THROMBOCYTES. (Plate 1, Figure 7).

These cells, which in fowl blood have been named hematoblasts by Hayem (1879) and by Goodall (1910), are usually oval, nucleated and are very unevenly distributed on the slide. They occur in pairs or groups of three or more, but are often seen singly. They measure usually about 8.8u. in length and 5.3u. in breadth. Occasionally a thrombocyte almost twice the normal size may be seen.

The nucleus is oval and usually occupies about three-quarters of the length and nearly the entire width of the cell. It is usually situated in the central part of the cell and is very basophilic, the basi-chromatin being arranged to form a coarse pattern.

The cytoplasm stains a pale grey and ordinarily there may be one or more acidophil granules. These are usually near the poles of the nucleus. The cytoplasmic rim is not always well marked. In films not prepared immediately after the vessel has been punctured the thrombocytes can be seen in masses in which it is difficult to distinguish the outline of individual cells. Both the cytoplasm and the nucleus become less distinct, the cytoplasm losing its structure first. Finally the cells appear merely as a mass of darkly stained roundish bodies. When thrombocytes have become thus transformed they may be mistaken for small lymphocytes but the cells can be differentiated by the following features: The outline of the lymphocyte, which is usually completely circular, is well marked, whereas that of the thrombocyte is very irregular and usually the cytoplasm of the lymphocyte does not appear to surround the nucleus completely.

In the counting chamber the thrombocytes when stained with Wiseman's solution, about which particulars are given under "Leucocyte Counts", look mottled

and.../

and the red granules in the cytoplasm can usually be seen. No attempt, however, was made to count the thrombocytes in the counting chamber, for it was felt that the criteria by which these cells may be discriminated from free nuclei and small lymphocytes are not prominent enough to make accurate counting possible.

LYMPHOCYTES (Plate 1, Figure ?).

These cells are spherical and vary in size from 5.3u. to 14.1u. They may be arbitrarily classed as small, intermediate and large lymphocytes, though the cells apparently do not belong to separate families but to one series. This was the conclusion arrived at by Magath and Higgins (1934) who, in order to test the validity of the theory that there are classes of lymphocytes based on size, measured the diameters of 100 lymphocytes from each of eight ducks, and plotted curves.

In the smaller cells the nucleus is usually round, but it may be oval or slightly indented and it may be situated centrally or eccentrically. It usually consists of heavy blotches of chromatin but sometimes its structure is almost homogeneous. The nucleus is stained purple. It occupies by far the greater part of the cell and in the smallest cells the cytoplasm is scareely seen. The cytoplasm is pale blue and usually takes the stain better at the periphery than towards the nucleus, with the result that there is a sort of "halo" near the nucleus. The cytoplasm forms only a very narrow layer and usually it does not appear ~~not~~ to surround the nucleus completely. A varying number of azurophil granules may sometimes be seen in the cytoplasm and occasionally lingulate processes may be seen to extend from the cytoplasmic rim. As already stated it

may..../

may be very difficult to differentiate between very small lymphocytes showing very little or no cytoplasm and thrombocytes which have lost their usual structure.

In the larger cells the proportion of cytoplasm to nucleus is much greater than in the small cells and the cytoplasmic colour varies from a pale blue to a distinct blue. Otherwise the cells are identical with the small forms. The large cells often have ~~nother~~ irregular shapes in smears on account of being wedged in between the evythrocytes.[1)]

MONOCYTES (Plate/ , Figure ?).

The cells, which may be considered homologous with the mammalian monocytes, vary in form from round to slightly oval and measure from 8.8u. to 12.4u. in diameter but in the oval forms the long axis may measure up to 21.2u.

The nucleus shows a loosely woven chromatin network, the chromatin being finely divided, and it is usually stained a paler purple than the nucleus of the lymphocyte ~~does~~. It may be nearly round, slightly indented, kidney-shaped or of irregular shape. The kidney-shaped nuclei are rare, however, and are usually seen only in the smaller cells. The nucleus usually occupies about half of the cell in the larger forms, whereas in the small cells it may form three-quarters or more of the cell. It is usually eccentric and usually the cytoplasm does not appear to surround it completely.

