Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 11, Number 2, October, 1938.

Printed in the Union of South Africa by the Government Printer, Pretoria.

The Blood of the Ostrich.*

By O. T. DE VILLIERS, M.R.C.V.S., D.V.Sc., Senior Professional Officer, Stellenbosch-Elsenburg College of Agriculture of the University of Stellenbosch.

INTRODUCTION.

Blood is subject to many diseases and the study of haematology not only yields information concerning these diseases but often also furnishes valuable information for use in the interpretation of disorders which primarily affect other parts of the body. However, all knowledge about the state of the blood during disease is useless without comparative data for healthy blood.

The purpose of this treatise is to present haematological data for use in the study of diseases of the ostrich, and to render possible a comparison of the data on ostrich blood with those on the blood of other animals.

In order to avoid repetition, the technique, results and discussions of each phase of the investigation are grouped together, whenever possible, and the work is presented under the following headings:—

1. Subjects used for determinations.
2. Marking of ostriches.
3. Collecting the blood.
4. Preventing the coagulation of the blood.
5. Preparation of smears.
6. Staining of smears.
7. Cell measurements.
8. Morphology of the blood.
9. Erythrocyte counts.
10. Relative volume of corpuscles and of plasma.

* Thesis approved for the degree of D.V.Sc. by the University of South Africa. (March, 1938.)

11. Minimum and maximum resistance of red cells.
12. Osmotic pressure on the red cells.
13. Haemoglobin content.
14. Leucocyte counts.
15. Differential counts.
16. Thrombocyte counts.
17. Viscosity.
18. Specific gravity.
19. Phosphorus, calcium, sodium, magnesium and potassium content of the blood.
20. Total blood volume.
21. General discussion.
22. Summary.

The blood of birds has been little investigated compared with that of domesticated mammals, and literature referring to the blood of the ostrich is very meagre indeed. Table 1 comprises all the records (relating to the blood of this bird) revealed by an exhaustive search carried out with the assistance of the Staff of the Imperial Bureau of Animal Health, Weybridge, Surrey, England. None of the six investigators mentioned in Table 1 indicated the technique employed for collecting the blood, neither was there any mention of the number of birds from which blood was collected, nor the number of blood samples examined.

It would appear, therefore, that the data were obtained incidentally in the course of other studies; and it will be observed that, although the erythrocyte counts given by Malassez (1872) and Hayem (1879 and 1889) are in fairly close agreement, they differ considerably from that of Venzlaff (1911). The cell lengths recorded by Malassez and Venzlaff also differ appreciably from those given by Gulliver (1875) and Hayem. But in any case, great accuracy can scarcely be expected in the cell counts and cell measurements by the early investigators, considering that in those days the apparatus was not so accurately calibrated as it is to-day.

The ostrich is a domesticated bird with a very ancient history and the following information given by Wormser (1930) may prove of interest. A specimen of the bird was found in a sepulchral chamber of the 18th dynasty which is supposed to be contemporary with Moses and frequent mention of the feather is made in Egyptian hieroglyphics. Arsenoe, an Egyptian queen before Cleopatra, caused to be erected a statue of herself seated on an ostrich, and in Roman public functions the feather was much worn and the bird was ridden by ladies of noble birth. The beauty of the feather has been praised in all ages and from time immemorial the plumes have been sought

TABLE 1.

Summary of Ostrich Blood Examinations Carried out by Different Investigators.

Author and Date.	Species of Ostrich.	Red Cells per c.mm.	White Cells per c.mm.	Hemato-blasts* per c.mm.	Maximum Measurements Red Cells.		Minimum Measurements Red Cells.		Average Measurements Red Cells.			Average Nuclear Measurements.		Phosphorus Content.	Agglutination titer of anti-lepiseptic Serum.	Agglutination titer of anti-pigeon R.B.C. Serum.
					Length.	Breadth.	Length.	Breadth.	Length.	Breadth.	Thickness.	Length.	Breadth.			
Mallassez, L., 1872	Not stated	1,600,000	—	—	—	—	—	—	18μ†	9μ	—	—	—	—	—	—
Gulliver, G., 1875	Struthio camelus	—	—	—	—	—	—	—	1″‡/1649	1″/3000	1″/9166	1″/3200	1″/9166	—	—	—
									15·33μ	8·47μ	2·77μ	7·94μ	2·77μ			
Hayem, G., 1879	Struthio camelus	1,581,000	9,000	11,600	—	—	—	—	14·38μ†	9·15μ	—	—	—	—	—	—
Hayem, G., 1889	Struthio camelus	1,620,000	9,000	11,500	—	—	—	—	14·30μ†	9·15μ	—	—	—	—	—	—
Venzlaff, W., 1911	Struthio camelus	2,560,000	—	—	19μ†	10μ	15μ	8μ	18μ	9μ	—	—	—	—	—	—
Malan, A. I., 1930	Not stated	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
														Total Phosphorus 109·0	—	—
														Lipoid Phosphorus 11·3	—	—
														Organic Phosphorus 39·0	—	—
														Inorganic Phosphorus 5·5	—	—
														Nucleo-protein 53·2	—	—
Buchbinder, 1934	Struthio Camelus	—	—	—	—	—	—	—	—	—	—	—	—	—	0	40

* These are evidently blood platelets; this is inferred from the author's discussion concerning the nature of the cells and the part which these cells take in the clotting of blood.
† Moist preparations.
‡ Not stated whether moist or dry preparations. Gulliver gave the measurements only in vulgar fractions of an inch.

TABLE 2.

Summary of Examinations by Different Investigators of the Blood of Normal Fowls.

Author and Date.	Number of Animals and Sex.	Number of Counts.	Haemoglobin per cent., and grams per 100 c.c.	Red Cells per c.mm. (Millions).			White Cells per c.mm. (Thousands).			Thrombocytes per c.mm. (Thousands).	Percentages of Varieties.					
				Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.		Lymphocytes.	Monocytes.	Polymorphonuclears with Eosinophilic Rods.	Polymorphonuclears with Eosinophilic Granules.	Basophiles	Unclassified.
Stolzing, 1856(1)	—	—	—	—	—	3·8	—	—	—	—	—	—	—	—	—	—
Malassez, 1872(1)	—	—	—	—	—	3·1	—	—	—	—	—	—	—	—	—	—
Hayem, 1889(1)	—	—	—	—	—	2·4	—	—	26·3	—	—	—	—	—	—	—
Albertoni and Mazzoni, 1891(1)	—	—	—	—	—	2·5	—	—	32·3	45·5	—	—	—	—	—	—
Moore, 1895–6(4), (7)	2	2	—	3·7	3·5	3·6	21·2	18·9	20·0	—	—	—	—	—	—	—
Heinz, 1901(7)	4	—	—	4·1	3·9	4·0	—	—	—	—	—	—	—	—	—	—
Ward, 1904(4)	—	—	—	4·0	2·4	3·3	61·0	24·0	36·2	—	—	—	—	—	—	—
Warthin, 1907	—	—	—	3·0	2·0	—	29·0	12·0	21·0	—	50 (small 35·5) (large 14·5)	—	21·5	10	2	16·5
Ellerman and Bang, 1908	—	—	50–65 Per cent. (Sahli)	—	—	3·0	—	—	30·0	30·0	40	23	37		—	—
Hirsfeld, 1909(4), (7)	—	—	60 Per cent. (Sahli)	3·9	2·6	3·0	—	—	30·0	—	70–80	—	22·5	—	—	—
Goodall, 1910(1), (7)	—	—	—	—	—	3·2	—	—	19·0	—	56	—	37	6	1	—
Hedfeld, 1911(4), (7)	M. 6	6	—	4·9	3·6	4·2	—	—	24·0	—	54·0	—	30·0	12·0	3·0	—
	F. 7	7	—	5·1	3·9	4·3	—	—	—	—	—	—	—	—	—	—
Kleineberger and Carl, 1912	6	6	79 Per cent. Method not stated	—	—	3·12	60·8	35·0	—	22·9–130·0	63·8 (small 12·3) (large 51·5)	—	29·5	4·5	2·2	—
Burckhardt, 1912	—	—	—	—	—	3·3	—	—	23·8	—	61·3 (small 48·5) (large 12·8)	—	27·9	7·9	2·9	—
Kozma, 1913	—	—	—	4·0	2·5	—	—	—	—	—	—	—	—	—	—	—
Launoy and Levy-Bruhl, 1913(1), (7)	—	—	—	3·16	2·18	—	35·0	20·0	28·0	—	—	—	—	—	—	—
Schmeisser, 1915	—	—	60–70 Per cent. (Sahli)	4·0	3·0	—	80·0	20·0	—	—	42·3	19·4	29·6	4·3	2·2	2·2
Pickens, 1915(5)	—	—	—	4·0	3·0	3·5	—	—	35·0	—	—	—	—	—	—	—
Fatham, 1916(4)	—	—	—	5·8	3·6	4·7	—	—	—	—	—	—	—	—	—	—
Taylor, 1916(1), (4)	—	—	—	—	—	—	30·0	17·0	23·67	—	51 (42–60)	9 (6–14)	33 (25–41)	3·6 (1·9–6·8)	3 (2·6–4·1)	—
Burnett, 1917	—	—	76 Per cent. Method not stated	—	—	3·3	—	—	—	17·9	58	5·5	28·8	3·3	4·3	—
Mack, (no date) (1)	—	—	87·3 Per cent. Method not stated	—	—	3·0	—	—	33·77	—	54·9	6·2	32·7	2·7	3·3	—
Kaupp, 1918(5)	—	—	—	4·0	3·0	—	35·0	28·0	—	—	—	—	—	—	—	—
Salomon, 1919(3), (4)	M. 1	—	—	3·4	3·1	3·2	49·0	28·0	—	—	61·0	1·0	28	7	3	—
	F. 3	—	—	—	—	—	57·0	41·0	—	—	69·0	2·0	18	9	1	—
Fritsch, 1920(3)	M. 5	5	12·3 Grams	—	—	3·24	—	—	—	Very few	40·0	2	49	5	3	—
	F. 5	5	9·6 Grams	—	—	2·77	—	—	—	,,	64·0	5	23	5	2	—
Kleineberger and Carl, 1927	M. 2	2	73–80 Per cent. Method not stated	3·91	3·44	—	32	23	—	25·3–28·0	24·27	—	60–71·5	1–2	0·5–14·0	—
	F. 5	5	67–80 Per cent. Method not stated	3·91	2·88	—	42·2	29·6	23	22·7–40·9	63·5–74	—	18·6–33·5	1·5–4·0	1·5–3·5	—
Ellerman, 1921	—	—	—	—	—	—	—	—	—	—	53·0	12	29		4	—
Romer, 1921	—	—	75 Per cent. (Sahli)	—	—	2·62	—	—	—	—	64·6 (small 52·7) (large 12·3)	1·4	26·5	5·4	1·9	—
Fink, 1924(2), (7)	—	—	—	3·35	2·61	2·84	26·2	16·7	21·5	—	—	—	—	—	—	—

[*Continued on next page.*

TABLE 2—(*continued*).

