with 3,000 larvae, which were given in one dose on 17.11.26. As will be seen on referring to Table 6 and Graph 6, this animal was not examined haematologically during the pre-infection period. The first counts, made 10 days after infection, are extremely high even for a young sheep. It cannot be stated positively that, on the day (27.11.26) when these counts were made, conditions such as polyuria, diarrhoea, lack of water, etc., which produce overconcentration of the blood, were absent. As a reult of the infestation, the red counts decreased rapidly until 22.12.26. This was accompanied by a corresponding decrease in the haemoglobin and the precipitate. After this date there is a gradual improvement in the anaemia. The helminthological records of this sheep are unfortunately not available, so that it is not possible to state definitely whether the animal continued to pass eggs in its faeces after 22.12.26 when recovery commenced. If one can draw any conclusions from the study of Graph 6, it would seem that the animal had not entirely lost its infection and that during the period from 7.1.27 to 11.1.27, the parasites again produced a decrease in the number of red cells, with a corresponding decrease in the haemoglobin and the percentage volume of red cells. Thereafter the blood again improved, until 10.2.27, when a second setback occurred. After that the animal made an uneventful recovery.

TABLE 6.

SHEEP No. 15699.

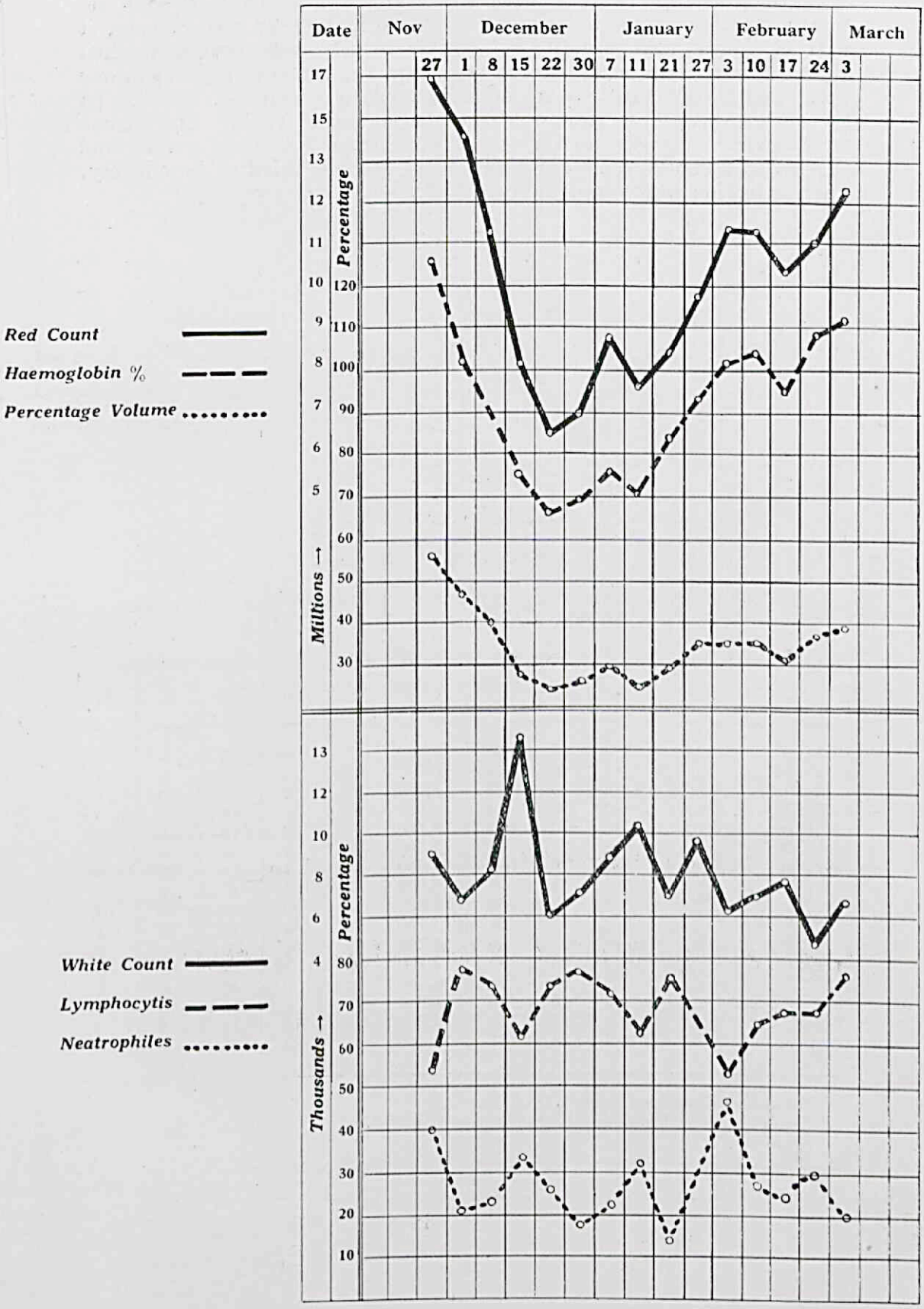
RECOVERY CASE.

Method of treatment: Infected with Haemonchus contortus, 3,000 *larvae, on* 17.11.26.

Date.	Source.	R.C.	R.P.	Hglb. %	W.C.	L.	M.	N.	E.	B.	V.C.I.	C.I.	V.I.	Remarks.
27/11/26	Jugular	17·1	56	126	9,000	54	1	40	3	2	—	—	—	No morphological changes in red cells.
1/12/26	,,	14·2	47	102	6,700	78	0	21	1	0	—	—	0·76	—
8/12/26	,,	11·3	40	86	8,200	74	0	23	2	1	—	—	0·75	—
15/12/26	,,	8·2	28	76	13,300	62	2	34	2	0	—	—	0·95	—
22/12/26	,,	6·6	24	67	6,000	74	0	26	0	0	—	—	0·98	—
30/12/26	,,	7·0	26	69	7,100	77	4	18	0	1	—	—	0·93	—
7/12/27	,,	8·8	30	76	8,700	72	3	22	2	1	—	—	0·88	Serum pale yellow and cloudy. White layer on ppt.
11/1/27	,,	7·7	24	71	10,200	63	1	32	4	0	—	—	1·00	—
21/1/27	,,	8·5	29	84	7,000	77	0	14	7	2	—	—	1·00	—
27/1/27	,,	9·8	35	93	9,800	Smear bad, unable to count.					—	—	0·93	—
3/2/27	,,	11·5	35	102	6,100	52	0	47	1	0	—	—	1·00	—
10/2/27	,,	11·4	35	104	6,900	65	1	28	1	5	—	—	1·00	—
17/2/27	,,	10·4	31	96	7,800	68	1	26	4	2	—	—	1·08	—
24/2/27	,,	11·0	37	108	4,700	68	0	30	1	1	—	—	1·02	—
3/3/27	,,	12·4	39	111	6,600	77	0	20	3	0	—	—	0·99	—

In calculating the volume index the average of the last 6 counts is regarded as normal.
For abbreviations, see Table 1.
For method of calculating colour indices, see Table 4.

GRAPH 6.
SHEEP No. 15699.
RECOVERY CASE.



On studying Table 7 with its Graph 7 and Table 8 with its Graph 8, which refer to Sheep Nos. 15680 and 11828 respectively, it will be found that practically similar results were obtained when 10,000 larvae were administered to Sheep No. 15680 (Graph 7) in one dose, and 20,600 larvae were administered to Sheep No. 11828. In the latter sheep infection was commenced on 16.11.25. 200 larvae were given every second day until 600 larvae had been given, and thereafter 2,000 larvae were given every second, third or fourth day, until infection was completed on 23.12.25.

TABLE 7.

SHEEP No. 15680.

RECOVERY CASE.

Method of treatment: Infected with Haemonchus contortus, 10,000 *larvae, from* 17.11.26.

Date.	Source.	R.C.	R.P.	Hglb. %	W.C.	L.	M.	N.	E.	B.
16/11/26...	Jugular	13·3	37	99	7,200	87	0	5	8	0
24/11/26...	,,	14·1	40	104	10,100	80	1	15	4	0
1/12/26....	,,	11·6	35	93	8,700	80	2	17	0	1
8/12/26....	,,	8·0	25	69	8,600	83	1	16	0	0
11/12/26.	,,	6·3	19	54	8,400	81	3	16	0	0
15/12/26...	,,	6·6	21	59	7,300	58	1	40	0	1
22/12/26...	,,	5·7	20	57	7,700	81	3	15	1	0
30/12/26...	,,	5·1	19	56	14,000	53	0	47	0	0
7/1/27.....	,,	6·1	17	52	6,300	75	2	23	0	0
11/1/27....	,,	6·9	19	59	7,700	62	4	33	0	1
21/1/27....	,,	6·8	24	65	9,700	69	1	30	0	0
27/1/27....	,,	8·2	25	69	7,600	75	3	20	2	0
3/2/27.....	,,	7·9	25	74	3,700	82	0	16	0	2
10/2/27....	,,	7·7	26	76	6,300	81	0	19	0	0
17/2/27....	,,	9·3	28	80	5,500	74	2	18	6	0
3/3/27...	,,	8·1	24	71	6,600	83	3	11	3	0

For abbreviations, see Table 1.
For method of calculating colour indices, see Table 4.

GRAPH 7.

SHEEP No. 15680.

RECOVERY CASE.

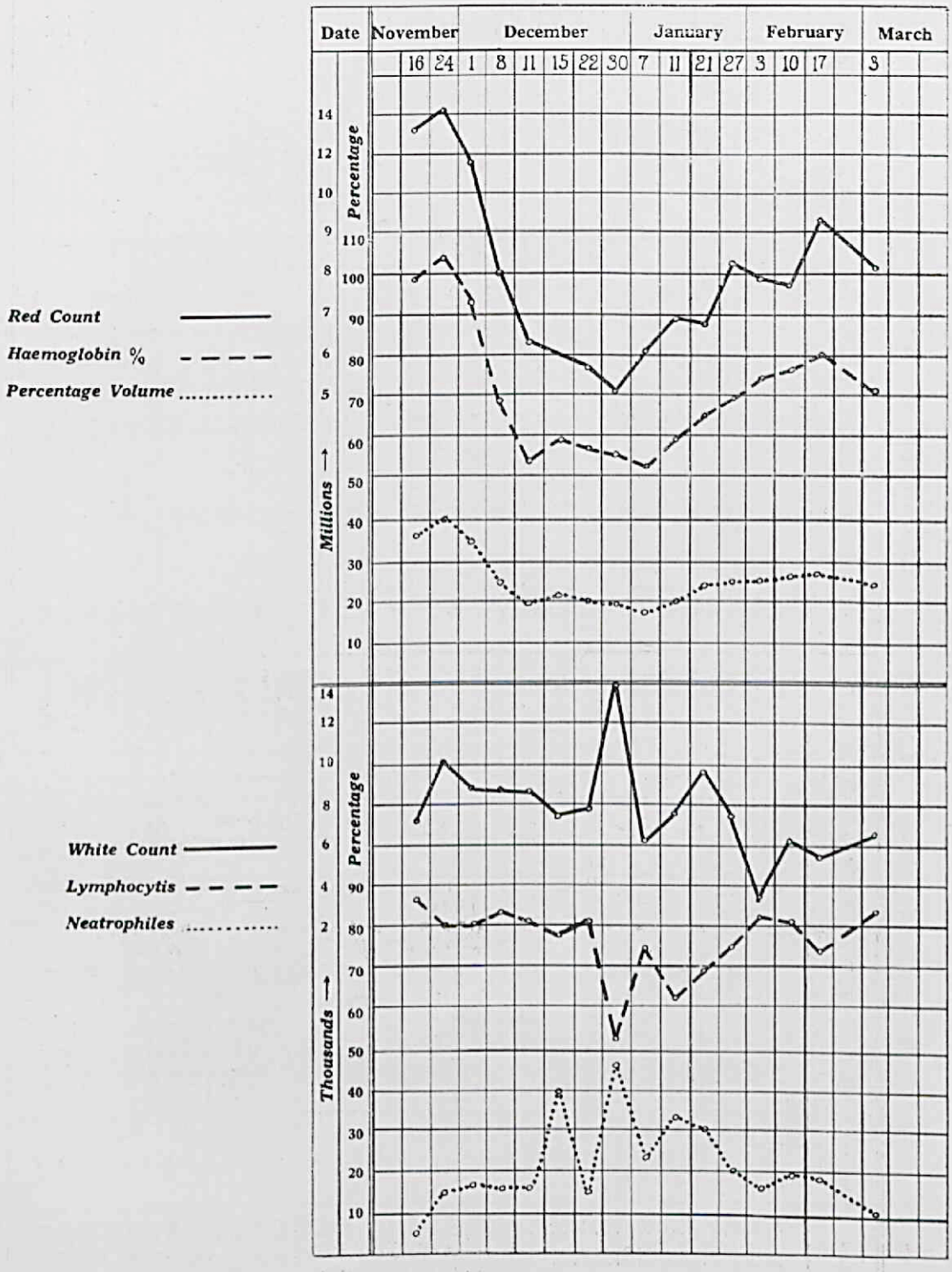


Table 8.