The cytoplasm has a greyish-blue colour and is more granular than that of the lymphocyte, having the

ground-glass..../

1) Although the blood forming organs are not being considered in the present work yet it may be mentioned that no lymphatic glands could be found in the ostrich.

ground-glass appearance usually seen in the monocytes of man. Sometimes the cytoplasm is dusted with innumerable minute azurophil granules. Small lingulate runners ~~may~~ sometimes ~~be seen to~~ extend from the cytoplasmic rim.

These cells can be studied best in thin smears prepared very quickly and immediately after the blood has left the vessel. Otherwise many damaged forms may be seen.

HETEROPHILES (Plate , Figure).

These cells measure from 8.9u to 17.7u. They are spherical and have polymorphous nuclei which stain a reddish violet colour. Sometimes the nucleus consists of two or three lobes connected either by a thin or a thick strand of chromatin and usually the lobes are more or less oval. In ordinary stained preparations the lobes may sometimes appear to be quite apart, but when smears are stained with the "Analine-water thionin solution" as recommended by Pepper and Farley (1933) the connection, which is sometimes only a very fine thread of chromatin, can be easily seen.

The cytoplasm is filled with large spindle and rod-shaped granules which measure about 1.75u in length. These stain a light red colour and give a negative oxydase reaction. In the young cells the granules are roundish. On a few occasions peculiar staining effects were observed. The granules of practically all the heterophiles became transformed into small round granules, as seen in plate ?, figure ?, whereas the granules of the eosinophiles looked the same as usual. It was shown that this was simply a fixation artefact, for, in smears prepared at the same time from the same bird, the granules

were..../

were as ordinarily seen. When Wright's stain was added to smears and the stain immediately washed off again, it was noticed that the granules also appeared rounded ~~like those which were produced accidentally~~. Many of the heterophilic granules were also round when vital staining was carried out either by the method described by Kolmer and Boerner (1931) or by that used by Magath and Higgins (1934) for the purpose of demonstrating reticulocytes.

These phenomena are of especial interest, as Lundquist and Hedlund (1925) express the opinion that the heterophilic granules of the fowl are naturally round, only becoming spindle-shaped as the result of fixation and staining. These writers consider the heterophiles and the cells with the round acidophil granules (eosinophiles) to be identical - the eosinophiles being the forms in which the granules retain their round shape. According to Ellerman (1921) the two cell types are distinct from each other as the heterophiles give a negative reaction to Graham's oxydase test, whereas the eosinophiles react positively. Can you confirm this ?

The transformation of haemocytoblasts into heterophilic and eosinophilic myelocytes may be favourably studied in the red marrow of the ostrich, and the independence of the two cell types should be apparent to anyone who examines these cells in the marrow [1]. Though eosinophilic myelocytes have to be searched for in the marrow of the fowl, they are found without difficulty in that of the ostrich.

A few heterophilic myelocytes were once seen in a smear prepared from the blood of a normal ostrich - No.5. The cells were round and measured about 17u in diameter..../

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1) It was observed that the femur of the ostrich is devoid of marrow.

How do these cells differ from lymphs?

diameter. They were completely filled with round coarse granules, some of which were basophilic in reaction. The nuclei were so obscured by the granules that the structure could not be judged. However, in the marrow it will be observed that the nuclei of these myelocytes are round or indented. They are eccentric and stained purple.

EOSINOPHILES (Plate , Figure).

These cells are also round and measure from 8.7u to 15.7u. They are, therefore, of about the same size as the heteropniles.

The nucleus, which stains a reddish violet, may be a single polymorphous structure or it may consist of two lobes connected by either a slender or a thick strand of chromatin. Sometimes the lobes appear to be entirely separate. Usually the nucleus is bi-lobed. The cytoplasm contains discrete round granules whose size and staining are uniform. They stain a bright pink colour in marked contrast to the dull red colour of the granules of the heteropniles and they are also much smaller than the granules of the heteropniles. The eosinophilic granules give a positive oxydase reaction.