Author and Date.	Number of Animals and Sex.	Number of Counts.	Haemoglobin per cent., and grams per 100 c.c.	Red Cells per c.mm. (Millions).			White Cells per c.mm. (Thousands).			Thrombocytes per c.mm. (Thousands).	Percentages of Varieties.					
				Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.		Lymphocytes.	Monocytes.	Polymorphonuclears with Eosinophilic Rods.	Polymorphonuclears with Eosinophilic Granules.	Basophiles.	Unclassified.
Niggermaier, 1925(2)	—	—	—	2·83	2·16	2·68	40	19	29	—	52·5 (40·4–64·7)	7·5 (4·3–10·6)	32·1 (24–41)	6 (3·7–10)	1·9 (0–3·4)	—
Kennett, 1926(5)	—	—	—	—	—	2·9	—	—	33·0	—	—	—	—	—	—	—
Chaudhuri, 1926(5)	—	—	—	M. 5·2	3·9	4·6	—	—	—	—	—	—	—	—	—	—
				F. 3·1	3·8	2·0	—	—	—	—	—	—	—	—	—	—
Blacher, 1926(5)	—	—	—	M. 4·4	3·0	3·8	—	—	—	—	—	—	—	—	—	—
				F. 3·7	2·1	2·9	—	—	—	—	—	—	—	—	—	—
Hayden, 1927	F. 9	9	74 Per cent. Method not stated	3·7	2·28	2·83	48	22	38·9	—	81	1·5	5·17	10	1·6	—
Breusch, 1928	—	—	—	—	—	3·47	—	—	33·3	—	66·5	9·2	17·7	4·1	2·5	—
Blain, 1928	6	75	—	—	—	—	29·5	10·1	18·6	None	32·8	5·7	49·4	8·7	3·6	—
Gohs, 1928	—	—	—	—	—	—	22·0	14·0	17	50–100	—	—	—	—	—	—
Yakimoff, and Raste-gaieff, 1929(7)	11	—	—	2·67	2·09	—	—	—	—	—	58·1 (41·6–69·9)	9·1 (4–15·6)	29·1 (18·8–41·0)	1·4 (0–6·0)	2·3 (0–5·0)	—
Kyes, 1929	—	—	—	—	—	—	13·0	8·0	—	—	—	—	—	—	—	—
Forkner, 1929	11	29	62·9 Per cent. (Newcomer)	4·6	2·3	3·27	74·0	6·8	24·6	35·0	41·8	17·1	34·7	1·8	4·2	—
Kaupp, 1929	—	—	—	—	—	3·5	30·0	25·0	—	50·0	45·0	18·0	30·0	6·0	1·0	—
Bayon, 1930	—	—	60 Per cent. (Sahli)	—	—	3·0	—	—	20·0	—	—	—	—	—	—	—
Thomsen and Engelbreth-Holm, 1931(3), (7)	—	—	50 Per cent. (Sahli)	—	—	3·0	—	—	30·0	—	65·0	5–10	25·0	2–4	1–2	—
Wirth, 1931	—	—	50–65 (Sahli)	4·0	3·0	—	—	—	—	—	60·0 (small 40–60) (large 5–15)	2 (1–4)	30 (20–50)	5 (2–8)	3 (1–5)	—
	—	—	10·2–10·6 grams	—	—	—	—	—	—		—	—	—	—	—	—
Fenstermacher, 1932	12	120	76 (Dare)	2·87	2·08	2·59	29·3	19·8	24·4	—	—	—	—	—	—	—
Seager, 1933	—	—	—	—	—	2·9	—	—	27·0	—	—	—	—	—	—	—
Landauer and David, 1933(5)	—	—	—	M. 4·6	4·0	4·3	—	—	—	—	—	—	—	—	—	—
				F. 3·7	3·4	3·2	—	—	—	—	—	—	—	—	—	—
Cook and Dearstyne, 1934	M. 5	80	10·8 grams	4·2	1·8	2·84	47·0	3·0	16·36	—	12–58 (small 8–54) (intermediate 0–18) (large 0–5)	4–33	22–78	0–18	0–18	0–3
	F. 75	—	—	—	—	—	—	—	—							
Gibbs, 1934(6)	15	20	85·3 (Wong)	—	—	3·2	—	—	24·0	—	—	—	—	—	—	—
Blakemore, 1934	19	19	—	—	—	2·8	36·0	13·3	25·1	—	69·4	7·5	21·5	—	—	1·6
Palmer and Biely, 1935	50	50	—	3·3	1·0	2·5	77·5	15·8	38·5	—	—	—	—	—	—	—
Morgan and Chichester, 1935	—	—	—	—	—	2·9	—	—	—	—	—	—	—	—	—	—
Biely and Palmer, 1935	F. 100	100	—	3·84	1·80	2·78	49·0	18·33	32·15	—	—	—	—	—	—	—
	47(8)	47	—	3·18	1·80	2·48	28·30	15·00	20·60	—	—	—	—	—	—	—
Olson, 1937	32(9)	—	10·12 grams	—	—	2·95	—	—	19·8	25·7	62·5	9·4	24·4	1·9	1·7	—
	89(10)	—	9·73 grams	—	—	2·74	—	—	32·7	29·4	66·0	8·1	20·9	1·9	3·1	—

(1) Listed by S. H. Burnett (1917).
(2) Listed by D. Wirth (1931).
(3) Listed by T. B. Magath and G. M. Higgins (1934).
(4) Listed by J. Palmer and E. I. Biely (1935), citing Scarborough (1931).
(5) Listed by J. Palmer and E. I. Biely (1935).
(6) Gibbs records the differential counts as follows: Eosinophilic leucocytes with rods 10·3 per cent. Eosinophilic leucocytes with granules 23·0 per cent. Polynuclear leucocytes 10·6 per cent. Neutrophilic leucocytes and mast cells 4·2 per cent. Small lymphocytes 40·3 per cent. Large lymphocytes and mononuclears 11·8 per cent.
(7) Listed by C. Olson (1937).
(8) One-to two day-old chicks.
(9) Adult fowls.
(10) Young chickens.

for personal adornment. Warriors wore them as early as 1350-1100 B.C. and ostrich eggs were greatly prized and used for ornamental and religious purposes. The plumes formed part of the tribute imposed by Egyptians on conquered countries.

In the light of these facts and in view of the important role which the ostrich has already played in the economic history of South Africa it is surprising that the haematology of this bird has not received more attention, particularly, from veterinarians in South Africa and that there is comparatively little scientific veterinary literature about the ostrich. This lack of information cannot be ascribed to the bird's immunity from disease, for, though the grown animal is very hardy, yet when young—particularly when under three months—it readily succumbs to disease.

That it is susceptible to a number of affections is well known, though to judge by the literature only a few of these have been scientifically investigated. This may be attributed partly to the fact that countries other than South Africa had little interest in the ostrich feather industry; for, during the years in which it flourished, it was almost entirely monopolised by South Africa, since the bird is indigenous to this country, and legislation prohibited the exportation of ostriches or even of fertilised eggs. Moreover at that time there were few veterinarians in South Africa and they were fully occupied with the many stock diseases then rampant, which owing to the havoc they were causing doubtless warranted more attention. Apparently no one qualified to do so had sufficient leisure to make a thorough study of the hygiene and diseases of the ostrich and to present the information for the guidance of others. Besides, veterinary haematology and especially avian haematology was then much less advanced than it is at present.

For almost half a century the ostrich industry was of very great importance to South Africa and formed the most lucrative branch of farming. At one time ostrich feathers were second only to wool as an agricultural export. From the year 1860, when 2,361 lbs. of feathers valued at £19,726 were exported from South Africa (Wormser 1930), the exports steadily increased, except for minor fluctuations, till 1913, the peak year of the industry, when 1,023,307 lbs. valued at £2,953,587 were exported. At the end of 1913 there were 776,313 ostriches in South Africa, and before the collapse of the industry in 1914 as much as £1,000 was paid for a good breeding pair, and the prices obtained for chicks varied from £5 to £50 (Official Year Book of the Union of South Africa, 1910-'17). Feathers realised as much as £100 per lb. during the boom (Laite 1915).

The ostrich feather industry forms at present one of the lesser branches of South Africa's agricultural activities, and may be said to have been moribund for many years in so far as the value of the feather as an article of adornment is concerned. Still, it does not seem likely that ostrich feathers will ever pass quite out of favour, though the price may fluctuate in accordance with the vagaries of fashion. The ostrich is being exploited also in other ways—its skin is used for wallets, handbags, attache cases, tobacco pouches, shoes,

etc., and the meat, particularly when dried (biltong), and the eggs are relished by many people. The following paragraph recently appeared in the press:—

> "Having discovered that ostrich meat has a delicious flavour, the Moscow Zoological Park has begun breeding ostriches on a large scale, with the object of establishing large flocks in the Southern Steppes of the Soviet Union. Because of its thick layer of fat, which originally served as a protection from the Semi-tropical sun, the ostrich can endure the cold Russian winter, even at the latitude of Moscow."