SHEEP No. 11828.

Recovery Case.

Method of treatment: Infected with Haemonchus contortus, 20,600 *larvae.*

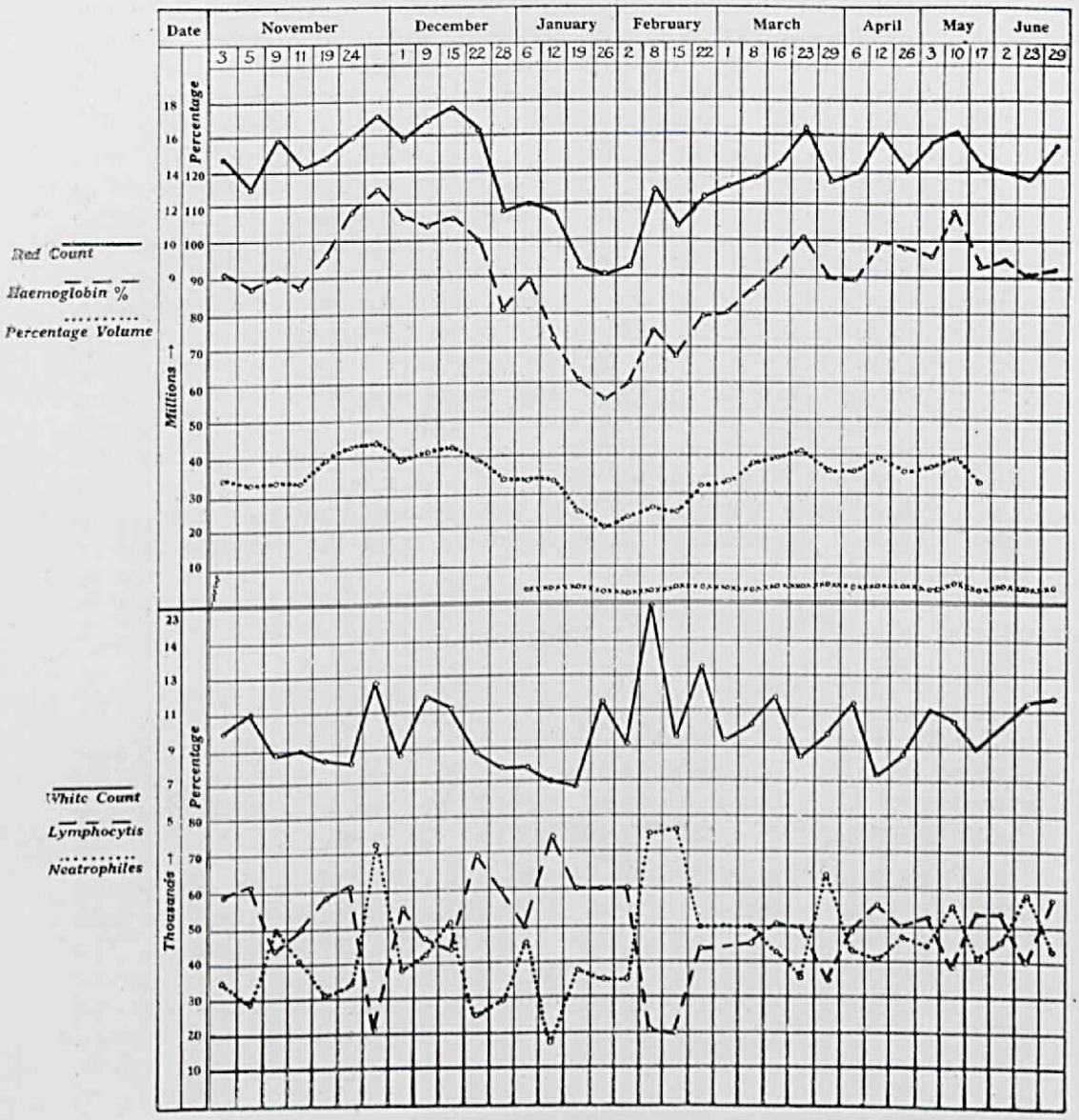
Date.	Source.	R.C.	R.P.	Hglb. %	Viscosity.	W.C.	L.	M.	N.	E.	B.	Remarks.
3/11/25	Jugular	14·6	35	91	—	10,000	59	2	35	4	0	1st dose.
5/11/25	,,	13·0	33	87	—	11,000	62	4	29	5	0	(16/11) 200, (18/11) 200, (20/11)
9/11/25	,,	15·7	34	90	—	8,800	44	5	50	1	0	300, (2/12) 2,000, (4/12) 2,000,
11/11/25	,,	14·3	33	87	—	9,000	50	3	41	6	0	(7/12) 2,000, (9/12) 2,000, (11/12)
19/11/25	,,	14·9*	39	96	—	8,600	58	2	31	9	0	2,000 (14/12) 2,000, (17/12)
24/11/25	,,	15·9	43	108	—	8,200	61	2	34	2	1	2,000 (19/12) 2,000, (21/12) 2,000
1/12/25	,,	17·2	44	114	—	12,700	21	3	72	4	0	(23/12) 2,000 = 20,600 larvae.
9/12/25	,,	15·7	39	107	—	8,700	56	2	38	4	0	—
15/12/25	,,	16·9	41	104	—	11,700	47	2	42	9	0	—
22/12/25	,,	17·4	42	107	—	11,200	44	3	51	2	0	—
28/12/25	,,	16·3	39	100	—	8,800	69	1	25	3	2	—
6/1/26	,,	11·6	34	81	3·2	7,800	60	2	29	8	1	—
12/1/26	,,	12·0	34	79	3·4	7,800	50	0	46	2	2	—
19/1/26	,,	11·5	33	73	3·5	7,000	74	3	17	6	0	—
26/1/26	,,	9·3	25	61	2·7	6,700	60	1	38	1	0	—
2/2/26	,,	9·1	20	56	2·0	11,400	60	3	35	2	0	—
8/2/26	,,	9·3	23	60	2·3	9,200	60	1	35	2	2	—
15/2/26	,,	12·8	26	75	3·2	23,000	21	2	75	2	0	—
22/2/26	,,	10·7	25	67	3·2	9,500	20	3	76	0	1	—
1/3/26	,,	12·4	33	79	3·2	13,400	44	3	50	2	1	—
8/3/26	,,	13·1	34	80	3·3	9,300	—	—	—	—	—	—
16/3/26	,,	13·5	38	87	3·2	10,100	46	2	50	2	0	—
23/3/26	,,	14·4	39	92	3·6	11,800	51	4	42	3	0	—
29/3/26	,,	16·3	41	101	3·7	8,500	50	1	36	13	0	—
6/4/26	,,	13·4	36	90	4·1	9,700	35	1	64	0	0	—
12/4/26	,,	13·8	36	89	3·9	11,500	49	3	43	5	0	—
26/4/26	,,	15·9	39	99	4·1	7,300	56	2	41	0	1	—
3/5/26	,,	14·0	36	98	4·3	8,700	50	1	47	2	0	—
10/5/26	,,	15·5	37	96	3·9	11,000	52	0	45	3	0	—
17/5/26	,,	16·1	39	108	4·8	10,600	38	2	56	4	0	—
2/6/26	,,	14·3	33	92	3·9	8,900	54	1	41	4	0	—
23/6/26	,,	—	—	95	4·7	—	54	0	46	0	0	—
29/6/26	,,	13·5	40	91	4·0	11,400	40	1	59	0	0	—
26/7/26	,,	15·4	40	92	4·11	11,700	51	1	43	5	0	—
18/9/26	,,	13·8	44	117	—	18,200	12	2	85	0	1	—

For abbreviations, see Table 1.
For method of calculating colour indices, see Table 4.

GRAPH 8.

SHEEP No. 11828.

RECOVERY CASE.



In all these three cases it is not possible to state whether the animals completely got rid of the infection or whether the infection remained and they developed an immunity as a result of which they were able to resist or completely to neutralize the effects of the parasites.

In view of the self cures reported by Stoll (1929), in the case of two sheep, it is necessary to refer to a further recovery case that was encountered during the coure of this investigation. This Sheep No. 11814, haematological records of which are presented in Table 11 and Graph 11, was at first used in a nodular worm experiment. The animal became at the same time accidentally, but naturally, infected with wireworms. This was diagnosed by means of faeces cultures. As a result of the wireworm infection, the animal developed a fairly severe degree of anaemia, the red counts decreasing from a normal of 12-14 million to 5.5 million red cells per c.mm. of blood (16.3.26). After this the anaemia gradually improved. When this was observed, the animal was reinfected with large doses of *Haemonchus contortus* larvae, but completely resisted the infection, and when the blood was finally examined, months later (27.11.26), the animal was, from a haematological point of view, quite normal. In this work no attempt was made to study the question of immunity in, or resistance to haemonchosis in sheep. This question of acquired immunity with metazoan and other parasites in becoming increasingly important, as was shown by Taliaferro (1929) and other authors quoted by him. On referring to the differential counts (Table 11 and Graph 11), it will be observed that an undoubted eosinophilia occurs, during the time the animal is recovering from the anaemia. It is noteworthy that de Kock and Quinlan (1926), also recorded an eosinophilia in splenectomized sheep at the time recovery from the anaemia due to Anaplasmosis was taking place.

(C) Controls.

As previously stated, quite a number of uninfected control sheep were always kept separate from the infected sheep, but otherwise under identical conditions of housing, feeding and watering. These control animals remained healthy and never developed any clinical symptoms. The haematological records of two of these sheep are given below.

Table 9 and Graph 9 refer to Sheep No. 11929. This is a young sheep free from worms. There is what is interpreted as a normal variation and fluctuation in the red counts, the haemoglobin and the percentage voume of red cells. In isolated cases it will be oberved that the haemoglobin and precipitate curves do not follow the red count curves. This is probably due to errors in the red counts. There is perhaps a slight downward trend of the curves in Graph 9. This is possibly due to the fact that in very young sheep the red cells decrease in number as such sheep grow older.

TABLE 9.

SHEEP No. 11929.

CONTROL.

Date.	Source.	R.C.	R.P.	Hglb. %	Viscosity.	W.C.	L.	M.	N.	E.	B.
4/11/25	Jugular	12·4	39	81	—	8,000	49	1	50	0	0
6/11/25	,,	11·6	32	73	—	9,500	51	5	43	1	0
10/11/25	,,	11·4	34	78	—	6,100	55	1	44	0	0
13/11/25	,,	14·8	42	87	—	6,400	76	2	22	0	0
20/11/25	,,	11·4	36	83	—	8,800	55	3	42	0	0
26/11/25	,,	12·1	34	78	—	5,500	74	4	21	1	0
3/12/25	,,	12·1	35	81	—	7,800	53	2	45	0	0
11/12/25	,,	10·6	34	75	—	5,600	58	5	36	1	0
18/12/25	,,	11·1	32	69	—	7,400	51	2	46	1	0
24/12/25	,,	12·0	37	81	—	9,300	51	2	47	0	0
30/12/25	,,	13·6	43	91	3·5	6,800	68	3	27	2	0
8/1/26	,,	—	40	79	3·2	7,100	61	0	39	0	0
14/1/26	,,	10·0	30	72	3·1	4,800	64	1	32	0	3
21/1/26	,,	10·3	31	76	4·0	4,200	66	1	33	0	0
23/1/26	,,	13·3	38	87	4·0	5,900	67	0	33	0	0
28/1/26	,,	11·0	30	76	3·0	5,500	60	5	32	3	0
3/2/26	,,	11·0	31	76	2·9	4,700	62	0	38	0	0
10/2/26	,,	11·7	35	84	2·5	5,900	38	0	60	1	1
17/2/26	,,	12·0	36	87	3·3	6,100	53	0	47	0	0
23/2/26	,,	11·9	31	74	3·2	6,300	58	1	41	0	0
2/3/26	,,	10·3	29	70	2·8	6,100	54	2	41	3	0
9/3/26	,,	11·1	32	75	—	4,300	50	0	50	0	0
19/3/26	,,	11·3	29	72	3·1	5,100	62	1	34	2	1
26/3/26	,,	9·8	25	62	2·6	4,700	72	3	24	0	1
30/3/26	,,	11·7	32	77	3·0	3,400	55	3	42	0	0
9/4/26	,,	10·5	28	70	2·8	5,600	47	1	52	0	0
16/4/26	,,	9·8	25	64	2·7	5,200	64	0	34	2	0
30/4/26	,,	11·8	33	81	3·1	4,500	68	3	29	0	0
5/5/26	,,	12·0	33	78	3·0	6,100	53	3	44	0	0
14/5/26	,,	10·6	—	73	2·8	6,200	50	0	48	0	2
21/5/26	,,	9·4	28	71	3·0	4,900	67	2	31	0	0
4/6/26	,,	12·2	33	84	3·0	3,000	59	1	38	0	2

For abbreviations, see Table 1.
For method of calculating colour indices, see Table 4.