BASOPHILES (Plate , Figure).

The basopniles measure from 8.8u to 10.6u in diameter. The cell has a simple round nucleus which is stained a purple colour and shows a diffuse chromatin arrangement. The simple round nucleus is very characteristic of the basophile of the ostrich. It is usually situated to one side of the cell and the cytoplasm does not appear to surround it completely. The cytoplasm contains large granules which stain a very dark purple colour, often with a reddish tinge, and there is considerable variation in the degree of concentration of the granules. Occasionally some of these may be seen scattered over the nucleus. These granules appear to be readily

dissolved.../

dissolved or washed out in the process of staining - for very often no granules will be seen - the cytoplasm which stains a faint blue presenting a reticular appearance. However, granules which are present in the cells after staining has been completed cannot be removed by washing the smears in water. Basophiles without granules can, nevertheless, be recognised by the round, eccentric nuclei and the reticular appearance of the cytoplasm. Oxydase reaction. ?

ERYTHROCYTE COUNTS.

Wiseman's solution was used as diluting fluid for the enumeration of both the red cells and the white cells. Further information about the technique employed in counting the red cells is given under "Leucocyte Counts" where it can be more conveniently considered.

Magath and Higgins (1934) state - with reference to their work on duck blood - "Counts of erythrocytes were made after the blood had been diluted with Hayem's solution to 200. A photographic method not yet reported was used; its accuracy is extremely high."

These workers used Wiseman's solution as diluting fluid in enumerating the leucocytes, but for the erythrocyte counts Hayem's solution was used because the red colour of Wiseman's solution interfered with the photographic process of counting.[1] This photographic method has been described by Berkson, Magath and Hurn (1935).

Elsdon-Dew (1937) describes an electrical apparatus for the performance of blood counts and mentions several advantages which this device has over the routine method.

Detailed data pertaining to the erythrocyte counts of twenty-two ostriches are shown in tables 3 - 10, and in table 12 a statistical analysis of the erythrocyte counts is given.

As.../

[1] Personal communication from Dr. T.B. Magath.

As stated on page 10, counts were made at intervals from each of the birds kept at Mariendahl, and the mean erythrocyte count of all the counts made during the period of about two years from the five normal birds (1 - 5) was 1,894,000 ± 14,939 (Standard deviation 124,000, Coefficient of variability 6.5 percent.). The range was 1,653,000 to 2,266,000. The counts ~~of~~ from bird No.5 gave a comparatively high coefficient of variability which, however, would have been appreciably less but for the low result shown by this bird on 21/1/36.

It is not apparent why this result deviated so much from the others. The bird appeared healthy. Its blood showed no evidence of haemolysis and the accuracy of the result can be vouched for. It will be seen that the red cell counts were not significantly affected by the age or the sex of the birds.

The results from bird No.6 also show a low coefficient of variability and the average count of this bird does not differ much from the mean figure obtained for birds numbered 1 to 5.

The results from ~~on~~ the majority of the clinically healthy birds 7 - 17 are low compared with those from birds 1 - 5, and the difference between the averages of the two groups is statistically significant. As previously stated the faeces of some of these birds were examined and ~~it~~ these contained many worm eggs The low counts may, therefore, be possibly correlated with verminosis as in the case of the worm-infested birds, 18-21, which showed marked oligocythemia.

The figures recorded by Malassez (1872), Hayem (1879, 1889) and by Venzlaff(1911)(table 1) differ appreciably from the average result shown by birds 1 to 5. But the results given by Malassez and by Hayem closely approximate the mean count obtained for birds 7 to 17.

Examination of table 2 shows that the average counts reported for the fowl are considerably higher than those obtained for the ostrich. Available results for other birds also greatly exceed those for the ostrich.

TABLE 12.

Statistical analysis of erythrocyte counts.