The knowledge gained from veterinary research work in connection with the ostrich would, therefore, serve the interests not only of science but of trade, especially if there should be a revival of the feather industry. It is hoped that these studies will add a link to the chain of that knowledge.

Incidently it may be mentioned that the literature discloses no data about the blood of any kind of South African bird—apart from the chemical analyses by Malan (1930)—and it will be seen from Table 2, which embraces most of the observations on the cellular elements of the normal blood of the domestic fowl, that the results show marked discrepancies, especially in respect of the total leucocyte and thrombocyte counts and the percentages of the types of leucocytes. Moreover, blood observations made overseas are not necessarily applicable to South African birds, for, as is well known, the blood picture varies more or less with breed, climate, geographical habitat, etc.

SUBJECTS USED FOR DETERMINATIONS.

As the primary object of this work was to obtain information about the blood of normal ostriches the intention was to utilise, for the purpose of research, birds hatched either in an incubator or naturally and removed immediately to an area where there was no risk of worm infection—to which the ostrich is very susceptible—and where they could be so attended to as to remain in perfect health. But incubator-hatched birds were unobtainable and the writer had to content himself with six chicks which had been hatched by a semi-wild ostrich, and which were already about two weeks old when they were caught. Luckily they proved later to be three hens and three cocks and consequently the blood of the sexes could be compared.

Before capture these birds had the run of a big farm on which there were few other ostriches and it was, therefore, considered that little, if any, worm infection could have occurred within so short a period. They were then reared in a camp on the Stellenbosch-Elsenburg Agricultural College farm, Mariendahl, Stellenbosch district, where they had considerable freedom and where there had been no ostriches for many years (perhaps never), not even within a radius of many miles. Furthermore, as the four species of worms known to be harboured by the ostrich, viz., the tape-worm, *Houttuynia struthionis*, and the nematodes, *Codiostomum struthionis*,

Ornithostrongylus douglasii and *Contortospiculum spicularia* (Mönnig 1929 and 1934), are not known to occur in any other species of animal, little, if any, worm infestation was anticipated. This expectation was fortunately realised, for faeces examinations made—by the centrifugal flotation method described by Mönnig (1934)—during the course of the research work proved negative and also on post-mortem examination no worms were found. The administration of vermicides was, therefore, unnecessary, and possible blood changes due to such treatment was accordingly eliminated. Care was taken also to ensure that the birds remained entirely free from ectoparasites. Five of the birds—numbers 1 to 5—showed no trace of disease, either during life or on post-mortem examination, and their blood may, therefore, be considered to have been normal.

The remaining bird, number 6, when about six months old, dislocated a metacarpo-phalangeal joint and the dislocation could not be remedied. In consequence the bird had a club-foot, and as the joint always came in contact with the ground a large tumour-like formation developed over the part exposed to the ground. Otherwise the bird was perfectly healthy, for on post-mortem examination it showed no internal evidence of disease. As it could not be considered perfectly normal, the results of the examinations of its blood are not included with those from the normal birds, but they are nevertheless recorded for the purpose of comparison.

It would, of course, have been better to have had a larger number of absolutely healthy birds for these blood observations, but sufficient lucerne-grazing, so essential to the well-being of ostriches kept in a limited area, was not available. Their other food consisted of a plentiful supply of chopped bones, oats, mangolds, etc. Some of the observations, such as cell-counts, etc., were repeated at intervals from the time when the birds were about seven months until they were about two years and eight months, viz., until they were fully mature.

The blood of sixteen other ostriches was also examined. Eleven of these—numbers 7 to 17—were semi-wild full-grown birds caught on the farm " Nagwag ", Bredasdorp District. They appeared healthy, but the writer was unfortunately not given an opportunity of conducting post-mortem examinations on them. Faeces from only a few of these birds could be examined and these contained many worm eggs. As none of the birds had ever received vermicidal treatment, it is most likely that all of them were worm-infested.

The remaining five birds, 18 to 22, were semi-wild ostrich chicks sent to the writer from the farm " van der Stelkraal ", Bredasdorp District. These birds showed marked unthriftiness, and on post-mortem examination they were all found to be severely infested with worm parasites, particularly tapeworms. As it is impossible to judge of normality from external appearance alone, only the results from birds 1 to 5 will be considered as results from normal birds, and those from numbers 7 to 17 and from numbers 18 to 22 will be classed respectively as from clinically healthy ostriches and from unthrifty ostrich chicks which on post-mortem examination showed marked verminosis.

It was difficult to make observations as the nearest farm—other than Mariendahl—on which ostriches were kept was over a hundred miles distant from the laboratory.

Classification of the Ostrich.

There are different opinions regarding the classification of the ostrich. Cronwright-Schreiner (1898) gives the following classification:—

1. North African bird—*Struthio camelus.*
2. South African bird—*Struthio australis.*
3. Somali Ostrich—*Struthio molybdophanus.*

In South Africa the industry was started with the *Struthio australis*, but as the feather of the North African bird, *Struthio camelus*, proved superior, a number of these birds were introduced at different times to improve the stock. Cross-breeding was successful and, as the hybrids are fertile, a certain percentage of the birds in South Africa are crosses between the two species, *Struthio camelus* and *Struthio australis*. The birds used in this work, however, had the characteristics of the true South African bird, namely, *Struthio australis*.

The Marking of Ostriches.

As ostriches look so much alike—even the sexes being difficult to distinguish until the birds are about a year old—it was necessary to mark the birds on which repeated observations were being made, so that they could be readily identified. They were, therefore, branded, for branding is a simple and most effective way of marking an ostrich. Clips in the wing were considered undesirable, as they sometimes cause inflammation and possibly leucocytosis.

A piece of wire, about 7 mm. in diameter and 18 inches long, was bent at right angles, so that the short arm of the wire was two and a half inches long. The six birds kept at Mariendahl were each branded on the right thigh in Roman letters, viz., I to VI, when they were four months old and the letters were placed sufficiently low and were big enough to be seen readily from a distance; thus any unnecessary handling of a bird was avoided. The wire, heated to a dull red, was applied just long enough to burn through the epidermis. Excessive burning, particularly when a small brand with ordinary lettering is used, sometimes results in so much scar tissue formation that the actual lettering is obliterated. If chicks under three months have to be branded, it is best to brand them on the side of the abdomen, for the thighs are then still covered with down. A fine wire should then be used. Healing is usually complete after five weeks and the mark probably remains for life. Determinations recorded were made only about two months after the wounds had completely healed.

Collecting the Blood.

From ostriches up to the age of about four months small amounts of blood, such as for cell counts, are most easily obtained by puncturing the brachial vein just above the shoulder joint. The bird is laid on its side on a table or box of convenient height, and is suitably controlled by one or two assistants. It is much easier to restrain troublesome birds if the hock joints are flexed and then immobilised by means of cords, or preferably soft linen bands, as the skin is easily chafed. The uppermost wing is held extended and the site is cleansed with a pledget of cotton wool moistened with alcohol. The vein, which is easily seen when the wing is extended, is then slightly compressed opposite the shoulder joint with the thumb of the left hand and when the site is quite dry the vessel is punctured, an ordinary hypodermic needle being quite suitable for the purpose. The needle should be held in a position vertical to the course of the vein ; if the puncture is made obliquely, the skin stretches slightly, with the result that when the needle is withdrawn the skin retracts, preventing the free flow of blood. The blood then collects on the fold of the skin which fills the angle between the humerus and the ribs, and the required amount of blood can then be correctly drawn up in a blood cell count or other kind of pipette. Blood cell count pipettes (Thoma-Zeiss and Trenner) were always used for diluting the blood in the case of young chicks, as much blood could not easily be obtained, nor was it desirable to extract much blood. Haemorrhage may be arrested by pressing a finger on the wound for about one minute, but after release of the bird bleeding generally stops quite soon.

When a fair quantity of blood was needed, as for the purpose of determining percentage volume, etc., it was drawn from the brachial vein by inserting into it a hypodermic needle with a bore of about 0·5 mm. and drawing off the required amount of blood with a syringe. As a needle of such fine bore has to be used the blood seldom flows satisfactorily of its own accord even when the vein is well compressed. (Differences between counts made of blood drawn off with a syringe and of blood which flowed out spontaneously were negligible). Very young chicks should be handled carefully as they readily succumb to injury.

In older birds, sufficient blood for ordinary haematological determinations can be obtained from either the right jugular or a brachial vein, for these veins are well developed. The blood was usually collected from the right brachial vein, which is easily accessible; the birds usually resent handling of their necks. They persist in swaying them about and they usually struggle if attempts are made to control the neck. The needle, therefore, easily becomes dislodged. The procedure adopted here was based on the method of Neser (1923) and is as follows:—

The birds are driven into a small enclosure and by means of a stick—about six feet long, to one end of which a thick piece of hard wire bent into the shape of a hook is attached—the bird is gripped at the back of its neck just below the head. The bird immediately rears and with the right hand it is then caught at the back of the neck. Very tame birds may be caught without the aid

of a stick. A black stocking or some other type of blinker porous enough to admit sufficient air but too dense for the bird to see through is pulled over the head. When once the head has been caught there is little danger of being kicked, and it is surprising how helpless and docile an ostrich becomes when it cannot see. The bird is then manoeuvred into a V shaped crush just long enough to accommodate it. The back of the ostrich is on a level with the top of the crush. (Fig. 1.)

Figure 1.

It is not desirable to use galvanised iron for making the crush, for noise caused by the kicking of the birds frightens them and they become very restless. Neither should the crush be built close to trees for their rustling on a windy day also excites the birds. Flooring boards were found very suitable. Their surfaces should be planed smooth otherwise the ostrich can get a hold on rough surfaces with its claws and thus lever its body backwards and upwards and make control difficult. Thinner boards might be broken by an adult bird.