GRAPH 9.

SHEEP No. 11929.

CONTROL YOUNG SHEEP.

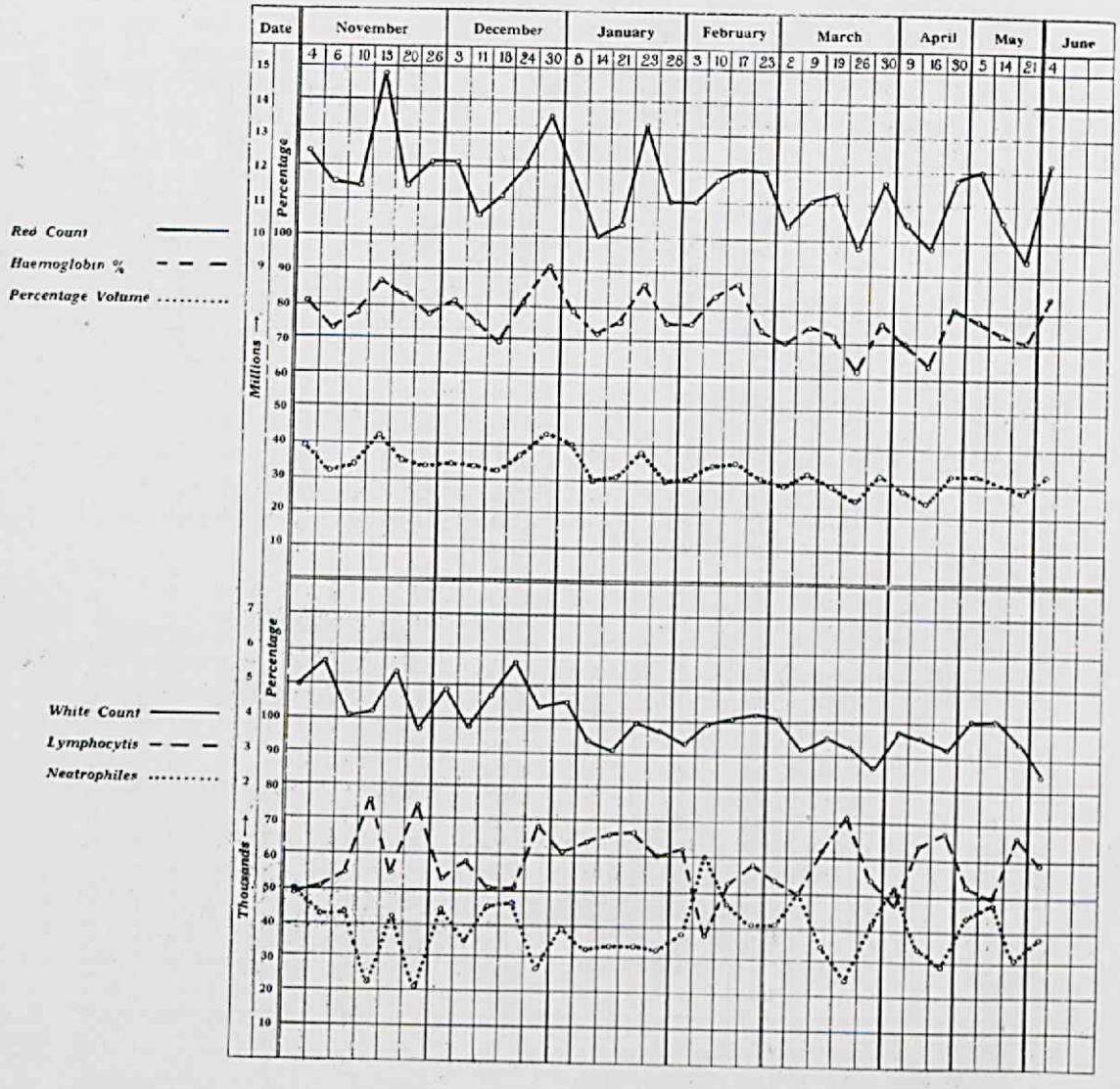


TABLE 10.

CONTROL SHEEP No. 13601.

Date.	Source.	R.C.	R.P.	Hglb. %	Visco-sity.	W.C.	L.	M.	N.	E.	B.
6/1/26	Jugular	8·6	31	79	3·0	8,100	81	2	16	1	0
7/1/26	,,	8·6	29	81	3·2	7,400	70	2	26	2	0
8/1/26	,,	9·3	33	78	3·0	6,400	73	5	18	2	2
11/1/26	,,	9·0	34	81	—	6,400	72	1	26	1	0
12/1/26	,,	9·3	32	81	3·2	6,500	63	5	28	3	1
13/1/26	,,	9·3	35	89	3·2	7,700	76	3	21	0	0
14/1/26	,,	8·2	30	79	2·9	6,700	71	1	26	1	1
18/1/26	,,	7·6	30	79	3·3	7,600	unable to count			—	—
19/1/26	,,	9·2	37	84	3·3	7,300	63	1	31	4	1
20/11/26	,,	10·0	39	81	3·0	6,000	unable to count			—	—
21/11/26	,,	9·3	34	84	3·0	8,600	69	2	25	3	1
22/1/26	,,	9·2	32	81	3·0	6,400	77	3	20	0	0
25/1/26	,,	8·0	27	79	3·0	5,700	76	2	20	1	1
26/1/26	,,	8·4	30	77	2·9	5,000	71	3	23	2	1
27/1/26	,,	9·1	30	80	3·0	8,900	68	1	28	3	0
28/1/26	,,	10·0	32	84	3·0	6,800	67	5	24	4	0
29/1/26	,,	10·0	36	87	3·0	7,100	72	4	22	0	2
1/2/26	,,	9·6	34	86	2·5	5,300	67	2	27	3	1
2/2/26	,,	9·0	32	77	2·2	4,900	77	0	21	2	0
2/2/26	,,	10·0	37	93	2·5	6,200	74	3	21	1	1
4/2/26	,,	9·0	32	84	2·4	6,400	70	1	23	6	0
5/2/26	,,	8·5	33	78	2·3	8,500	74	1	22	3	0
8/2/26	,,	9·6	32	80	2·4	10,300	55	0	39	5	1
9/2/26	,,	9·0	32	80	2·1	10,600	63	0	30	7	0
10/2/26	,,	10·0	37	96	2·4	7,300	unable to count			—	—
12/2/26	,,	10·2	34	90	2·5	5,900	56	0	34	8	2
15/2/26	,,	10·2	34	87	2·9	5,700	72	4	16	5	3
16/2/26	,,	8·4	29	76	2·9	6,000	62	2	32	3	1
17/2/26	,,	9·4	36	81	2·9	7,300	55	2	39	4	1
18/2/26	,,	9·3	30	84	3·0	8,700	78	0	14	5	3
19/2/26	,,	9·9	33	79	3·4	7,500	68	2	24	4	2
22/2/26	,,	10·7	35	95	3·1	7,000	68	2	25	2	3
23/2/26	,,	8·8	30	78	2·9	4,300	68	2	26	4	0
24/2/26	,,	9·5	32	84	3·0	5,100	75	4	18	1	2
25/2/26	,,	10·9	33	84	3·0	7,100	77	0	21	1	1
26/2/26	,,	9·1	30	75	2·8	6,600	83	0	16	0	1
1/3/26	,,	10·9	36	91	3·0	8,300	69	1	28	1	1
2/3/26	,,	9·3	30	80	2·9	5,500	69	5	24	2	0
3/3/26	,,	11·2	33	89	3·1	6,100	70	2	22	5	1
4/3/26	,,	11·0	35	90	3·1	8,500	66	3	26	3	2
8/3/26	,,	9·4	33	81	2·8	4,000	71	0	25	3	1
9/3/26	,,	11·7	34	83	2·8	6,900	68	1	23	4	4
10/3/26	,,	9·4	30	78	3·0	6,000	62	2	34	1	1

For abbreviations, see Table 1.
For method of calculating colour indices, see Table 4.

GRAPH 10.

SHEEP No. 13601.

CONTROL ADULT SHEEP.

NORMAL SHEEP.

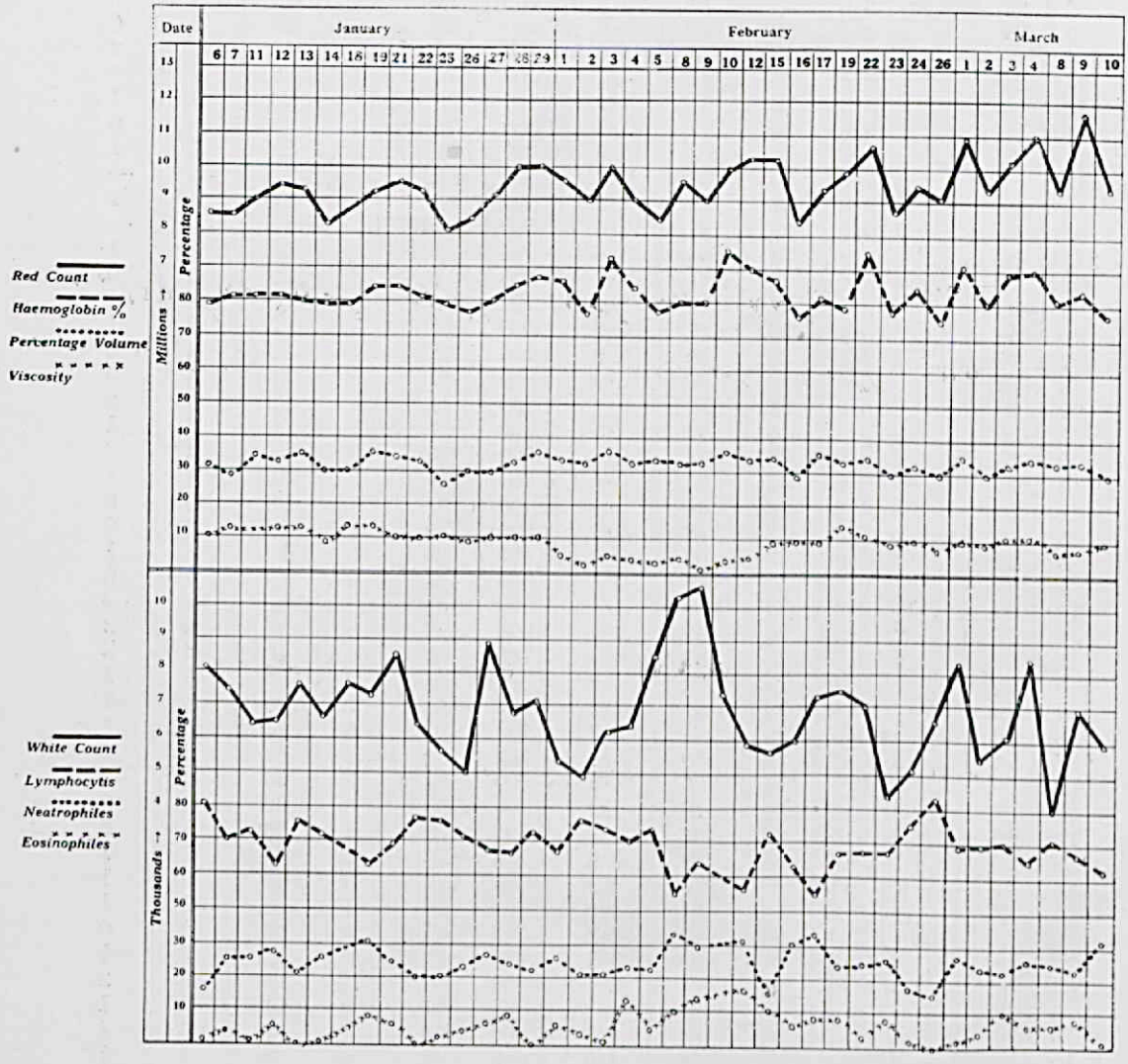


TABLE 11.

SHEEP No. 11814.

RECOVERY CASE.