Bird No. [1]	Sex	No. of counts	Maximum count per c.mm.	Minimum count per c.mm.	Mean count per c.mm.	Standard error of the mean	Standard deviation.	Coefficient of variability.
1	Male	15	2,064,000	1,840,000	1,976,000	24,090	93,230	4.7
2	"	15	1,903,000	1,653,000	1,790,000	22,435	86,827	4.8
3	"	12	2,017,000	1,806,000	1,920,000	18,349	63,490	3.3
4	Female	15	1,993,000	1,693,000	1,882,000	23,160	89,632	4.7
5	"	13	2,266,000	1,676,000	1,981,000	48,055	173,000	8.7
1,2 and 3	Males	42	2,064,000	1,653,000	1,893,000	17,746	115,000	6.0
4 and 5	Females	28	2,266,000	1,676,000	1,928,000	26,654	141,000	7.3
1 to 5	Males & Females.	70	2,266,000	1,653,000	1,894,000	14,939	124,000	6.5
6	Female	14	2,053,000	1,669,000	1,956,000	27,005	101,000	5.1
7 to 11	Males	5	1,790,000	1,403,000	1,567,000	64,545	142,000	9.0
12 to 17	Females	6	1,790,000	1,430,000	1,595,000	50,000	120,000	7.5
7 to 17	Males & Females	11	1,790,000	1,403,000	1,583,000	37,575	124,000	7.8
18 to 22	Males & Females.	6	1,707,000	806,000	1,331,000	132,500	318,000	23,8

[1] For particulars of birds see pages 9 - 11.

RELATIVE VOLUME OF CORPUSCLES AND OF PLASMA.

An electric centrifuge was used for the purpose of precipitating the red cells, and the precipitation tubes (Zeiss) were 12 cms. long and 0.6 cm. in diameter. As the centrifuge tubes were much wider than the precipitation tubes, the latter were fitted vertically into the centrifuge tubes in this way : In the centrifuge tube two corks were fitted, one at the bottom and one at the mouth. A precipitation tube was fitted into the centre of each cork through a hole made just big enough for it. The tubes were all tested before use, and two tubes were used for each sample so as to check the one against the other.

The arm length of the centrifuge was 19 cm. when measured to the bottom of a centrifuge tube, and, at a top speed of 2,800 revolutions per minute, a limiting value of the red cell volume of ostrich blood was obtained in from 45 minutes to one hour. However, the centrifuge was always allowed to run for at least one and a half hours to make quite sure of the final reading at that speed. On many occasions the centrifuge was run at speeds lower than 2,800 r.p.m. so that results obtained at slower speeds could be compared with those given at top speed. The tubes were spun for 30 minutes at the lowest speed and the results read. The Centrifuging was repeated for another half hour and the readings again noted. This procedure was carried out until two successive readings had the same value. At 1700 r.p.m. the same readings were obtained as at top speed of 2,800 r.p.m. and the results at the lowest speed of 600 r.p.m. differed very little from those shown at the highest speed. But at the lowest speed a period of 5 to 6 hours was usually required before limiting values were obtained. Six typical results are recorded. Samples numbers 1, 2 and 3 were

/whole .....

whole blood and 4, 5 and 6 heparinised blood.

Sample numbers and percentage volume readings.

Speed in r.p.m.	1	2	3	4	5	6
600	54	49.5	48.8	49	54	48
1700	53.6	49	48.5	48.9	53.5	47.5
2800	53.4	49	48.5	48.9	53.5	47.5

Neser (1923), using horse blood, found that the results obtained in 15 minutes were the same whether the centrifuge ran at 250 or 3000 revolutions per minute, and he remarked : "It is not contended that very high speeds may not result in a closer packing of the red corpuscles or that very low centrifuge speeds will give rise to the same degree of packing as very high speeds. But the results point to this fact that, within certain limits of speed, the volume of the red corpuscles is in no way influenced by the speed. The readings obtained by the centrifuge are reliable and in no way dependent upon the speed of the centrifuge for all moderate speeds."