In the crush the bird is controlled by one or more assistants applying their hands to the ischial bones and pushing the bird forward, while others push him downwards by applying their hands to the sacral region, and at the same time another assistant lifts the right wing. The operator stands on the left side of the bird, and during the operation of inserting the needle into the vein and collecting the blood he presses with his fore-arms on the bird's back thereby also helping to prevent its moving upward. Occasionally a bird will flop down and refuse to get up. It is best then to drag it out of the crush and to press it down so that it cannot get up while the blood is being drawn. At least three men are required to keep an adult

ostrich down. Attempts were made to control birds in the crush by tying ropes round them to obviate the use of many assistants, for at least four are required to control a restless bird. This method, however, proved undesirable, for the feathers were easily dislodged and the skin was badly chafed by the ropes.

The semi-wild birds from which blood was collected in the Bredasdorp district, when once they were caught, struggled much less than the six birds kept at Mariendahl, and they did not very vigorously resist the drawing of blood from the jugular vein; no doubt they were too scared to do so. Of the six " Mariendahl " birds five soon became used to being handled and scarcely resented the insertion of the needle, so that less assistance was required. But the remaining bird, No. 6, always put up a struggle.

In an adult ostrich the brachial vein measures about 6 mm. in diameter, and a hypodermic needle about 4 cm. long and with a bore of about 1 mm. in diameter was found quite suitable for insertion into the vein of a full-grown bird. For younger birds a needle with a diameter of 0·7 mm. was used. The wings were used alternately when fairly frequent collections were made and sometimes the blood was obtained from the jugular in order to eliminate the possibility of contamination by inflammatory products. But it would appear that phlebitis is not easily induced in the ostrich. It was never once observed and it is surprising how soon the puncture wound, or indeed any wound, heals in the ostrich.

In the adult bird the skin overlying the vein is fairly thick and its colour is blue like that of the vein; the vessel cannot, therefore, be seen until the blood flow is slightly impeded by gentle compression, which is also necessary in order to obtain a free flow of blood. When the needle is inserted it should be pushed in at a very acute angle and in a direction against the flow of blood, so that its point is at least 0·5 cm. from the point of entry into the vein; otherwise the opposite wall of the vessel may be penetrated or, if not inserted deeply enough, it may slip out if the bird struggles slightly.

A 20 per cent. solution of lithium citrate and powdered heparin, which are again referred to under " Preventing Coagulation of the Blood ", were used as anticoagulants. Lithium citrate was used in the early part of the work, but was later replaced by heparin, and the blood was collected in bottles with necks about 0·5 centimetre in diameter and graduated as described by Neser (1923). Each bottle contained such an amount of the lithium citrate solution, or of the powdered heparin, that when the bottle was filled to the mark on the neck, there was 1 part of lithium citrate to 199 parts of blood, or 1 part heparin to about 5,000 parts of blood. It is not always easy to fill the bottle accurately to the level of the mark but since such a small proportion of anticoagulant is used a slight error in the level is not significant when a fair quantity of blood is drawn. The blood was, however, always collected in such small amounts and at such intervals that the observations may be regarded as always having been made on normal blood.

According to the observations by Neser (1923), the rate of the circulation is a very important factor in the distribution of the red cells, a sluggish peripheral circulation resulting in a concentration of the red cells in the periphery and the reverse in the jugular vein. He considered the jugular vein in domestic mammals the best source from which to collect blood, and his reasons may with advantage be repeated here:—

1. "The whole circuit is comparatively short and the flow of blood is fairly rapid.
2. The circulation of the head is relatively larger than that of any other accessible part of the body, and for this reason the relative change in the blood for a given activity will be least.
3. There is little, if any, obstruction to the flow of blood in the jugular vein owing to the action of gravity. Stagnation is, therefore, impossible here under normal circumstances, and the blood is consequently thoroughly mixed."

Ponder (1934), on the other hand, remarks: "In spite of occasional statements to the contrary, it seems to be established that the red cell count is the same in normal arterial, venous, and freely flowing capillary blood, and that it is the same in normal blood taken from central and peripheral vessels (Rud, 1922-23, McCay, 1928)."

The wing vein of the ostrich may, for the reasons mentioned by Neser, be considered more suitable than the jugular, for the circulation is much shorter in the wing than in the neck, while the other two reasons are equally applicable to the wing vein. Besides, as already stated, blood is more easily obtained from this source.

Counts and percentage volume determinations were made from blood extracted from the jugular and also from the wing vein, but they differed only within limits of error, so that, both for theoretical and practical reasons, the brachial vein may be considered the most suitable in the ostrich.

Preventing Coagulation of the Blood.

In testing ostrich blood to determine its coagulation time, it was observed that clotting is usually much delayed, and often samples of whole blood, still uncoagulated, were discarded days later. Usually samples did not coagulate for several hours after they were collected but sometimes it did happen that coagulation occurred within an hour. The great advantage of this unusual property of ostrich blood was that observations could also easily be made on whole blood and these could be compared with those made on blood containing anticoagulants.

Bainbridge and Menzies (1919) state: "The blood of birds contains no platelets, and will not clot, if it is drawn directly from a blood vessel without contact with the tissues. If, however, it is allowed to flow over the adjacent tissues in its passage from the vessel, it will coagulate readily."

This statement requires modification, for, though the blood of the ostrich does not readily clot, this is not true of fowl blood. The writer has on many occasions drawn blood—by means of a syringe—direct from the hearts of fowls according to the method of Sloan and Wilgus (1930), and also from their wing veins, and coagulation usually occurred within a short period even when the inside of the syringe barrel was coated with vaseline. According to Dukes (1934) the period of coagulation for fowl blood is 4½ minutes, and Johnson and Connor (1933) who determined the coagulation time of the blood of 12 fowls found that it varied from 1 minute 10 seconds to 14 minutes 15 seconds with an average of 6 minutes 21 seconds. Kaupp (1929) states the period to be 30 seconds. It is, of course, generally agreed that, though platelets as seen in mammalian blood do not occur in avian blood, the thrombocytes in birds' blood serve the same purpose as the platelets.

Ostrich blood sometimes clots quickly, especially in warm weather, once it has been in contact with the tissues or if brought in contact with blood that has already coagulated. It was also noticed that the blood coagulated more readily when the bottles were filled slowly because of obstruction in the needle or when a needle with a very fine bore was used.

It would appear that the prolonged coagulation time of ostrich blood cannot be correlated with a low calcium content of the blood. From Table 29 it will be noted that the calcium content of ostrich blood averages 10·1 mgms. per 100 c.c. blood. This figure is high compared with the values given for some animals whose blood clots very readily. For example—to give only a few figures as recorded by Dukes (1934)—the calcium content of the blood of the ox is 7·1 mgms. per 100 c.c. and the coagulation time 6½ minutes, the corresponding figures for the dog being 6·6 mgms. and 2½ minutes and for the cat 5·5 mgms. and 1 to 3 minutes. Calcium values, reported by Knowles (1934) for hens not in lay, vary from 8 to 12·3 mgms. per 100 c.c. blood.

As coagulation of samples sometimes did take place before it was possible to complete the observations, it was necessary to use an anticoagulant. Neser used a 7½ per cent. sodium citrate solution as anticoagulant in the proportion of 1 part anticoagulant to 9 parts of blood, and the writer's intention was to use, also in the same proportion, an isotonic solution of a suitable anticoagulant so that true percentage volume readings could be obtained. It was, however, soon realised that, whereas it is possible to fill up the bottle containing the anticoagulant to a definite mark when collecting blood from a docile animal like the horse, it is difficult to do so when extracting blood from an ostrich. As a rule, the birds are restless, and as the operator has to collect blood from the under side of the wing while usually standing on the opposite side, it is often impossible to fill up the bottle exactly to the mark. This necessitates the subsequent addition of saline or the removal of blood, and therefore additional calculations have to be made.

It was, therefore, decided to find an anticoagulant of which such a minute quantity need be used, either in solid or liquid form, that corrections need not be made even if the bottle is filled up slightly

above or below the mark. Sodium citrate, potassium citrate, lithium citrate, sodium oxalate, potassium oxalate and sodium fluoride were tried in solid form. But it was found that, when these were used in such quantities as would invariably prevent coagulation, slight haemolysis sometimes resulted, as was evidenced by tinged plasma when the blood was centrifuged. The aforementioned salts in powder form were, therefore, considered unsatisfactory, as the degree of haemolysis could not be controlled. It was finally found that 0·5 c.c. of a 20 per cent. solution of lithium citrate to 99·5 c.c. of blood invariably prevented coagulation and never caused any haemolysis. Moreover, as lithium citrate is a salt that does not occur in the blood, corrections need not be made in chemical analyses of the blood.

The lithium citrate solution, however, causes slight crenation of the cells, and consequently a true percentage volume reading is not obtained. But it will be shown under "Relative Volume of Corpuscles and of Plasma" that, by applying a small correction factor to percentage volume readings obtained with citrated blood, such readings may for practical purposes be regarded as identical with those of whole blood.

Heparin, which was unprocurable in South Africa at the time it was first required, was tried later and proved an even more suitable anticoagulant, for, when used in the proportion of 1 mg. to 5 c.c. blood as was used by Wiseman and Bierbaum (1932) for human blood, it also invariably prevented coagulation. Haemolysis was never observed and differences, when present in the percentage volume readings of whole blood and of heparinised blood, were insignificant.

The aforementioned workers state: "After weighing out this quantity a few times to visualise the approximate volume involved, the quantity of heparin added to each tube may be estimated without disturbing the accuracy of the test". This procedure was adopted by the writer and proved satisfactory.

Preparation of Smears.

Opinion differs considerably as to the most satisfactory method of making blood smears suitable for differential counts. According to Neser (1923) the distribution of the leucocytes is often most irregular when smears are prepared by the slide method, viz., by placing a drop of blood on one slide and causing it to spread by pushing it with the edge of another slide. He states that as a rule the leucocytes are evenly distributed throughout the smear by his coverslip method in which the blood is spread between a slide and a coverslip, instead of between two coverslips, as is customary in determinations on human blood. However, he does not record differential counts made from smears prepared in both ways, indicating the differences in the counts. De Kock (1931) slightly modified Neser's method by substituting a squarely cut portion of slide glass for the coverslip and states that the method gave more uniform results than smears prepared in any other way. Many other workers also favour the coverslip method.