Date.	Source.	R.C.	R.P.	Hglb. %	Vis-cosity.	W.C.	L.	M.	N.	E.	B.
3/11/25	Jugular	13·8	39	96	—	13,300	58	2	35	4	1
5/11/25	,,	14·5	39	99	—	10,600	55	3	41	1	0
9/11/25	,,	12·8	35	91	—	6,700	70	3	27	0	0
11/11/25	,,	14·4	40	95	—	7,600	53	2	44	1	0
17/11/25	,,	14·4	41	100	—	8,000	58	6	35	1	0
23/11/25	,,	13·9	37	96	—	10,100	63	5	31	1	0
30/11/25	,,	14·2	40	100	—	10,700	54	3	38	3	2
14/12/25	,,	13·4	37	95	—	10,300	52	1	38	9	0
28/12/25	,,	13·8	—	96	—	12,200	46	3	49	2	0
5/1/26	,,	10·8	32	77	3·2	17,700	47	1	40	12	0
11/1/26	,,	9·2	27	69	3·0	9,700	48	2	37	8	5
18/1/26	,,	9·7	25	65	3·0	8,100	—	—	—	—	—
1/2/26	,,	8·1	25	57	2·3	12,000	52	4	37	6	1
5/2/26	,,	7·1	21	54	2·2	9,400	49	3	42	2	4
8/2/26	,,	7·4	24	57	2·2	11,600	60	2	28	6	4
15/2/26	,,	7·6	—	58	2·8	11,800	58	3	29	9	1
22/2/26	,,	6·6	17	49	2·6	8,900	52	2	37	5	4
1/3/26	,,	7·6	24	57	2·6	11,200	44	2	45	6	3
8/3/26	,,	6·4	22	51	2·5	10,400	41	2	44	9	4
16/3/26	,,	—	23	52	2·7	11,800	43	6	43	6	0
22/3/26	,,	7·3	23	52	2·1	7,100	36	5	45	10	4
29/3/26	,,	7·6	23	55	2·6	12,900	37	3	54	4	2
8/4/26	,,	7·3	22	53	2·6	9,900	51	5	37	4	3
12/4/26	,,	7·6	23	50	2·7	10,700	46	5	38	11	0
26/4/26	,,	6·5	20	53	3·1	12,000	47	3	42	7	1
3/5/26	,,	—	25	58	3·1	12,900	45	0	40	10	5
10/5/26	,,	8·8	28	63	3·4	11,700	49	1	42	7	1
17/5/26	,,	9·9	32	75	3·6	15,200	35	2	60	3	0
26/5/26	,,	6·9	23	66	3·6	9,700	46	2	35	14	3
9/6/26	,,	8·6	26	65	3·4	12,800	47	4	31	16	2
23/6/26	,,	8·7	25	63	—	11,600	50	1	33	11	5
29/6/26	,,	8·5	27	60	3·5	11,000	54	3	34	7	2
26/7/26	,,	10·6	33	74	3·9	10,800	47	4	33	14	2
27/11/26	,,	12·2	37	96	—	13,700	47	5	39	9	0

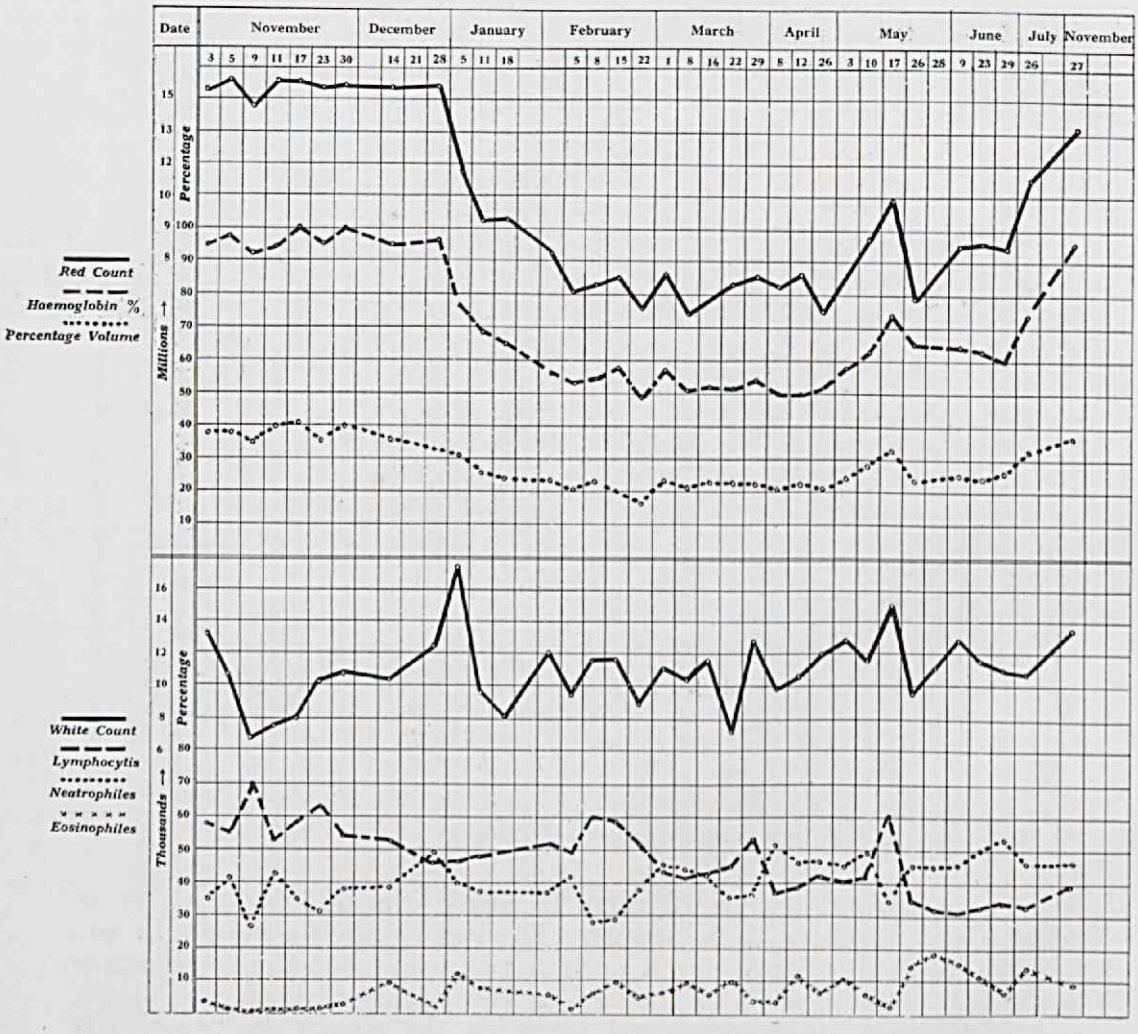
For abbreviations, see Table 1.
For method of calculating colour indices, see Table 4.

Table 10 and Graph 10 refer to Sheep No. 13601. This is an adult sheep also free from worms. The red count, haemoglobin and precipitate curves also show a normal variation, but the fluctuations are not so ponounced as in the case of the younger Sheep No. 11929, and within the limits of normal variation the curves are practically straight.

PATHOGENESIS OF THE ANAEMIA IN HAEMONCHOSIS OF SHEEP.

It is obvious that the outstanding pathological effects of wireworms on sheep is the very severe and often fatal anaemia that is produced, and it is of fundamental importance to establish definitely the manner in which this is brought about. It is remarkable that

GRAPH 11.
SHEEP No. 11814.
RECOVERY CASE.



these parasites, spending as they do their entire parasitic life in the abomasum of sheep, can produce such profound changes in the blood of their hosts.

Theoretically, anaemia can be due to: (1) The destruction and disintegration of the red cells by (*a*) parasites such as occur in the piroplasmoses, or (*b*) toxic or other principles which can conveniently be classified as haemolytic substances; (2) A disturbance or disease of the blood-forming organs as a result of which the full ontogenitic development of the red cells is interfered with and the normal replacement of red cells cannot keep pace with those which are being daily eliminated in the process of normal wear and tear; (3) The removal of the red cells due to phagocytic activity of certain cells in the body. The anaemia is then due to excessive erythrophagocytosis. Since the reticulo-endothelial system plays an active part in the disintegration of red cells, e.g. in anaplasmosis in splenectomised sheep, according to de Kock (1923), erythrophagocytosis could perhaps more conveniently have been grouped and discussed under (1) above; (4) The removal of red cells as occurs in haemorrhage.

Before considering in detail into which of these groups the anaemia of haemonchosis can be placed form the known facts of the condition, and from the new evidence that is presented in this work, an attempt will be made to review very briefly, and without pretending to quote exhaustively the tremendous literature that has grown around this subject, the views generally held as to the manner in which parasites produce their effects on the hosts. In general the injurious effects of parasites on their hosts can be grouped into one or other of the following:—

(*A*) *Nutritive Disturbances.*—Either by depriving the host of food, which the gastro-intestinal parasites may assimilate, before this can become absorbed by the host, or by producing lesions in the alimentary canal, as a result of which large portions of the digestive tract are rendered valueless for digestion and absorption.

(*B*) *Mechanical injury inflicted during the migration of the parasites and bacterial infection through wounds and injuries.*—Such infection may have remote effects on various organs and tissues of the body.

(*C*) *Toxic Substances.*—There is the possibility that toxic substances, which are absorbed, may have a general or specific effect on the body as a whole, or on particular systems, such as the circulation, respiration, nervous system, endocrine organs, etc.

(*D*) *Anaemia.*—This may be due to (1) mechanical injury inflicted on the mucous membrane and underlying tissues by numerous parasites, particularly those equipped with mouth parts such as lancets designed for cutting, etc., and which secrete substances which prevent the coagulation of the blood; (2) the action of toxic principles, (*a*) haemolytic substances secreted by the parasites, which when absorbed will produce haemolysis, possibly also the action of haemolytic organisms which can gain entrance, through mechanical injuries and wounds; (*b*) toxic substances which may cause injury to the blood-forming organs.

In considering to what extent any of these conditions are involved in haemonchosis of sheep and in some other parasitic diseases, as reported in the literature, they can be dealt with individually.

(A) No attempt was made to determine to what extent, if any, nutritive disturbances are associated with the injurious effects of wireworms. It is not likely that this can be an important factor in haemonchosis, as very often sheep which are heavily infested with wireworms are in fat condition, and it is doubtful if this deposition of fat can be entirely ascribed as secondary to the anaemia.

(B) The conditions enumerated under this heading may be involved on rare occasions in so far as haemonchosis is concerned, and will be referred to when some unusual lesions associated with wireworm infection are described subsequently.

(C) No such effects were observed or have been reported in the literature that was studied.

(D) Anaemia in Haemonchosis.

As previously stated, a systematic haematological study of the anaemia due to wireworm infestation in sheep is not recorded in the literature. On the question of toxic substances secreted by parasites, a vast literature has, however, accumulated.

Schwartz (1921) very fully discusses what he calls hematoxins from parasitic worms. He gives a comprehensive review of the literature, and mentions 75 papers. The period covered by this review is from 1865-1921. No attempt will be made to cover the same ground in the literature, but some of the conclusions in the author's summary of the recorded investigations on hemotoxins from parasitic worms, will be referred to. He states that: (1) certain parasitic worms secrete harmful toxic substances, which are named hemotoxins; these hemotoxins are in general of a non-specific nature and may be active on the blood of animals other than their normal hosts; (2) of the cestodes, *Diphyllobothrium latum*, definitely contains haemolytic substances; (3) *Schistostoma japonicum* and worms belonging to the genus *Ascaris* contain haemolysins; (4) hookworms, worms of the genus *Strongylus* and *Gastrophilus* larvae, secrete haemolysins and anticoagulins. *Haemonchus contortus* is specifically mentioned as apparently secreting a weak haemolysin and further whipworms as apparently secreting a haemolysin.

In concluding this summary, the author makes this statement: " Owing to the fact that the direct abstraction of blood by parasites appears to be inadequate as an explanation of the causes of anaemia in parasitic diseases, and in view of the fact that in tapeworm infections, which are accompanied by anaemia due entirely to the presence of the parasites, the direct abstraction theory is inapplicable, the view that haemolysins from parasites are of aetiological significance in parasitic diseases appeared to be entirely justified."

In his own work on salt solution extracts of *Haemonchus contortus* on sheep and cattle erythrocytes, Schwartz (1921) concludes that the weakly positive haemolytic effects obtained do not favour very strongly the view which has been commonly accepted as regards the secretion of a haemolysin by this parasite.

Hookworm disease in man and some of the domesticated animals has been extensively studied and, although Schwartz, just previously quoted, concludes from his study of the literature that haemolysins and anticoagulins are present in hookworms, Flue (1922) submits evidence, in connection with hookworm disease of dogs, from which he deduces: (1) the presence of anticoagulins in the cranial portions of these hookworms; (2) the absence of haemolytic substances; (3) that the worms are able to produce not only chronic, but also profuse, haemorrhage, which is sometimes fatal. He further concludes that these facts favour the theory of chronic haemorrhage as the cause of anaemia in hookworm disease of dogs.