Millar (1925a) by centrifuging human blood at 4350 r.p.m. obtained a final reading of 39.0. At 5700 r.p.m. the final result was 37.1 and at 11600 r.p.m. 35.0. Ponder (1934), using rabbit's blood, obtained a final result of 31.5 at 1700 r.p.m. and at 14000 r.p.m. the reading was 29.5. He remarks: "The attainment of "constant volume" consequently cannot be used as a test for the correct percentage volume having been reached unless we know which speed of the haematocrite produces neither incomplete packing nor compression of the cells, i.e., unless we solve a second problem very similar to the one whose solution is

/being .....

being sought. The speed of 4000 r.p.m. is supposed to be such a speed, but why this particular rate is selected I have not been able to discover. There is certainly no evidence that it is as ideal as it is thought to be."
The observations by Millar and Ponder stress the importance, when recording percentage volume results, of stating both the arm-length of the centrifuge and the speed at which it rotated. This is, unfortunately, not often done.
Facilities did not exist for testing the effects on the corpuscles of ostrich blood of speeds higher than 2800 r.p m., but from repeated observations made it may be stated with confidence that with a centrifuge as specified the same final results may be expected within a speed range of 1700 to 2800 r.p.m.

Special care was always taken to shake the blood in the bottle ~~well~~ thoroughly before it was drawn off with the pipette for the purpose of determining the percentage volume, and to fill the tubes exactly to the 100 mark. The blood was also run from the pipette into the percentage volume tubes with as little delay as possible to prevent sedimentation in the lower part of the pipette. Nevertheless there were sometimes differences in the readings of samples from the same bottle, although the maximum difference never exceeded 1.5.

The recorded percentage volume readings include the white cell volume, for this never once exceeded more than 2 per cent of the blood volume of fresh blood and often constituted even less than 0.5 per cent. Often also it was impossible to observe a well defined margin between the red cell column and the white cell layer.

Neser (1923) made tests to ascertain in how far the centrifuge readings could be relied upon. For this purpose a large quantity of blood was drawn and citrated and this citrated blood was then centrifuged at definite

/intervals .....

intervals until there was evidence of haemolysis. He found that with horse blood the results remained constant for 36 hours and that the percentage volume decreased as soon as any sign of haemolysis appeared. The writer made similar tests with ostrich blood using heparinised, citrated, and also whole blood. The blood was kept in well stoppered bottles and successive fillings of the precipitation tubes were always made from the same sample. Most of the samples were kept at room temperature, which varied from 15°C. to 25 C. according to the time of the year, as the tests were conducted at different times. The other samples, when not required, were kept in cold storage. The results are given in table (13) and it will be observed that contrary to the findings of Neser (1923), who used horse blood, the percentage volume readings of heparinised, citrated, and also whole ostrich blood invariably increase with age and the older the sample the greater the increase. For example, this increase averaged 9.6 in the heparinised samples five days old and kept at room temperature; and the average increase in the five day old heparinised samples kept in cold storage was 6.9 Haemolysis was usually well marked in samples a few days old. It would appear that the plasma of ostrich blood becomes so viscid after a time as to prevent such close packing of the cells as results when the plasma is fresh. This problem is being further investigated. Attempts were made to obtain more results with whole blood kept in cold storage but the blood usually coagulated within 24 hours.

It was noticed that in old samples the increase in volume was particularly marked in the white cell volume. For example, in sample number 4, which is typical, the white cell layer constituted only 1 per cent. of the blood volume when the blood was fresh, but it formed 6.9 per cent. of the

blood/.....

blood volume when the cells were precipitated again five days later.

Although the results show that significant increases in the readings may not be expected even when the blood is 11 hours old, yet in these studies the precipitation of the blood from ostriches 1 - 6 and 18 - 22 was always completed within 3½ hours from the time that the blood was collected. The precipitation of the blood samples from birds 7 - 17 was completed within 6 hours after they were collected, for they had first to be conveyed a distance of over 100 miles.