On the other hand, Schilling (1935) describes only the slide method. Wirth (1931) is of opinion that the distribution of leucocytes is as even with the slide method as with the coverslip method. Lucia and Lucia (1928) give counts made in different ways from smears prepared by the slide method and state:—

"It was found that the counts in every case fell within one standard deviation of the group. If the probability of variation around the mean (standard deviation) is taken into consideration, no advantage can be ascribed to the coverslip over the slide method, as far as distribution of cells is concerned."

Kolmer and Boerner (1931) describe both methods, and their only comment is:—"The coverslip method is preferred by many clinical pathologists, although it tends to produce rupture of the leucocytes." In other text books consulted usually both methods were also described, but no preference was mentioned. The writer also consulted several well-known haematologists, and they prepare smears for differential counts only by the slide method.

Messrs. Arthur Thomas & Co., Philadelphia U.S.A. have placed on the market a "Differential Counting and Staining Outfit" invented by Hausser. With this outfit, smears are prepared as in the coverslip method, the blood being spread with a small rectangular block of glass on a specially thick glass slide. The following remarks from the firm's 1931 Catalogue edition, in which the outfit is advertised, may be quoted here: "It is difficult to obtain blood smears of uniform thickness over a large area with any technique when ordinary microscopic slides and coverglasses are used, because of the curvature in the surfaces of both. It has been stated that this usually amounts to a full wave-length in a linear distance of 1mm. Since a wave-length is about 600 microns, and a blood corpuscle measures about 10 microns, it is evident that, while at one point absolute contact may be possible, there may be sufficient unequality of surface at a distance of only 1 mm. from such a point to pile up a layer of not less than sixty corpuscles, when smears are so made."

Therefore, it does not appear to have been definitely established yet that the coverslip method gives a more even distribution of the leucocytes or in any case a so much more regular distribution as to be of practical importance.

Smears may be easily and satisfactorily prepared, by the coverslip method, from the blood of animals which can be brought into a laboratory or sheltered place in which the air can be kept free from dust, but in the case of the ostrich this is not so easy. Usually ostriches are not stabled, but are allowed to pick up their food on the veld, and they can only with difficulty be driven into a stable. Under ordinary field conditions, therefore, smears have to be prepared in the open, and as the surface of the glasses have to be scrupulously clean in the coverslip method it is difficult to prepare such smears on a windy day as the dust so quickly settles on the glasses. Moreover, the numerous fine scales on the feathers of the ostrich easily become dislodged and settle on the glasses.

The coverslip method of Neser as modified by De Kock was tried and, apart from the above-mentioned disadvantage associated with the coverslip method when applied to the ostrich, the writer often had to make a number of smears before a satisfactory one was obtained even on quiet days when the surfaces of the glasses were as clean as could be expected. The slides were always cleaned with bichromate of potash and nitric acid and kept before use in absolute alcohol but owing to the unevenness of the slides—though high quality polished slides were always used—the blood would not spread properly on many of them, and consequently the smears were too thick. Smears could, therefore, not always be prepared quickly with the result that the flow of blood from the needle had to be unduly prolonged—a matter of considerable inconvenience when dealing with a restive animal. Apart from the loss of blood, the bird soon becomes uneasy and the needle is either dislodged or the blood-flow ceases and the needle has to be reinserted.

Thin smears from ostriches can be easily prepared under field conditions by the slide method described by Neser (1923), slightly modified as indicated below;

A platinum loop is attached by means of sealing-wax or other suitable adhesive to the end of the slide used for spreading the blood and one of the corners of the opposite end of the same slide is cut off, so that the length of the spreading edge is about 1·5 centimetres. (Fig. 2.)

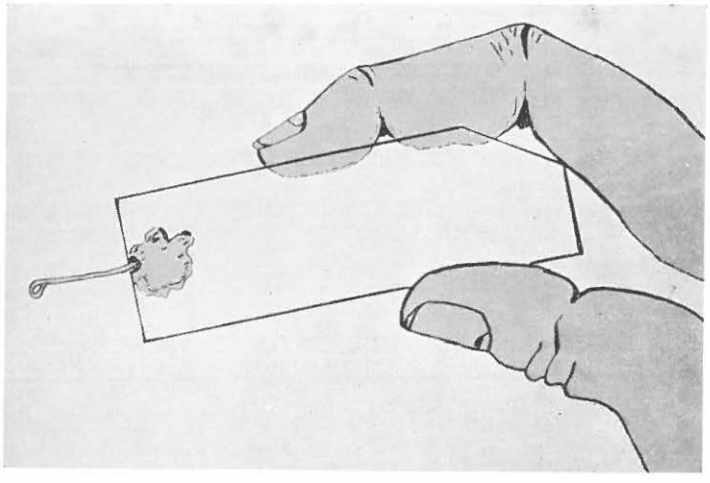


Figure 2.

The slide to which the loop is affixed is held at its edges with the thumb and forefinger and about midway between its ends, and a drop of blood is then taken up with the loop. After the drop has been deposited on to another slide held in the left hand, the slide carrying the platinum loop is simply tilted and with its narrow edge the blood is spread in the customary manner of pushing the spreader towards the other end of the under slide.

The process of first putting the loop down and taking up the slide for spreading the blood is thus eliminated and the smear is, therefore, prepared more quickly. In making smears from ostrich blood in which not only the thrombocytes but also the leucocytes clump very soon after the blood is drawn, a delay of some seconds in spreading the blood may vitiate the making of a smear satisfactory for differential counts.

By using a narrower spreader the blood is prevented from spreading over the long edges of the under slide and thus leucocyte counts can be satisfactorily made vertically across the smear, as the edges of the smear can be included in the fields examined. The size of the loop used for taking up the blood was always such that the length of the smear was about 3·5 cm. when the drop had been spread and as already indicated the breadth was about 1·5 cm. Dispensing with the use of the loop by taking up blood as it issues from the needle with the edge of the spreader proved unsatisfactory, for usually too much blood adhered to the edge with the result that the smears were too thick.

Staining of Smears.

May-Grunewald and Giemsa stain (as used by Pappenheim) Giemsa's, Leishman's, Jenner's and Wright's stain were all tried as recommended in text books and by individual workers. Wright's stain was finally adopted.

Staining with Wright's stain entails little work and the smear is usually ready for examination within ten minutes. This stain gave as good and uniform results as any of the others, and proved more satisfactory than some of them for the purpose of differentiating between the lymphocytes and the monocytes. The stain was prepared as prescribed by Carleton (1926) and used as recommended by Slider and Downey (1929).

In properly stained smears the cytoplasm of the red cells is a fawn colour and sometimes fine azurophil granules can be seen in the monocytes. With some samples of stain the diluted stain need only be left on the smear for 5 minutes whereas with others 10 minutes or more are necessary. Experience is the best guide as to how long to stain and to wash.

Pepper and Farley (1933) state: "The difficulty with methylene azur-eosin stains is to obtain the proper balance of eosin and basic stain. Muddy blue preparations are the result of too much alkali in the stain. To keep the stain at just the proper reaction is difficult."

For diluting Wright's stain McJunkin (1920) uses, instead of distilled water, a buffer solution made from monopotassium phosphate and dibasic sodium phosphate. This solution has a PH of 6·4 and will absorb excess alkali without change in the reaction of the solution.

The best results were usually obtained when Wright's stain was used immediately after the smears were prepared.

Cell Measurements.

The cells were measured with a Leitz ocular micrometer that had been carefully calibrated, and their measurements are given under "Morphology of the Blood". Ponder (1924) states that this method has been used with variations for the last seventy years and that nearly all tables of the average sizes of erythrocytes contain values determined in this way. He considers that at times results so obtained may have attached errors amounting to 10 per cent. and he remarks as follows: "When a body so small as the red cell is examined under the microscope owing to the fact that there is a limit of resolution to even the most perfect optical system the edge of the body is not seen distinctly but as a blurred band, the true edge being located somewhere in this "spurious disc" its position depending on the refractive indices of cell and surrounding fluid respectively. However carefully the system is adjusted a blurred band of at least $0{\cdot}25\mu$will replace the cell edge and if no special precautions are taken the band may be as wide as $0{\cdot}4\mu$."

There were no facilities for applying the photographic method described by Ponder (1934) and this author states in his work (dated 1924) that Pyper's (1919) diffraction method cannot be applied to avian red cells because of their oval shape.

Morphology of Ostrich Blood.

The cells in the blood of the ostrich closely resemble the corresponding types of cells in the blood of the fowl, and they may be best considered by first reviewing the literature on the morphology of fowl blood.

Most investigators are agreed that the following types of cells are present in the normal blood of the fowl; red cells, thrombocytes and five varieties of white cells, viz., lymphocytes, monocytes, cells with polymorphus nuclei and spindle-shaped acidophil granules in their cytoplasm, cells with polymorphous nuclei and round acidophil granules in their cytoplasm, and basophiles.

Gibbs (1934) refers to a "polymorphonuclear leucocyte without rods and granules" as also being present in normal fowl blood in addition to the above-mentioned types of cells, while several workers saw no monocytes, and the findings of those who do record the presence of monocytes differ considerably. Thus, to quote only some of the workers listed in Table (2):—

Warthin (1907), Hedfeld (1911), Burchardt (1912) and Kleineberger and Carl (1912) found no monocytes in fowl blood. According to Wirth (1931), Steen (1913) is of opinion that they do occur, but only seldom, Salomon (1919) records 1 to 2 per cent., Fritsch (1920) 2 per cent. and Romer (1921) 1·4 per cent. Wirth (1931) records 2 per cent. On the other hand, Ellerman and Bang (1908) counted 23 per cent., Schmeisser (1915) 19·4 per cent., Breusch (1928) 9·2 per cent., Forkner (1929) (using supravital technique) 17·1 per cent. and Cook and Dearstyne (1934) (using supravital technique) 4 to 33 per cent.

These discrepancies serve to emphasise the difficulty of differentiating between lymphocytes and monocytes in ordinary stained preparations of fowl blood; in ostrich blood it is as difficult.