Recently Chandler (1929) states that it is generally agreed that hookworms do not cause anaemia by the secretion of a haemolysin. Hookworms suck blood, and as a result of the action of anticoagulins, the wounds keep bleeding for some time after the worms have abandoned them; but the amount of blood in the faeces, even in severe hookworm infestation, is usually very small. He concludes: "It seems fair to assume, therefore, that chronic loss of blood from the intestine is at most a minor factor in hookworm disease, and certainly cannot account for the severe anaemias so frequently seen in heavy infestations." He states further that "there seems to be little ground for doubting that the anaemia is due primarily to a toxic effect on the blood-forming organs."

Chandler quotes de Lange as stating that hookworm anaemia is primarily an aplastic one, caused by loss of normal regenerative power on the part of the blood-forming organs, due to toxins derived directly or indirectly from the worms in the intestine.

Nicoll (1914) maintains that hookworm disease in dogs "is generally accompanied by distinct though not profuse haemorrhage, which is most marked in the early stages, but tends to disappear. Evidence of blood regeneration was furnished by the appearance of large numbers of erythroblasts (normoblasts), which increased with the progress of the disease."

Mhaskar (1924) discusses the hookworm problem very fully, with the exception of the pathology and haemotology of the condition. He uses the Duncan Whytes Phenolphthalein test to determine the presence of occult blood, and finds that: (1) occult blood is absent in cases free from hookworm infection; (2) it is present in 39 per cent. of infected cases; (3) it is present in 17.8 per cent. of cases for periods varying from 12 days to 4 months after the complete expulsion of hookworms; (4) the intensity of the reaction has no correlation to the number of hookworms harboured in the intestine.

All the cases examined were carefully selected and were free from all causes known to lead to evacuation of blood with faeces. It was supposed that the occult blood detected was due to haemorrhage consequent upon ulceration of the intestinal wall, brought about by hookworms, but the author states that he had no direct proof of this.

Scott (1930) believes that some of the acute anaemias in hookworm disease may be due to haemorrhage, but that the chronic anaemia does not seem to be fully explained on the basis of blood

loss. The remaining explanation, then, is a toxic depressing action on the bonemarrow and other blood-forming organs, but he points out that the pathology of these organs in chronic cases has not been adequately studied.

Referring now to the available literature in connection with the anaemia of haemonchosis in sheep, Hall (1920) mentions that the stomach worms of sheep have the habit of attaching at one point for a time and then moving away and attaching at another point, leaving the old point of attachment bleeding for some time. This causes anaemia, dry wool, oedematous swellings, and general unthriftiness. The anaemia produced by stomach worms resembles that caused by hookworms in man, dog, and sheep. The only way to differentiate between the anaemia of hookworm and wireworm infections in sheep is by post-mortem examination.

Donatien and Lestoquard state that anaemia of sheep and goats may to-day be attributed to (1) worms, (2) blood parasites, (3) bacteria, (4) pernicious anaemia. Group (1) is discussed as parasitic anaemia which can be caused by (*a*) distomiasis, (*b*) gastro-intestinal strongylosis which is a progressive anaemia, with loss of appetite and diarrhoea. The manner in which this anaemia develops is not discussed, neither is any evidence submitted in regard to changes produced in the blood by uncomplicated infection with the individual strongyles; (*c*) trichocephalosis, and (*d*) ovine bunostomiasis.

Hutyra and Marek (1926) state that the *strongylidae* bore into the gastric mucosa and such blood from it. In this way they disturb the nutrition of the host to a degree proportionate to their number. More detrimental probably than the loss of blood is, however, the absorption of toxic metabolic products of the parasites.

The conclusion that one comes to in studying the literature on hookworm disease is that in spite of the great deal of work that has been done, it is not definitely known what the pathogenesis of the anaemia is. It seems to be generally agreed that direct haemolysis does not occur. There are quite a number of workers who believe that the anaemia is due to haemorrhage from the intestinal tract, but others maintain that, although this may be a somewhat insignificant contributory factor, the anaemia is mainly caused by the action of toxic products from the parasites themselves, or toxic bacterial products, or the combined action of these toxins on the blood-forming organs, although it is difficult to reconcile a depressing effect caused by toxins on the blood-forming organs with the leucocytosis, which is described by Sarless (1929) in some dogs experimentally infected *per os* with *Ankylostoma caninum*. Wintrobe (1931) suggests a classification of the anaemias on the basis of differences in the size and haemoglobin content of the red corpuscles. The anaemias in his Class 4 largely comprise those resulting from chronic blood loss. The characteristic feature of these anaemias, according to this author, is the decrease in the size of the red cells, accompanied by a relatively greater decrease in the haemoglobin content. On this account he suggests the term hypochromic anaemia. He found that the anaemia in hookworm disease is of this type. With such an anaemia one would expect to find a decrease in the colour, volume, and volume colour indices.

In the case of haemonchosis in sheep, the anaemia-due-to-haemorrhage theory has its adherents. Other workers state that haemorrhage, as well as toxins, causes the anaemia, but no evidence is adduced as to how exactly the toxins are supposed to produce this effect.

The four main causes of anaemia previously referred to, will now be individually examined, with the object of determining, from the available haematological and pathological facts that are already known or have been established in this investigation, into which of these groups the anaemia of haemonchosis can be classified.

(1) *The Destruction and Disintegration of the Red Cells.*

If haemolytic toxins from wireworms or bacterial products be absorbed, the degree of haemolysis that is produced will depend on the haemolytic potency of the toxin and on the amount of toxin absorbed. When haemolysis occurs the haemoglobin will be dissolved in the plasma and produce haemoglobinaemia and, depending on various factors, this may lead to haemoglobinuria and possibly to haemolytic icterus. Irrespective of whether the haemolysis is of such a degree as to produce haemoglobinuria or icterus, derivatives of haemoglobin such as occurs in haemosiderosis, must be found in the tissues, especially in those cases where the haematological examination reveals a marked decrease over a short period of time in the number of the red cells and in the absolute haemoglobin content. There is no evidence of an erythrorhexis or an erythrolysis such as is described by de Kock (1923) for enzootic icterus and anaplasmosis in splenectomize sheep respectively.

How do the results obtained accord with these postulates? Clinical icterus or haemoglobinuria never occurs in haemonchosis. Haemoglobinaemia has never been observed. If haemolysis does occur, one would expect, especially in those cases where the red cells decrease to the extent of over 5 millions per c.mm. of blood, within a week that haemoglobinaemia could be diagnosed by examining the plasma after centrifuging the blood. This was always very carefully done, and on no occasion was evidence of haemoglobinaemia observed.

In all the fatal experimental cases of haemonchosis, the liver, lung, spleen, and kidney were specifically stained for iron but none could be demonstrated in these organs, with the exception of the spleen. A good deal of iron-containing pigment is normally present in the spleen, but in this organ, which in quite a number of cases was very much atrophied, it was even difficult to demonstrate the presence of any iron-containing pigment at all. It can, therefore, be definitely stated that no haemolysis occurs which can explain the anaemia in haemonchosis in sheep. It is generally agreed that this holds for hookworm disease also.

(2) *A Disturbance or Disease of the Blood-forming Organs.*

If this occurs, one must assume that the blood-forming organs will reveal some morphological evidence of such disturbance and sometimes, at any rate, a compensatory myeloid metaplasia in organs like the spleen and the liver, as occurs in lymphomatosis, leucaemia,

and in carcinoma of the bonemarrow, according to Naegeli (1923), and as occurs in lymphatic aleucaemia in the dog, described by Fourie and Ziehn (1930). It may, however, reasonably be argued that the toxins are carried in the bloodstream and their effect on the spleen and the liver would also be such as to prevent the development of any myeloid metaplasia.

In all the fatal cases of haemonchosis, the bonemarrow, including the fat bonemarrow, was carefully examined histologically and the only changes that were observed were those not of decreased activity but rather those suggestive of increased activity. Numerous neutrophile and eosinophile myelocytes, as well as immature forms of erythrocytes, were easily recognized in the sections. In the fat bonemarrow the fat spaces were extensively replaced by myeloid tissue, in which young forms of erythrocytes were frequent and in which the other usual marrow cells, such asmegakaryocytes, neutrophile and eosinophile myelocytes, etc., could be identified. The neutrophilia, which occurs in nearly all cases towards the end of the disease, is evidence of an actively functioning myeloid tissue. Should the animal live long enough, with such a severe anaemia in chronic cases one would expect to find eventually exhaustion of the blood-forming tissues, and the severe poikilocytosis and the " pessarformen " that occurs in some cases may be evidence that such exhaustion is taking place, although Naegeli (1923) points out that poikilocytosis is of small diagnostic significance and is probably dependent on changes in isotonicity of the blood. In the cases studied, there was a fatal termination before well defined exhaustion occurred.

That compensation was taking place is proved by the myeloid hyperplasia of the fat bonemarrow, contrary to what one would expect where such a circulating toxin causing disturbances in the blood-forming organs is postulated, such toxin should also tend to prevent the formation of any compensatory myeloid hyperplasia in the fat bonemarrow.

There is further definite evidence that, in a number of these fatal cases of haemonchosis, an increase in the size of the cells occurs and, according to Naegeli (1923), the presence of small cells (microcytes) is the most significant and distinctive feature of a bonemarrow insufficiency (Knochenmarksinsuffizienz). Schilling (1922) describes microcytes as degenerative forms of erythrocytes which indicate degenerative changes in the bonemarrow.

It can, therefore, be concluded that, in the fatal cases of haemonchosis studied, there is no evidence of a disturbance in the blood-forming organs interferes with the normal replacement of red cells, which are daily being eliminated in the process of normal wear and tear. There is certainly a disturbance in the blood-forming organs, but this is caused by and is secondary to the anaemia and not the primary cause of it.

The possibility that the lesions described in the liver and other organs, can be caused by toxic substances, will be discussed under section (4) below.

(3) *Erythrophagocytosis.*

Only on very rare occasions were cells, in which erythrophagocytosis had occurred, encountered; this, as a cause of the anaemia, can therefore be discarded.

(4) *Removal of the Red Cells as occurs with Haemorrhage.*

The mouth parts and the buccal capsule of wireworms are not developed to the same extent as in hookworms, but nevertheless these parasites cut and lacerate the mucous membrane and such blood. One hundred live wireworms were collected from a sheep that was destroyed, were carefully washed in saline, then transferred to and washed in distilled water, and finally transferred to a third receptacle, also containing distilled water. In this they were ground up and, after filtration the extract was examined spectroscopically, when typical haemoglobin bands were identified. Hall, previously quoted, states that when the parasite detaches, as it has a habit of doing, it leaves the old point of attachment bleeding for some time, and when hundreds of worms are present in heavily infested individuals, it is not inconceivable that serious effects will be produced.

In typical and fatal cases of haemonchosis, the abomasal contents are of a brownish chocolate colour, and are strongly positive for haemoglobin, when tested with tincture of guaiaconic acid. Positive tests with this reagent may even be obtained with the intestinal contents. If one takes into consideration the large amount of food and water that normally pass through the abomasum a great deal of blood must continuously flow into the abomasum, in order to produce this discolouration of the abomasal and even the intestinal contents.

In most cases the anaemia begins to develop from the third to the sixth week after artificial infection. If it is considered that it takes three weeks for the parasites to become adult and allowance is made for an initial stimulating effect resulting in an increase in the number of red cells, it seems doubtful that toxins could produce the anaemia in such a short period of time. Owing to the relatively few cases in which a progressive anaemia developed, no attempt was made to destroy the parasites in an animal in the later stages of the disease, in order systematically to trace the regenerative changes in the blood during recovery, but from practical experience it can be stated that infested animals nearly always recover and rapidly improve after appropriate medicinal treatment. It seems unlikely that a bone-marrow, which has been so severely damaged by toxins that it cannot maintain even two million cells per c.mm. of blood, should immediately the toxins are removed be capable of producing sufficient cells to meet the ordinary requirements of the body.

It is now necessary to consider whether or not (1) the morphological changes in the red cells, (2) the changes in the various indices recorded, and (3) the pathological lesions described support the contention, that the anaemia is due to haemorrhage along the alimentary canal.

(1) *Morphological Changes.*

These are, briefly, poikilocytosis, polychromasia, punctate basophilia, and "pessarformen."

Poikilocytosis.—It seems to be generally agreed that this condition is of no great diagnostic value, although Piney (1927) regards it as a more definitely abnormal mode of regeneration that is anisocytosis, as it never occurs in normal individuals.

Polychromasia.—Naegeli (1923), Schilling (1922), Krumbhaar (1928), and other authors recognize this as a sign of youth of the cell, and as such it must be associated with an actively functioning bonemarrow. Piney (1927) states that polychromasia is simply a condition of slight immaturity of the red corpuscles.