As already stated under the heading "Preventing Coagulation of the blood", 0.5 c.c. of a 20 per cent. lithium citrate solution to 199.5 c.c. of blood was at first used as anticoagulant but this was later replaced by heparin - 1 mg. powdered heparin to 5 c.c. blood. As the lithium citrate rendered the plasma slightly hypertonic and as the blood was also slightly diluted, observations were made to ascertain to what extent the percentage volume readings of citrated blood differed from those of whole blood. On thirty-five occasions whole blood was collected and centrifuged along with blood collected immediately afterwards in a bottle containing lithium citrate solution. In no case did the reading of the whole blood vary from that of the citrated blood by more than 2 and as will be noted from table 14 the average difference was only 1.36. A reading, very near to that of whole blood, was therefore obtained by adding 1.4 to the percentage volume reading of citrated blood and in this work all percentage volume readings of citrated blood were corrected accordingly.

TABLE 13

PERCENTAGE VOLUME INCREASES.

Sample No.	Temperature	Anticoagulant added	Period which elapsed from time that sample was collected till percentage volume was obtained. 2 hrs	3½ hrs.	5 hrs.	6½ hrs	8 hrs	9½ hrs	11 hrs.	24 hrs.	36 hrs.	48 hrs.	56 hrs.	4 days	5 days	6 days	15 days	30 days
1	15°C-25°C	Heparin	47.1	47	47.5	47.8		48	48	48.2	48							
2	" "	"	50.5	51	51.1	51.1		51	51	52	51.1							
3	" "	"	54	54	54.2	54.1		54.2	55	55	55							
4	" "	"	49.1	49.1		49.8			49.8	51.5	51.5			54	56			
5	" "	"	49	50.5		49.5			49.9	51.6	51			53.1	58.6			
6	" "	"	47.2							50								
7	" "	"	48							50								
8	" "	"	48					49		50			52.5		63			
9	" "	"	47.2							50					60			
10	" "	"	51							54					60.6			
11	" "	"	54.8	54.8						57								
12	" "	"	54.5		54													
13	" "	"	53.6		54													
14	" "	"	53		54										57			
Av. Increase															9.6			
15	Kept in ice chest		48.4			48.9				50.2	50				55.3			
16	" " " "		48.4			49.5				49.5	50				57			
17	" " " "		48.5					48.5		49.5		49			54			
18	" " " "		48							48.5					55			68
19	" " " "		47.5												54.1			66
20	" " " "		50.5												56.2		67.5	
Av. Increase															6.7			
21	15°C-25°C	Lithium citrate	44.3		44.2	44.6		44.5		46.5								
22	" "	" "	47.5		48.6	48.6		48.5		50								
23	" "	" "	48		48.2	48.5		49		50.2								
24	" "	" "	51		51.3	51.5		52										
25	" "	" "	51			51												
26	" "	" "	49.5			49.2												
27	" "	" "	51			51.5												
28	" "	" "	48.5			49.5												
29	" "	" "	51			51												
30	" "	" "	42.4							47					53.3			
31	" "	" "	49.2							52					58			
32	" "	" "	50									53.5			59.1			
33	" "	" "	44							47.5								
34	" "	" "	44.3							47								
35	" "	" "	46.8							48		50						
36	" "	" "	49.1							50.1								
37	" "	" "	48							49								
38	" "	" "	52							53								
39	" "	" "	48.2							49								
40	" "	" "	47.4							50								
41	" "	No anticoagulant	49.3		49.6		50	50										
42	" "		50.2		51		51	51.3										
43	" "		45.7		46		46	47										
44	" "		49		49.2		49											
45	" "		53.5		54													
46	" "		54	54.5	54	54.1		54.1	54.1	54								
47	" "		48.7		49					52		52.2			54			
48	" "		49		47													
49	" "		53.5		54													
50	" "		46.5		46.8													
51	" "		46.8							47.4								
52	Kept in ice chest		48.1					48.9		49								
53	" " " "		48							49								
54	" " " "		50					51		51								