There can be no dispute about the presence of the elements which Sugiyama (no date) and others consider to be thrombocytes, but Blain (1928) states that he identified no structures corresponding to the platelets of mammals and he does not mention the thrombocytes.

Haematological terminology also gives rise to some confusion, entirely different cytological entities being called by the same name, or the same cytological entity by a variety of terms. Especially is this true of the cells with the spindle-shaped acidophil granules and those with the round acidophil granules, as will be observed from Magath's (1934) table given below.

Polymorphonuclear Leukocytes in Birds.

Author.	Date.	Eosinophilic rods.	Eosinophilic granules.
		Eosinophilic leukocytes.	
Bizzozero and Torre	1880	" Little spindleshaped bodies "	Granular forms.
		Eosinophilic leukocytes.	
Grunberg.....	1901	Crystalloid.....	Globular-like granules.
		Eosinophilic leukocytes.	
Cullen.....	1903	Crystalloid.....	Granular forms.
		Polymorphonuclear eosinophils.	
Warthin.....	1907	Crystalloid type.....	Granular type.
Goodall.....	1910	Polymorphonuclear leucocyte with rods	Eosinophil.
Kleineberger and Carl	1912	Pseudo-eosinophil.....	Eosinophil (with small granules)
		Polymorphonuclears.	
Schmeisser.....	1916	Eosinophilic rods.....	Eosinophilic granules.
Burnett.....	1917	" Polymorphs ".....	" Eosins."
Fritsch.....	1920	Pseudo-eosinophils.....	Eosinophils.
Hayden.....	1927	Polymorphonuclears.....	Eosinophils.
		Polymorphonuclears.	
Blain.....	1928	Eosinophilic rods.....	Eosinophilic granules.
Forkner.....	1929	Eosinophils.....	Pseudo-eosinophils.
		Polymorphonuclears.	
Thompsen and Engelbrethholm	1931	Pseudo-eosinophils.....	Eosinophils.
Maximow and * Bloom	1931	Heterophil.....	Eosinophil.
Wirth*.....	1934	Pseudo-eosinophiles.....	Eosinophiles.

* Investigators listed by the writer.

Burnett (1917) considers that the name " polymorphonuclear leucocyte with eosinophilic rods " is inexcusable. A " polymorphonuclear leucocyte " in his opinion signifies not merely a leucocyte with a polymorphous nucleus but the specific name of a certain kind of leucocyte, otherwise called " neutrophil ".

Maximow and Bloom (1931) adopted the term " heterophil ", first used by Kyes, as a comprehensive term to include the group of cells whose granules, though constant in a particular species, differ in form, size and staining reaction according to species. Hence the cell with the spindle-shaped granules was named " heterophil ", and in avian haematology investigators have apparently applied this term only to that particular cell.

The names " heterophile " and " eosinophile " for the cells with the spindle-shaped granules and for those with the round granules respectively appear to the writer the most appropriate and they will be so used henceforth.

In smears prepared from ostrich blood and stained with Wright's stain the cells are differentiated by the following characters:

Erythrocytes.

Special interest attaches to the erythrocytes of the ostrich, for in smears of the blood of normal birds these cells may sometimes be seen in various stages of development. In this study detailed descriptions will be given only of the mature cells, the cells which are almost mature (polychrome erythrocytes) and those closest to the stem cell or haemocytoblast (basophil erythroblasts—Ferrata cited by Furth 1931).

Mature erythrocytes.—(Fig. 1 on plate.) These cells vary in shape from elliptical to almost circular, the younger forms tending to be round, but in smears many irregular shapes may occur owing to pressure. Occasionally a cell may be seen without a nucleus. The cytoplasm is homogeneous and as stated under " Staining of Smears " when correctly stained with Wright's stain it is a fawn colour.

The nucleus which is usually oval is an extremely pachychromatic and trachychromatic type. The basichromatin is arranged to form a coarse pattern in angular or roughly rounded blotches which frequently cause irregular elevations of the nuclear membrane and between which the oxychromatin forms a lighter meshwork. Very occasionally a mature cell may be seen with two nuclei or one in which the long axis of the nucleus is situated transversely to the long axis of the cell. The nucleus may also be small and pycnotic.

Two hundred regularly shaped and fully developed cells were measured at random in dried, stained smears and the length varied from 11·5μ to 15·93μ and the breadth from 7·08μ to 9·73μ, the average length being 13·98μ and the breadth 7·92μ. The measurements of the nuclei were as follows: Length 4·07μ–7·96μ, average length 6·33μ, breadth 1·77μ- 2·65μ, average breadth 2·19μ.

In practically every smear there may be seen roundish structures which are light purple in colour and irregular in outline. They are of loose structure and measure about 8μ in diameter. These are injured nuclei of ruptured erythrocytes (smudges). Sometimes part of the cytoplasmic rim enclosing a certain amount of cytoplasm similarly stained to the cytoplasm of the ripe erythrocyte may still be seen partly surrounding the body. These structures occur no matter in what way or how carefully smears are prepared.

In moist preparations the erythrocytes of the ostrich have a buff yellow colour and the nuclei appear indistinct against the colour of the haemoglobin. The nuclear structure is almost invisible but in a cell with little haemoglobin the oval shape and the clumps of chromatin of the nucleus can be seen. Cells with little haemoglobin cannot always be easily recognised in the counting chamber and they may be mistaken for free nuclei, thrombocytes or even small lymphocytes. Only by close observation and suitable manipulation of the condenser can the faint cell membrane be discerned.

Neser (1923) states with reference to equine erythrocytes: " In smears, however, the cells are more or less stretched out and the measurements are in consequence larger than those obtained in moist preparations ", but Ponder (1934) remarks, " Although the point has been the subject of considerable controversy in the past I think it can fairly be said that it is now established that the red cell diameter is from 8–16 per cent. less when the cell is dried than when it is floating in plasma or serum ". The writer also made measurements of ostrich red cells bathed in plasma, the following procedure being followed: Fresh heparinised blood was diluted a hundred times with its own plasma. The diluted blood was then introduced into a red cell counting chamber and the preparation ringed with vaseline. Thus the cells were not subjected to any pressure and the preparation remained suitable for a long time. Two hundred cells were measured and the dimensions were as follows: Length 15μ–16·5μ, average length 15·25μ, breadth 7·75μ–10·25μ, average breadth 9·25μ. From Table 1 it will be seen that these measurements approximate closely the figures given by Gulliver and by Hayem.

Polychrome Erythrocytes.—(Fig. 2 on plate.) The cells approaching maturity resemble closely in shape and size the fully developed cell. The cytoplasm shows only slight polychromasia, staining a light grey colour. The nuclei are larger and more oval than those of the mature cells and they show large angular clumps of chromatin which stain a slightly paler colour than the nuclear chromatin of the ripe cell.

Basophil Erythroblasts (Fig. 3 on plate). These cells are usually almost round but may be oval and they vary considerably in size. The larger forms usually measure about 12·4μ in diameter. The cytoplasm is very basophilic and the cytoplasmic layer around the nucleus is narrower than that of the polychrome erythrocyte. There may sometimes be seen in the cytoplasm granules which appear in staining reaction and size very similar to the azurophil granules of lymphocytes. The nuclei are as a rule circular and large and though the chromatin particles which stain a dark purple colour may be

denser than in the more mature cells yet the tendency toward a checkerboard distribution of angular particles of chromatin is apparent.

Between the polychrome erythrocytes and the basophil erythroblasts may be seen various intermediate forms varying in shape, size and staining reaction. Some may be round and others oval. Round forms measuring only 7·9μ also occur. Immature forms are very occasionally seen in a state of mitosis and the cytoplasm of such cells is usually less basophilic than that of basophil erythroblasts. (Fig. 4 on plate.)

Reticulation can be favourably studied in the immature cells by staining them by the method used by Magath and Higgins (1934). The reticulations are heavy and look like a network in the very young cells, whereas in the older cells only a few strands or dots are to be seen.

The percentage of immature red cells which can be recognised in smears stained with Romanowsky stains was determined by counting, with the use of an Ehrlich eyepiece, 400 red cells. The results are shown in Tables 3-10 and a statistical analysis of them is given in Table 11.

The data were analysed statistically by the following formulae:—

(*a*) Standard deviation (σ)..... $= \sqrt{\frac{\Sigma d^2}{n-1}}$

(*b*) Standard error..... $= \frac{\sigma}{\sqrt{n}}$

(*c*) Coefficient of variability..... $= \frac{\sigma\ 100}{\mathrm{M}}$

Σd^2 = Sum of the differences squared.

n = Number of observations.

M = Mean.

In the smears from the normal ostriches (1–5) these cells constituted 2·5±0·1 per cent. (Standard deviation 1·1; Coefficient of variability 47·2 per cent.) of the red cells, ranging from 0 per cent. to 7·0 per cent. It will be seen that the results from birds 6–17 closely agree with those from the normal birds, but it is noteworthy that only one immature erythrocyte was seen in the smears from the worm-infested birds 18–22.

The number of basophil erythroblasts per c.mm. was estimated by determining from the smear the ratio between these and the leucocytes and applying this ratio to the total leucocyte count. The ratio was computed from the number of basophil erythroblasts enumerated each time 200 leucocytes were counted.

TABLE 3.