Punctate basophilia.—Naegeli and other authors maintain that cells so affected are products of embryological or pathological reactions on the part of the bonemarrow, and are clinical manifestations of pathological regenerative changes; but Krumbhaar states that " it is still doubtful if they are signs of youth or, as most authorities believe, of degeneration." The same author quotes Lehmann, who believes that basophile granules may be manifestations of either condition.

As punctate basophilia is absent from the blood in aplastic anaemia, Piney (1927) concludes that it is of regenerative significance, but the fact that these cells showing this do not occur in embryonic blood indicates that they are the products of abnormal regeneration.

" Pessarformen."—These are large pale cells, which according to Naegeli indicate degenerative forms or an insufficiency in the formation of red cells in the bonemarrow. Such cells occur in the so-called secondary anaemias, and in those stages of haemonchosis when the limits of compensation have been reached. The presence of such cells may possibly be explained by the haemoglobin deficiency developed on account of removal of blood along the alimentary canal.

(2) *Changes in the Volume Index, Colour Index, and Volume Colour Index.*

In a number of cases there is a definite increase in the volume index and in the colour index. The increase in these indices is due to a nincrease in the size of the red cells. Some of these large cells are fully haemoglobiniferous and can appropriately be classified as megalocytes. Many of the macrocytes show polychromatic staining and others contain punctate basophilic granules. These macrocytes must be regarded as young cells and are the output of an actively functioning bonemarrow, which is reaching the limits of compensation, and is no longer capable of turning out fully mature cells with the result that young cells must be pushed into the circulation. That the blood-forming organs are showing signs of exhaustion in the later stages of the disease is confirmed by the decrease in the volume colour index, and by the presence of pale cells, both of which indicate that the cells are deficient in haemoglobin. These changes in the morphology of the red cells and in the various indices are exactly what one would expect when internal haemorrhage occurs. On referring to Table 12 below, it will be seen that almost identical changes occurred in the red cells of a sheep which had been bled from the jugular vein until a severe anaemia developed.

TABLE 12.

SHEEP No. 20715.

Bled from jugular vein until anaemia devoleped.

Date.	Source.	R.C.	R.P.	Hglb. %	W.C.	V.I.	C.I.	V.C.I.		Remarks.
4/11/30	Jugular	13·2	46	113	9,200	1·00	1·00	1·00	bled 600 c.cs.	—
5/11/30	,,	10·5	40	99	7,500	1·09	1·10	1·00	,, 600 ,,	—
6/11/30	,,	8·3	29	81	10,900	1·00	1·17	1·11	,, 500 ,,	—
7/11/30	,,	6·0	21	—	9,700	—	—	—	,, 400 ,,	—
8/11/30	,,	5·6	19	52	7,300	0·97	1·13	1·06	,, 400 ,,	—
10/11/30	,,	4·6	18	51*	4,700	1·12	1·35	1·15	,, 500 ,,	Some megalocytes present.
11/11/30	,,	4·0	15	41	8,000	1·04	1·20	1·11	,, 200 ,,	Cells are well preserved, megalocytes present.
12/11/30	,,	2·7	12	33	8,000	1·27	1·51	1·12	,, 200 ,,	No smears.
13/11/30	,,	3·3	15	41	8,200	1·30	1·56	1·11	not bled	Microcytes, macrocytes, polychromasia, and punctate basophilia.
14/11/30	,,	3·6	16	42	9,700	1·28	1·36	1·07	bled 400 c.cs.	Many large cells less marked, polychromasian and punctate basophilia.
15/11/30	,,	2·9	15	38	9,700	1·48	1·53	1·03	,, 200 ,,	No smears made.
17/11/30	,,	3·5	16	43	7,900	1·30	1·44	1·09	not bled	Reticulocytes with vital staining.
18/11/30	,,	3·7	17	45	4,100	1·32	1·42	1·08	killed	Punctate basophilia and polychromasia reticulocytes numerous.

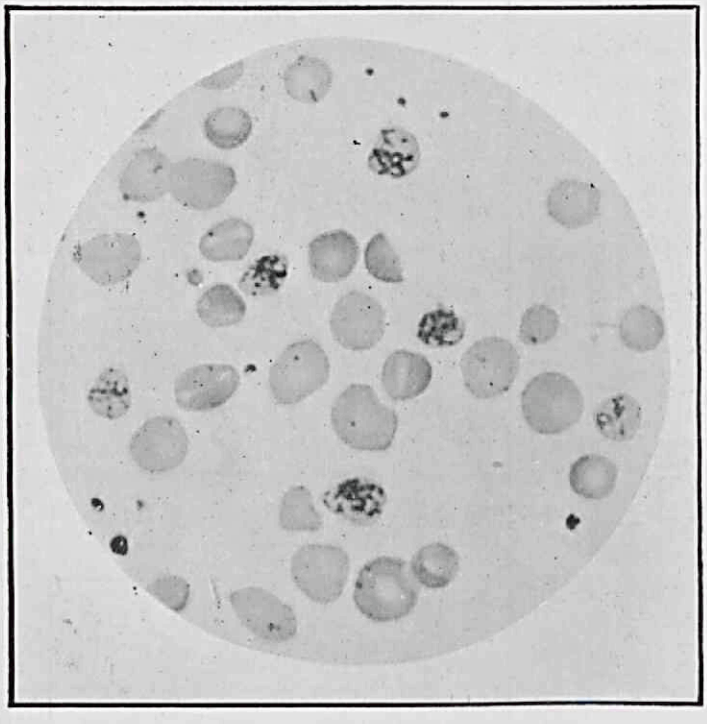
* Hemoglobin determination not reliable.

For abbreviations, see Table 1.

For method of calculating colour indices, see Table 4.

This sheep was bled almost every day, up to 600 c.cm. of blood being removed from the jugular vein at a time. Although large cells such as megalocytes were easily recognized microscopically previous to 13.11.29 macrocytes with polychromasia and punctate basophilia were for the first time numerous on this date, cells measuring up to 8.1μ being present. On 17.11.29, the blood was vitally stained and numerous reticulocytes were recognized. Just before the animal was destroyed on 18.11.29, the blood was again vitally stained, and the reticulocytes were exceedingly numerous on this date. The coverslips were removed from some of these vitally stained preparations, the blood was spread on the slide and after quick drying was stained in

FIG. 14.
SHEEP No. 20715.



×1350

Reticulocytes with vital staining.

the usual way with Giemsa. In these permanent preparations, a microphotograph of which is reproduced (Fig. 14), no polychromasia is present, but the reticulocytes are numerous. In the ordinary smears, stained by Giemsa, there are numerous cells showing polychromasia, and it would seem that the polychromatic cells in smears not vitally stained are the same as the reticulocytes in the vitally stained smears. This would tend to support those haematologists such as Ferrata (1909), Schilling (1922), and others quoted by Krumphaar (1928), who believe that the " vitally stained reticulum is an artificial clumping of the same substance that with Romanowsky methods stains diffusely as polychromatophilia."

The colour index and the volume index in this case of external bleeding is definitely increased. This is due, as in haemonchosis, to an increase in the size of the red cells. The volume colour index remains normal, whereas in haemonchosis it is decreased. If the animal could have been bled over a longer period of time, a deficiency in haemoglobin, with a decrease in the volume colour index would probably also have occurred.

It is noteworthy that the colour index, the volume index, and the volume colour index in some of these cases of haemonchosis are exactly like those of pernicious anaemia in the human subject. There is also a similarity in the morphological changes that occur in the red cells, e.g. anisocytosis, poikilocytosis, polychromasia and punctate basophilia, although undoubted megaloblasts, as such, were never identified in cases of haemonchosis. In pernicious anaemia there is a leucopenia and a relative lymphocytosis and Piney (1927) regards this as evidence of defective myeloid function. There is, on the contrary, a tendency towards a leucocytosis in haemonchosis, which would tend to support the view that in haemonchosis the bonemarrow is not defective.

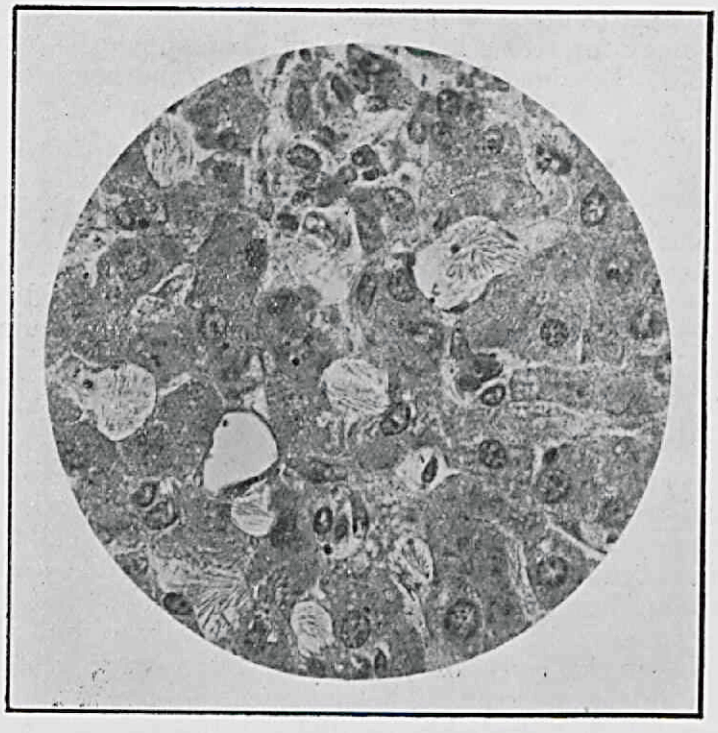
(3) *Pathological Changes.*

Except for the myeloid hyperplasia in the fat bonemarrow and other evidence of active erythropoiesis, the lesions in pernicious anaemia of the human subject are entirely dissimilar to those of haemonchosis in sheep. The erythrophagocytosis, myeloid metaplasia, and haemosiderosis, which occur in pernicious anaemia, find no counterpart in the lesions of haemonchosis. From this it can be concluded that the injury to or the disturbance in the red cells must be different in the two conditions.

The lesions that are most consistently encountered in haemonchosis of sheep are (1) lesions resembling necrosis around the central veins of the liver, and (2) the presence in the cells around the central veins of substances which stain with Sudan III and with Nile Blue Sulphate, but which are soluble in alcohol; and further the presence in some of the cells, not only of the liver, but also of most of the organs of the body, of peculiar crystals, which resemble those which occur in fat necrosis. The presence of these substances which are not true fats but probably related substances, may be associated with changes in fat metabolism in the liver and elsewhere. All these lesions are probably dependent on circulatory and nutritional disturbances, due to the anaemia. The peculiar distribution of the lesions just around the central veins of the liver suggest this. Owing to the anaemia, the total amount of oxygen is deficient. In the case of the liver, those parts first reached by the blood will readily absorb much of the available already depleted oxygen, and by the time the blood has flowed to the region of the central veins, sufficient oxygen is no longer available, and these peculiar lesions of characteristic distribution develop. MacCallum (1922) mentions a number of conditions of the liver, e.g., chronic passive congestion, chloroform poisoning, etc., where the lesions are confined to the cells around the central veins, and he makes the statement that " one cannot doubt the determining influence of the bloodstream in producing these

differences of distribution, although it is not always easy to understand it." This author believes that, in chronic venous congestion, the peculiar distribution of the lesions around the central veins can probably be explained on the basis of a circulatory disturbance, and that those cells which receive the blood last are poorly nourished and do not receive the proper supply of oxygen. It is nevertheless of importance to consider whether such lesions can be produced by toxic substances circulating in the bloodstream. In this connection one would expect that those portions of the liver, namely at the periphery of the lobules, which are first reached by the blood, should show, in a greater measure, the effects of the injury; but Opie, quoted by

SHEEP No. 15786: SPEC. No. 6833.
FIG. 15.



×450.

Crystals in Cells of Liver.

MacCallum (1922), maintains that in intense infections, and especially where a toxic injury is combined with bacterial infection, necrosis may occur in a part of the lobule midway between its periphery and the central vein. It seems, therefore, that on the basis of the distribution of the lesions alone, the possibility of toxic action cannot be absolutely excluded.

The crystals described are ~~most~~ not specific for wireworm infestation. They are present in the cells of the livers from sheep which had been splenectomized by De Kock and Quilan (1926), and which subsequently developed anaemia due to anaplasmosis. They are also present in the liver cells of sheep that were bled until a severe degree of anaemia developed. The section shown in Fig. 15 is from such a

case. Their presence seems to be consistently associated with anaemia of the sheep, although they were also recognized in the liver of some cases of heartwater, but were absent in other cases of this disease. It is, however, not by any means certain that all the sheep which died from heartwater were entirely free from parasitic infestation. The crystals were demonstrable in organs which were cut and examined within a few days after formalin fixation and also when these organs after preliminary fixation in formalin had been kept for months in Kaiserling. That they are not artefacts produced in the Kaiserling is further proved by their absence from the cells of many other organs that were so preserved.