Blood Cell Counts, etc., of Ostrich clinically healthy and found free from Disease on Post-Mortem Examination. Bird reared on Farm Mariendahl, Stellenbosch District. *(See pages 428-429.)*

No.	Sex.	Date Bled.	Age.		R.P.*	Erythrocyter per c.mm.	Percentage erythrocytes which in Romanowsky Stained Smears showed either Polychromatic or basophilic cytoplasm.	Basophil-erythroblasts per c.mm.	R.P. / R.C.	Thrombocytes per c.mm.	Leucocytes per c.mm.	Per Cent.				
												L.	M.	H.	E.	B.
			Mths.	*Days.*												
1	Male...	25/ 5/35	6	25	45·9	1,840,000	2·5	0	24·94	12,235	21,049	21·7	3·7	54·0	11·7	8·7
		17/ 6/35	7	17	45·8	1,944,000	2·0	0	23·60	13,860	25,301	23·0	2·2	55·7	10·5	8·5
		29/ 8/35	9	29	47	1,927,000	2·0	0	24·35	6,223	25,444	32·0	2·0	57·5	4·5	3·5
		25/ 9/35	10	25	47	1,910,000	4·0	0	24·60	11,869	28,672	21·2	2·7	60·0	5·0	8·0
		30/11/35	13	—	48	1,880,000	1·0	0	25·53	12,337	15,578	21·0	2·7	64·0	4·2	8·0
		30/ 1/36	15	—	50·2	1,950,000	1·5	0	25·74	7,526	21,344	21·2	3·5	58·2	10·7	6·2
		13/ 2/36	15	13	49·2	2,064,000	2·2	0	23·88	14,076	27,671	29·0	2·7	44·5	15·3	8·3
		15/ 4/36	17	15	50·6	1,970,000	2·0	0	25·68	6,136	20,408	32·5	3·7	48·5	8·0	7·2
		9/ 5/36	18	9	51·4	2,117,000	1·0	0	24·36	9,234	22,852	26·5	3·0	56·0	4·0	10·5
		20/ 7/36	20	20	51	1,920,000	3·0	146	26·56	12,264	29,255	23·7	8·5	58·7	2·7	6·2
		26/ 8/36	21	26	54	2,199,000	2·2	0	24·59	8,925	21,059	29·2	1·7	41·5	19·7	7·7
		22/ 9/36	22	22	52	2,000,000	2·0	0	26	12,190	22,956	23·2	5·0	58·5	3·4	9·7
		1/12/36	25	1	52	1,927,000	4·0	183	26·94	6,014	12,326	30·6	4·3	51·2	4·5	9·2
		4/ 2/37	27	4	52·4	1,967,000	4·7	258	26·59	8,944	17,253	27·7	3·2	52·7	6·5	9·7
		19/ 5/37	30	19	54	2,020,000	3·0	465	26·73	9,207	18,600	28·1	4·1	51·5	5·7	10·3

* Abbreviations :—

R.P. for percentage volume of the erythrocytes.
R.C. for number of erythrocytes per c.mm. of blood—expressed in millions.
L. for lymphocytes.
M. for monocytes.
H. for heterophiles.
E. for eosinophiles.
B. for basophiles.

TABLE 4.

Blood Cell Counts, etc., of Ostrich clinically healthy and found free from Disease on Post-Mortem Examination. Bird reared on Farm Mariendahl, Stellenbosch District. (*See pages* 428-429.)

No.	Sex.	Date Bled.	Age.		R.P.*	Erythrocyter per c.mm.	Percentage erythrocytes which in Romanowsky Stained Smears showed either polychromatic or basophilic cytoplasm.	Basophil-erythroblasts per c.mm.	R.P. / R.C.	Thrombocytes per c.mm.	Leucocytes per c.mm.	Per Cent.				
												L.	M.	H.	E.	B.
			Mths.	*Days.*												
2	Male...	9/ 7/35	8	9	44·4	1,720,000	2·5	246	25·81	12,160	24,751	27·0	2·5	60·0	8·7	1·7
		29/ 7/35	8	29	46	1,756,000	1·0	0	26·13	2,478	23,617	30·7	2·2	50·5	14·7	1·7
		25/ 9/35	10	25	44·4	1,729,000	2·2	0	25·66	8,554	18,251	27·0	4·7	58·2	8·2	1·7
		26/10/35	11	26	45	1,749,000	1·5	0	25·71	10,509	18,729	33·0	3·5	50·5	9·0	4·0
		7/12/35	13	7	43·8	1,653,000	1·5	0	26·54	10,608	15,543	32·5	4·5	48·2	9·5	5·2
		19/ 2/36	15	19	45·1	1,757,000	2·0	111	25·77	13,328	22,333	37·0	3·1	46·3	7·2	6·2
		15/ 4/36	17	15	43·8	1,700,000	1·7	0	25·76	9,660	23,103	34·5	2·5	52·0	4·5	6·5
		22/ 5/36	18	22	49	1,840,000	4·0	184	26·63	19,623	18,571	26·7	3·2	63·0	3·5	3·5
		14/ 7/36	20	14	49·1	1,830,000	1·0	0	26·77	7,020	21,567	22·2	3·0	60·0	9·7	5·0
		26/ 8/36	21	26	47·4	1,844,000	2·7	0	25·76	9,114	19,768	25·5	3·5	53·7	10·5	6·7
		26/ 9/36	22	26	46	1,983,000	1·0	0	23·23	13,746	17,461	25·0	3·0	62·5	4·7	4·7
		27/11/36	24	27	46·4	1,710,000	1·0	88	27·13	8,272	17,665	25·0	3·2	63·2	4·0	4·5
		21/12/36	25	21	47·1	1,831,000	2·2	318	25·73	11,554	21,312	25·5	6·0	59·2	6·0	3·2
		4/ 2/37	27	4	47·5	1,845,000	5·2	660	25·81	9,130	22,070	27·0	1·7	55·2	7·5	8·5
		19/ 5/37	30	19	50	1,903,000	2·2	195	26·31	11,088	13,157	25·5	6·0	59·2	6·0	3·2

* Abbreviations:—

R.P. for percentage volume of the erythrocytes.
R.C. for number of erythrocytes per c.mm. of blood—expressed in millions.
L. for lymphocytes.
M. for monocytes.
H. for heterophiles.
E. for eosinophiles.
B. for basophiles.

TABLE 5.

Blood Cell Counts, etc., of Ostrich clinically healthy and found free from Disease on Post-Mortem Examination. Bird reared on Farm Mariendahl, Stellenbosch District. (*See pages* 428-429.)

No.	Sex.	Date Bled.	Age.		R.P.*	Erythrocyter per c.mm.	Percentage erythrocytes which in Romanowsky Stained Smears showed either Polychromatic or basophilic cytoplasm.	Basophil-erythroblasts per c.mm.	R.P. / R.C.	Thrombocytes per c.mm.	Leucocytes per c.mm.	Per Cent.				
												L.	M.	H.	E.	B.
			Mths.	*Days.*												
3	Male...	12/ 7/35	8	12	48·9	1,916,000	1·2	156	24·45	9,984	31,303	26·7	2·5	60·7	8·2	1·7
		26/ 9/35	10	26	48	1,862,000	2·7	110	25·80	10,560	22,098	25·2	2·7	63·2	7·7	1·0
		9/12/35	13	9	46·5	1,806,000	2·2	200	25·69	14,600	20,044	26·5	3·7	59·0	7·7	3·0
		21/ 2/36	15	21	47	1,837,000	4·0	336	25·68	14,803	22,548	36·2	2·5	54·2	4·2	2·7
		15/ 4/36	17	15	45·4	1,873,000	2·5	0	24·27	14,060	29,596	26·5	3·2	65·5	3·0	1·7
		27/ 5/36	18	27	49·4	1,940,000	2·0	392	25·46	9,800	19,673	29·7	1·2	62·0	5·2	1·7
		14/ 7/36	20	14	52	1,920,000	3·0	488	27·08	10,248	24,495	28·7	1·7	59·0	7·2	3·2
		28/ 9/36	22	28	52	2,017,000	3·0	721	25·87	9,064	20,611	28·0	3·0	60·0	6·5	2·5
		27/11/36	24	27	48·5	1,980,000	3·0	206	24·49	5,562	20,618	28·5	3·5	55·2	8·7	4·0
		21/12/36	25	21	50·5	1,963,000	2·0	220	25·76	12,210	22,077	23·2	2·7	64·2	6·5	3·2
		4/ 2/37	27	4	50·5	1,962,000	4·0	792	25·76	10,593	19,994	28·5	3·2	57·2	7·0	4·0
		19/ 5/37	30	19	51	1,963,000	6·0	388	26·02	8,148	19,436	23·0	4·2	64·7	4·7	3·2

* Abbreviations :—

R.P. for percentage volume of the erythrocytes.
R.C. for number of erythrocytes per c.mm. of blood—expressed in millions.
L. for lymphocytes.
M. for monocytes.
H. for heterophiles.
E. for eosinophiles.
B. for basophiles.

TABLE 6.

Blood Cell Counts, etc., of Ostrich clinically healthy and found free from Disease on Post-Mortem Examination. Bird reared on Farm Mariendahl, Stellenbosch District. (*See pages* 428-429.)

No.	Sex.	Date Bled.	Age.		R.P.*	Erythrocyter per c.mm.	Percentage erythrocytes which in Romanowsky Stained Smears showed either polychromatic or basophilic cytoplasm.	Basophil-erythroblasts per c.mm.	R.P. / R.C.	Thrombocytes per c.mm.	Leucocytes per c.mm.	Per Cent.				
												L.	M.	H.	E.	B.
			Mths.	*Days.*												
4	Female.	21/ 5/35	6	21	45·4	1,693,000	3·0	0	26·86	20,086	24,296	27	2·7	56·2	12·7	1·2
		26/ 8/35	9	26	47	1,870,000	3·0	0	25·13	4,752	19,871	31	0·5	59·2	7·7	1·5
		24/ 9/35	10	24	48	1,750,000	3·2	0	27·42	6,018	20,459	27·5	2·7	62·5	4·7	2·5
		30/11/35	13	—	49·9	1,926,000	2·7	0	25·85	12,500	20,148	31	1·0	50·7	11·7	5·5
		27/ 1/36	14	27	47·9	1,948,000	2·5	0	24·56	17,191	23,496	31·5	2·1	46·5	14·6	5·1
		15/ 4/36	17	15	48·9	1,840,000	2·5	0	26·57	8,814	15,663	35·5	3·5	40·5	16·5	4·0
		9/ 5/36	18	9	49·9	1,930,000	0	0	25·85	9,625	15,325	30·5	2·7	52·2	8·0	6·5
		3/ 6/36	19	3	50	1,920,000	3·0	0	26·04	12,274	23,529	19·2	2·5	71·5	3·2	3·5
		20/ 7/36	20	20	47·8	1,740,000	2·5	161	27·47	16,422	32,273	25·2	5·5	61·7	6·0	1·5
		26/ 8/36	21	26	51	1,993,000	2·2	106	25·62	12,190	21,252	26·7	3·0	63·7	3·2	3·2
		22/ 9/36	22	22	51	1,890,000	4·2	140	26·98	15,194	14,157	25	2·7	64·7	2·0	5·5
		24/11/36	24	24	49	1,920,000	4·0	114	25·52	7,980	22,866	26·8	0·6	64·8	3·5	4·1
		19/12/36	25	19	49·6	1,926,000	2·7	71	25·84	10,008	14,399	30·5	1·5	59·2	3·2	5·5
		4/ 2/37	27	4	50·8	1,981,000	2·0	0	25·65	10,476	21,759	28·2	3·2	55·7	7·2	6·2
		17/ 5/37	30	17	50·6	1,900,000	3·5	0	26·63	12,852	21,514	27·7	3·0	60·5	4·7	4·0

* Abbreviations :—

R.P. for percentage volume of the erythrocytes.
R.C. for number of erythrocytes per c.mm. of blood—expressed in millions.
L. for lymphocytes.
M. for monocytes.
H. for heterophiles.
E. for eosinophiles.
B. for basophiles.