It can, therefore, be concluded that: (1) the wireworms ingest blood and pierce and cut the mucous membrane and underlying tissues of the abomasum, as a result of which there is constant haemorrhage into the abomasum. This causes discolouration of the abomasal and intestinal contents.

(2) There is a rapid production of the anaemia in severe cases of wireworm infestation, with prompt and complete recovery when the parasites have been destroyed.

(3) The anisocytosis, the polychromasia, the Jolly bodies and probably also the punctate basophilia, the active erythropoiesis with myeloid hyperplasia, the plus colour index, and the plus volume index indicate active regenerative changes and must be associated with an actively functioning bonemarrow.

(4) The poikilocytosis, the pale cells (pessarformen), and the decrease in the volume colour index indicate commencing exhaustion of the blood-forming tissues.

(5) The lesions in the liver and elsewhere are probably due to circulatory and nutritional disturbances and changes in fat metabolism, dependent on the anaemia, but the possibility that toxins may produce such lesions cannot be entirely excluded.

(6) With the exception of the lesions, like those of necrosis, occurring around the central veins and the decrease in the volume colour index, identical haematological and pathological changes are present in acute cases of *Haemonchus contortus* infestation in sheep, as also in sheep bled daily from the jugular vein until a severe degree of anaemia has been produced.

In view, therefore, of the facts that: (*a*) there is no haemolysis, (*b*) there is no evidence of a primary disturbance in the blood-forming organs, which interferes with the normal replacement of red cells daily eliminated in the process of normal wear and tear, (*c*) there is very little, if any, erythrophagocytosis, and (*d*) there is positive evidence that the worms ingest blood and undoubtedly cause considerable haemorrhage into the abomasum, it is justifiable to conclude that the anaemia is due (1) to the haemorrhages which occur at the points where the worms attach and detach, and (2) to probably a much smaller extent to the ingestion of blood by the parasites.

When numerous parasites are present, a marked anaemia can be produced in a relatively short period of time. The initial effect of this removal of blood is to stimulate the blood-forming organs to

increased activity, resulting in a temporary polycythaemia or erythrocytosis. Very soon, however, a progressive decrease in the number of red cells takes place and when the bonemarrow can no longer meet these enormous demands for red cells, a compensatory myeloid hyperplasia, which also involves the fatty bonemarrow, takes place. Eventually a state of affairs is reached when the blood-forming organs cannot supply sufficient adult cells for the circulation, and young immature forms are released into the circulation. This explains the anisocytosis, the polychromasia, the Jolly bodies, the plus volume index, the plus colour index, and probably also the punctate basophilia. During the further course of the disease, however, the bonemarrow gradually becomes exhausted, degenerative forms, such as poikilocytes and pale cells, make their appearance, and with the enormous elimination of haemoglobin along the alimentary canal, there is eventually also a haemoglobin dificiency in the cells, as is indicated by the decrease in the volume colour index. As a result of the anaemia there is probably a disturbance in the fat metabolism of the body. This leads to the appearance of related fatty substances and crystals in the cells of various organs, but particularly of the liver. Further, lesions like those of necrosis, dependent probably on circulatory and nutritional disturbances due to the anaemia, develop around the central veins of the liver.

That somewhat similar lesions and practically identical haematological changes occur when sheep are bled from the jugular vein until a severe degree of anaemia develops is very strong confirmatory evidence that the changes in the blood picture of haemonchosis in sheep are not due to a primary disturbance in the bonemarrow, but are the result of, and secondary to, the anaemia.

SOME UNUSUAL LESIONS OF HAEMONCHOSIS.

On three occasions infarcts were found to be present in the hearts of sheep that had a severe wireworm infestation. In the one case an embolus or thrombus was located in the left coronary artery, in which it formed a nodule that could easily be felt with the finger. On the vessel being opened and embolus or thrombus removed, two smaller branches of the left coronary artery were found to be completely occluded at this point, and that portion of the myocard supplied by these two branches was of a dark red colour. The area involved in the infarction measured 5 by 2 cm. Fig. 16. No emboli or thrombi could be detected in the lungs. The valves and endocardium of both the left and right portions of the heart were in no way diseased. In this case the origin of the emboli could therefore not be traced. Even theoretically it is difficult to conceive in what way the wireworm infestation, or even the anaemia produced by it, could cause the formation of emboli or thrombi in the coronary arteries.

In this same animal there were numerous ulcers in the abomasum. In many cases, as will be seen from the accompanying photograph (Fig. 17), numerous wireworms are attached to these ulcers. In some cases the ulcers are elliptical and measure as much as 3 by 2 cm., in other cases they are very irregular in outline. The depth to which the ulcers extend into the wall of the stomach, varies very much:

FIG. 16.—SPECIMEN No. 9461.

1. Left coronary artery.
2. Branches of left coronary artery completely occluded.
3. Infarction resulting from occlusion of vessels. (See 2.)

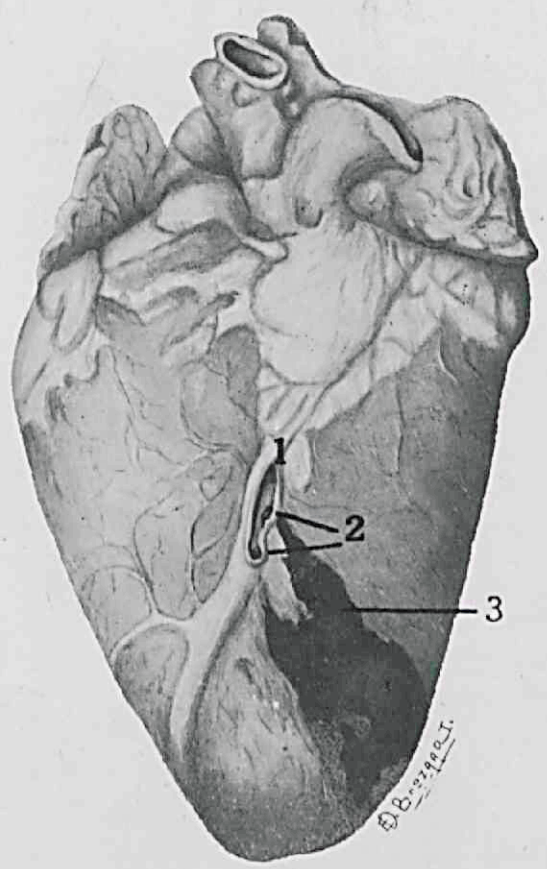
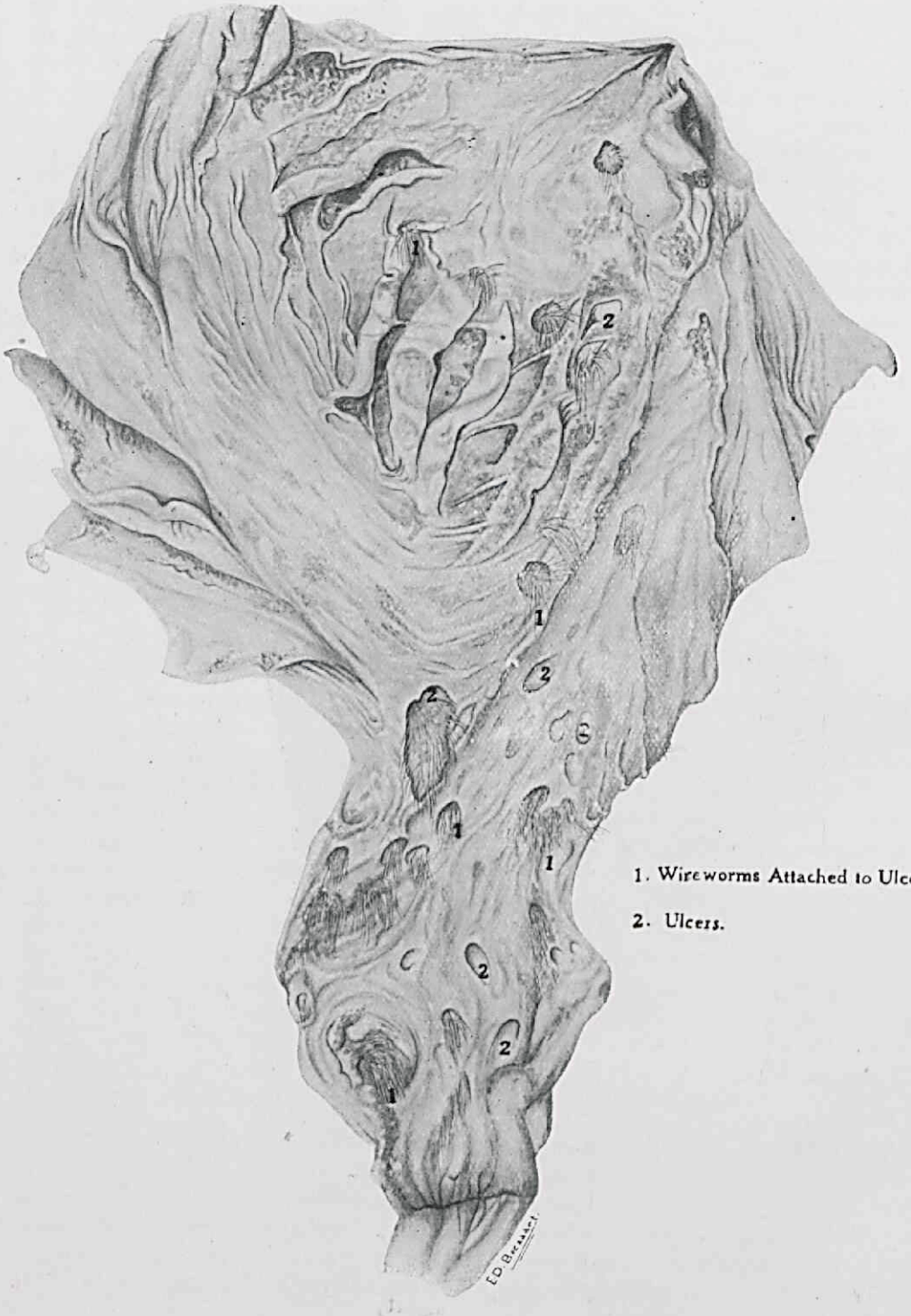


FIG. 17.—SPECIMEN No. 9461.

1. Wireworms attached to ulcers.
2. Ulcers.



some are superficial while others penetrate deeper into the submucosa, and in the more severe cases they may even extend through the muscular layers and are then apparently only limited by the serosa.

I am not aware that the view has ever been advanced that wireworms can be responsible for ulcers in the abomasum. It seems probable that the injury inflicted on the mucous membrane by these parasites may, in some cases, upset its normal protective mechanism, and there may then develop peptic digestion of the stomach wall; or certain bacteria may be responsible for the destruction of the issues in a secondary manner. That the worms themselves destroy the tissues and cause the ulcers, is most unlikely. It is more difficult to understand the pathogenesis of the infarction of the heart. No thrombi could be detected in the lungs, nor were the valves and endocardium in any way diseased. It is possible that bacteria gained entrance to the circulation through the ulcers in the abomasum, and were able to produce injury to the intima of the coronary artery, with thrombus formation in a heart whose resistance was decreased as a result of the anaemia. In anaemia the heart is undoubtedly called upon to do a great deal of extra work, as the smaller number of red cells must circulate so much more frequently in order to supply the necessary amount of oxygen. This would occur in spite of the presence of certain compensatory factors. It is, for example, well known that sheep with severe anaemia from wireworm infestation are easily fatigued; and the probalitiy is that these animals are less active than normal, and consequently do not require the same amount of oxygen as do normal animals.

SUMMARY.

(1) Sheep blood takes longer to precipitate than horse blood. When centrifuging normal citrated sheep blood in an electric centrifuge running at 1,500 revolutions per minute, one obtains constant readings in 60-70 minutes time. Under similar conditions, the blood of sheep with severe anaemia will give constant readings in 20-30 minutes.

(2) Uniform relative results are obtained when haemoglobin determinations on sheep blood are made in a Dubosq colorimeter, using a Newcomer disc as standard.

(3) In order that reliable colour, volume, and volume colour indices may be calculated, it is advocated that normality for each individual should first of all be established during the pre-infection period.

(4) Of 38 worm-free sheep that were artificially infected with pure faeces cultures of *Haemonchus contortus* larvae, 9 developed a fatal progressive anaemia, and 6 developed anaemia from which they recovered without any medicinal treatment (so-called " recovery cases "). One further recovery case resisted repeated attempts at infestation with large doses of wireworm larvae. The remaining 23 sheep either resisted infection or did not show any effects of infestation

(5) In haemonchosis there is (*a*) no icterus, haemoglobinaemia or haemoglobinuria, because haemolysis does not occur; (*b*) no evidence of a primary disturbance in the blood-forming organs, interfering with the normal replacement of red cells which daily are being eliminated in the process of normal wear and tear. Any disturbance that is present in the bonemarrow is secondary to the anaemia; and (*c*) very little, if any, erythrophagocytosis.

(6) The wireworms ingest blood and cause haemorrhage into the abomasum, resulting in a chocolate discolouration of the abomasal contents. This haemorrhage and the ingestion of blood by the parasites is the cause of the anaemia, which shows the following characteristics: (*a*) there is probably an initial polycythaemia or more particularly an erythrocytosis due to the stimulating effect produced by the haemorrhage, etc. (*b*) Regenerative forms of erythrocytes are present, e.g., cells showing anisocytosis, polychromasia and Jolly bodies. Punctate basophilia is also present and can probably also be included with regenerative forms. When such regenerative forms and megalocytes predominate, there is an increase in the colour index and the volume index, due mainly to the increase in the size of the red cells. (*c*) Degenerative forms are present, e.g., pale cells (pessaformen), poikilocytes, and possibly punctate basophilic cells. These indicate commencing exhaustion of the blood-forming organs and associated with them is a decrease in the volume colour (saturation) index, pointing to a relative deficiency in haemoglobin.

(7) No lesions which can be regarded as specific for haemonchosis are present. A myeloid hyperplasia is present which also involves the fat bonemarrow, and is secondary to the anaemia. Lesions like those of necrosis immediately around the central veins and the presence of related fatty substances and crystals in the cells of the liver, as well as in cells of other organs, are probably due to circulatory and nutritional disturbances, and to disturbances in the fat metabolism of the body as a result of the anaemia.

(8) With the exception of the lesions, like those of necrosis, occurring around the central veins of the liver, and the decrease in the volume colour index, identical haematological and pathological changes are present in acute cases of *Haemonchus contortus* infestation in sheep and in sheep bled daily from the jugular vein, until a severe degree of anaemia has been produced.

(9) There is a neutrophile leucocytosis towards the end of the disease.

(10) The colour index, the volume index, and the volume colour index of haemonchosis are exactly like those of many cases of pernicious anaemia in the human subject. The morphological changes of the red cells are very similar to those of the latter disease except that, in the fatal cases of haemonchosis studied, no megaloblasts were identified. The lesions, however, are entirely dissimilar, and haemosiderosis, erythrophagocytosis, and the myeloid metaplasia of pernicious anaemia are not present in haemonchosis. From this it is evident that the nature of the injury in or the disturbance to the red cells must be different in the two conditions.

(11) Some unusual lesions, e.g., infarcts in the heart and ulceration of the abomasum, were on rare occasions found in fatal cases of haemonchosis.

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LITERATURE.

BRYUCHONENKO, S., UND STEPPUHN, O. (1927). Experimentelles zur Anwendung von "Germanin" (Bayer 205) bei Bluttransfusionen. *Munchener Medizin Wochensch.* No. 31, p. 1316.

CANHAM, A. S. (1930). The blood of cattle. *16th Report of the Director of Veterinary Services and Animal Industry.* Onderstepoort. p. 531-566.

CHANDLER, ASA C. (1929). Hookworm Disease. The MacMillan Co., New York, p. 249-251.

DE KOCK, G. (1923). A Study of the Reticulo-endothelial System of the Sheep. *13th and 14th Reports of the Director of Vet. Educ. and Res.*, Onderstepoort, prt. 2, p. 647-723.

DE KOCK, G., AND QUINLAN, J. (1926). Splenectomy in Domesticated Animals and its Sequelae, with special reference to Anaplasmosis in Sheep. *11th and 12th Reports of the Director of Veterinary Education and Research.* Onderstepoort. Part I, p. 369-405.

DONATIEN ET LESTOQUARD. Abstract on Revue Gle de Medicine Veterinaire J. 37, No. 434.

FERRATA, A. (1909). Uber die klinische und morphologische Bedeutung der *vitalfärbbaren Substanz und die basophile Punktierung der Erythrozyten. Folia Haemotologica.* Bd. 9, p. 253-273.

FLU, P. C. (1922). Onderzoekingen over de oorzaken der anaemie bij Ankylostomiasis. *Tijdsch. voor Vergelijkende Geneeskunde, enz.* Nieuwe Reeks, Deel 2, aflevering 1, Deel 7, p. 47-72.

FOURIE, P. J. J., AND ZIEHN, T. (1930). A study of a case of Aleucaemia in the Dog. *16th Report of the Director of Veterinary Services and Animal Industry.* Union of South Africa, p. 337-360.

HADEN RUSSEL, L. (1925). The volume and haemoglobin content of the erythrocytes in health and disease. *Folia Haematologica*, Bd. 31, Heft 2, p. 116 (p. 113-135).

HADWEN, S. (1925). Ascariases in Horses. *The Jnl. of Parasitology.* Vol. 12, No. 1, p. 1-10.

HALL, M. C. (1920). Parasites and parasitic diseases of sheep. *Farmers' Bulletin No.* 1150, U.S. Dept. Agric., pp. 1-53.

HUTYRA AND MAREK (1926). Special Pathology and Therapeutics of the Diseases of Domestic Animals. Vol. 2, p. 325.

KRUMBHAAR, E. B. (1928). The Erythrocytes Special Cytology. Vol. 1, edited by E. V. Cowdry, p. 275-317.

MACCALLUM (1922). A Textbook of Pathology. Second Edition. W. B. Saunders Co., p. 313-315.

MALLORY & WRIGHT (1924). Pathological Technique. 8th Ed., p. 182-185.

MHASKAR, K. S. (1924). Report of the Ankylostomiasis Enquiry. Madras Indian Medical Research Memoirs, Vol. I.

MÖNNIG, H. O. (1930). Studies on the Bionomics of the Free-living Stages of Trichostrongylus spp. and other Parasitic Nematodes. *16th Report of the Dir. of Veterinary Services and Animal Industry.* Onderstepoort. p. 175-198.

NAEGELI, O. (1912). Blutkrankheiten und Blutdiagnostik, p. 140.

NAEGELI, O. (1923). Blutkrankheiten und Blutdiagnostik. 4 Auflage. Julius Springer, Berlin, p. 109 and 437-439.

NESER, C. P. (1923). The Blood of Equines. *9th and 10th Rept. of the Director of Veterinary Education and Res.* Onderstepoort, p. 481-556. (From a thesis for D.Sc. presented in 1921 in the University of South Africa.)

NEWCOMER, H. S. (1919). Absorption of spectra of acid haematin, oxy-haemoglobin and carbon-monoxide haemoglobin. A new haemoglobinometer. *Jnl. of Biol. Chemistry,* Vol. 37, p. 465-496.

NEWCOMER, H. S. (1923). A new optical instrument for the determination of haemoglobin. *Jnl. of Biol. Chemistry,* Vol. 55, p. 564-574.

NICOLL, W. (1914). The blood volume in Ankylostomiasis. *The Jnl. of Hygiene,* Vol. 13, No. 4, p. 369-392.

PINEY (1927). Recent advances in Haematology. I. & A. Churchill, London, p. 1-272.

PYPER, A. (1929). An improved diffraction method for diagnosing and following the course of pernicious and other anaemias. *Rept. of the British Medical Journal,* p. 1-10.

ROSSDALE, C. (1923). Observations with the Haematocrit, Volume-Colour-Index. *Quarterly Jnl. of Medicine,* Vol. 16 (1922-1923), p. 245-262.

SARLESS, M. P. (1929). Studies of the blood changes occurring in young and old dogs during cutaneous and oral infection with the Dog Hookworm *Ancylostoma caninum. The Amer. Jnl. of Hyg.,* Vol. 10, No. 3, p. 693-704.

SCHILLING, V. (1922). Das Blutbild und seine Klinische Verwertung. Zweite Auflage, p. 48.

SCHRIDDE, H., UND NAEGELI, O. (1921). Die Haematologische Technik. Zweite Auflage, p. 123-127.

SCHWARTZ, B. (1921). Hematoxins from parasitic worms. Reprinted from the *Jnl. of Agric. Research,* Vol. 22, No. 8, Washington, D.C., p. 379-432.

SCOTT, J. A. (1930). The Biology of Hookworms in their Hosts. *The Quarterly Review of Biology,* Vol. 5, No. 1, p. 79-97.

STOLL, N. R. (1929). Studies with the Strongyloid Nematode. *Haemonchus contortus. The Amer. Jnl. of Hyg.,* Vol. 10, No. 2, p. 384-418.

TALIAFERRO, W. (1929). The Immunology of Parasitic Infections. The Century Co., New York-London, p. 269-273.

TERRILL, E. H. (1922). On the colorimetric determination of haemoglobin, with special reference to the production of stable standards. *Jnl. of Biol. Chem.,* Vol. 53, p. 179-191.

VEGLIA, F. (1915). The Anatomy and Life-history of the *Haemonchus contortus* (Rud.). *3rd and 4th Repts. of the Dir. of Vet. Res.,* Union of South Africa, p. 432.

VEGLIA, F. (1918). Chemotherapy of Haemonchosis in Sheep. *5th and 6th Repts. of Dir. of Vet. Res.,* Union of South Africa, p. 420.

VEGLIA, F. (1928). Oesophagostomiasis in Sheep (Preliminary Report). *13th and 14th Repts. of the Dir. of Vet. Educ. and Res.,* Part 2, p. 756-759.

WINTROBE, M. H. (1930). Classification of the anaemias on the basis of differences in the size and haemoglobin-content of the red corpuscles. *Proceedings of the Society for Experimental Biology and Medicine,* Vol. 27, No. 91, p. 1071-1073.

STELLINGEN

I.

De toediening aan vee van oplosbare phosphaten in het drinkwater, in phosphor-arme streken van Zuid-Afrika, heeft vele voordeelen boven andere methoden van toediening van deze stoffen.

ERRATA

Page 12, 3rd last paragraph, 3rd last line read *of* for *on*.

Page 15, 2nd paragraph, 3rd line read *conjunctival* and *anal* for *conjunctivat* and *anae*.

Page 37, last line read *as* for *at*.

Page 54, 2nd paragraph, 3rd line read *course* for *coure*; 8th last line read *is* for *in* before *becoming*.

Page 61, 2rd paragraph, 5th line read *ontogenetic* for *ontogenitic*.

Page 66, last paragraph, 3rd line, read *interfering* for *interferes*.

Page 72, last paragraph, 1st line read *not* for *most*.

Bij de behandeling van parese van de voormagen van het rund is de toediening van ammoniumcarbonaat en nux vomica van bijzondere waarde.

VI.

De nieuwere physiologische gegevens omtrent de slokdarmsleuf *(Wester)* kunnen dienen als uitgangspunt voor een rationeele bestrijding van gastro-intestinale wormziekten bij de herkauwers.

STELLINGEN

I.

De toediening aan vee van oplosbare phosphaten in het drinkwater, in phosphor-arme streken van Zuid-Afrika, heeft vele voordeelen boven andere methoden van toediening van deze stoffen.

II.

De aanwezigheid van morphologisch niet van Koch'sche lichaampjes te onderscheiden vormsels is niet altijd een zekere aanwijzing voor Oostkust-koorts.

III.

Een sporenhoudende miltvuurentstof is in het algemeen meer betrouwbaar en doeltreffend, dan miltvuurentstoffen, die bestaan uit de bacillen.

IV.

In sommige gevallen van aleucaemische lymphatische leucose van honden treedt niettegenstaande een uitgebreide vernietiging van beenmerg, slechts een geringe anaemie op. Dit is het gevolg van een myeloïde metaplasie in de milt, met overwegende vorming van erythrocyten.

V.

Bij de behandeling van parese van de voormagen van het rund is de toediening van ammoniumcarbonaat en nux vomica van bijzondere waarde.

VI.

De nieuwere physiologische gegevens omtrent de slokdarmsleuf *(Wester)* kunnen dienen als uitgangspunt voor een rationeele bestrijding van gastro-intestinale wormziekten bij de herkauwers.

VII.

De functioneele toestand van het maagdarmkanaal is van principieele beteekenis voor de werking van therapeutische clysmata bij de oesophagostomiasis van schapen.

VIII.

De optimale doseering van een sporenhoudende miltvuurentstof kan beter bepaald worden langs biologischen, dan langs morphologischen weg (tellen der sporen).

IX.

De normale ontwikkeling van Oesophagostomum columbianum bij het schaap verloopt in de darmwand vrijwel zonder reactie.