Table 7.

Blood Cell Counts, etc., of Ostrich clinically healthy and found free from Disease on Post-Mortem Examination. Bird reared on Farm Mariendahl, Stellenbosch District. (*See pages* 428-429.)

No.	Sex.	Date Bled.	Age.		R.P.*	Erythrocyter per c.mm.	Percentage erythrocytes which in Romanowsky Stained Smears showed either Polychromatic or basophilic cytoplasm.	Basophil-erythroblasts per c.mm.	R.P. / R.C.	Thrombocytes per c.mm.	Leucocytes per c.mm.	Per Cent.				
												L.	M.	H.	E.	B.
			Mths.	*Days.*												
5	Female.	15/ 7/35	8	15	53	2,266,000	1·5	395	23·34	2,686	15,880	21·5	3·5	72·0	1·2	1·7
		28/ 9/35	10	28	47·9	1,980,000	3·7	116	24·19	10,374	23,329	21·2	2·5	74·5	0·7	1·0
		20/11/35	12	20	48	1,856,000	1·0	0	25·80	6,439	27,495	23·2	2·2	68·5	1·7	4·2
		21/ 1/36	14	21	40·6	1,676,000	3·0	0	25·94	8,613	19,959	17·8	2·5	75·5	1·0	3·1
		24/ 2/36	15	24	50	1,863,000	1·0	0	26·88	13,000	20,862	23·7	4·2	63·2	2·2	6·5
		15/ 4/36	17	15	45·7	1,853,000	1·0	0	24·70	4,646	20,164	17·0	2·0	78·5	0	2·5
		27/ 5/36	18	27	53·4	2,110,000	2·0	0	25·30	7,426	18,041	24·5	1·5	69·7	0·7	3·5
		14/ 7/36	20	14	53·8	2,096,000	2·2	182	25·61	15,168	19,204	25·5	1·5	65·7	1·7	5·5
		28/ 9/36	22	28	50·9	1,883,000	3·0	1,717	27·07	15,958	20,214	24·7	2·0	66·2	2·7	4·2
		24/11/36	24	24	53·5	1,853,000	7·0	6,283	28·91	7,072	20,733	33·5	3·7	53·0	3·2	6·5
		12/12/36	25	21	54	2,195,000	3·0	1,339	25·54	9,476	20,704	23·7	3·0	68·5	2·0	2·7
		4/ 2/37	27	21	53·2	2,167,000	1·2	890	25·64	12,905	17,890	26·5	2·8	62·0	3·6	5·0
		15/ 5/37	30	15	52	1,953,000	3·0	89	26·66	8,811	17,857	18	3·5	68·7	3·7	6·0

* Abbreviations :—

R.P. for percentage volume of the erythrocytes.
R.C. for number of erythrocytes per c.mm. of blood—expressed in millions.
L. for lymphocytes.
M. for monocytes.
H. for heterophiles.
E. for eosinophiles.
B. for basophiles.

TABLE 8.

Blood Cell Counts, etc., of Ostrich with Club Foot. (*See pages* 428-429.)

No.	Sex.	Date Bled.	Age.		R.P.*	Erythrocyter per c.mm.	Percentage erythrocytes which in Romanowsky Stained Smears showed either polychromatic or basophilic cytoplasm.	Basophil-erythroblasts per c.mm.	R.P. / R.C.	Thrombocytes per c.mm.	Leucocytes per c.mm.	Per Cent.				
												L.	M.	H.	E.	B.
			Mths.	*Days.*												
6	Female.	21/ 7/35	8	21	47	2,053,000	3·0	524	22·92	4,061	26,210	28·2	1·5	66·0	3·2	1·0
		10/ 8/35	9	10	49	1,921,000	2·2	296	25·52	12,580	29,668	28·2	3·2	62·5	4·0	2·0
		25/ 9/35	10	25	43	1,669,000	3·0	219	25·74	14,235	43,914	21·7	4·5	67·5	4·5	1·7
		20/11/35	12	20	51	2,010,000	1·2	452	25·37	9,266	45,372	25·7	3·0	64·7	3·2	3·2
		24/ 2/36	15	24	49	1,943,000	1·5	290	25·25	18,415	29,039	26·2	3·2	65·7	0·5	4·2
		15/ 4/36	17	15	48·2	2,023,000	1·5	0	23·86	12,862	23,598	21	2·2	71·5	0·7	4·5
		20/ 5/36	19	—	46·9	1,863,000	2·0	0	25·21	26,650	40,986	14·2	6·0	78·5	0·2	1·0
		20/ 7/36	20	20	48	2,033,000	3·0	595	23·64	10,948	23,842	26·7	6·2	61·0	3·7	2·2
		7/ 9/36	22	7	52·3	2,034,000	2·5	364	25·76	19,838	36,463	15·7	0·7	80·0	0·5	3·0
		28/ 9/36	22	28	52·8	2,053,000	1·7	0	25·75	15,480	25,808	20	3·7	73·7	0·5	2·0
		1/12/36	25	1	50·1	1,913,000	2·2	192	26·23	5,238	19,323	14·6	2·5	81·0	0·3	1·5
		23/12/36	25	23	50·5	1,961,000	1·7	0	25·76	10,956	26,510	16	4·2	77·7	0·2	1·7
		9/ 2/37	27	9	50	1,940,000	2·5	0	25·77	6,400	25,623	15·5	4·7	75·0	1·5	3·2
		17/ 5/37	30	17	49	1,970,000	2·5	87	24·87	7,221	17,422	25·5	1·7	70·5	0·7	1·5

* Abbreviations :—

R.P. for percentage volume of the erythrocytes.
R.C. for number of erythrocytes per c.mm. of blood—expressed in millions.
L. for lymphocytes.
M. for monocytes.
H. for heterophiles.
E. for eosinophiles.
B. for basophiles.

TABLE 9.

Blood Cell Counts, etc., of clinically healthy Ostriches on which Post-Mortem Examinations were not conducted. Kept on Farm Nagwag, Bredasdorp District. (*See page* 429.)

No.	Sex.	Date Bled.	Age.	R.P.*	Erythrocyter per c.mm.	Percentage erythrocytes which in Romanowsky Stained Smears showed either Polychromatic or basophilic cytoplasm.	Basophil-erythroblasts per c.mm.	R.P. / R.C.	Thrombocytes per c.mm.	Leucocytes per c.mm.	Per Cent.				
											L.	M.	H.	E.	B.
7	Male...	1/ 5/36	Over 3 years	38·4	1,403,000	3·2	0	27·42	12,444	12,239	14·2	4·0	73·0	6·0	2·7
8	,,	,,	,, ,,	47·4	1,790,000	0	0	26·48	8,946	8,409	11·7	1·0	69·0	14·2	4·0
9	,,	,,	,, ,,	40·4	1,580,000	1·0	0	25·56	17,415	16,146	15·7	3·0	62·0	18·0	1·2
10	,,	,,	,, ,,	41·7	1,510,000	3·0	330	27·61	13,002	13,271	8·7	3·2	68·2	15·2	4·5
11	,,	,,	,, ,,	42·3	1,553,000	0·5	77	27·29	13,650	15,560	14·0	4·5	69·7	9·0	2·7
12	Female.	,,	,, ,,	41·4	1,563,000	3·0	0	26·53	12,300	24,570	5·2	2·0	73·5	18·7	0·5
13	,,	,,	,, ,,	42·3	1,620,000	1·7	0	26·11	12,118	14,336	12·2	4·0	69·0	9·5	5·2
14	,,	,,	,, ,,	41·5	1,533,000	1·5	0	27·12	7,720	7,868	13·7	4·2	68·7	3·2	10·0
15	,,	,,	,, ,,	41·4	1,637,000	0·7	0	25·24	15,330	14,122	8·0	3·0	79·5	7·2	2·2
16	,,	,,	,, ,,	49·4	1,790,000	2·0	0	27·59	13,536	9,347	4·0	2·0	83·2	9·5	1·2
17	,,	,,	,, ,,	42·4	1,430,000	4·0	70	29·65	15,336	14,154	6·7	4·5	72·7	12·5	3·5

* Abbreviations :—

R.P. for percentage volume of the erythrocytes.
R.C. for number of erythrocytes per c.mm. of blood—expressed in millions.
L. for lymphocytes.
M. for monocytes.
H. for heterophiles.
E. for eosinophiles.
B. for basophiles.

TABLE 10.

Blood Cell Counts, etc., of unthrifty Ostrich Chicks which on Post-Mortem Examination showed marked Verminosis. From Farm Vanderstelskraal, Bredasdorp District. (*See page* 429.)

No.	Sex.	Date Bled.	Age.		R.P.*	Erythrocyter per c.mm.	Percentage erythrocytes which in Romanowsky Stained Smears showed either polychromatic or basophilic cytoplasm.	Basophil-erythroblasts per c.mm.	R.P. / R.C.	Thrombocytes per c.mm.	Leucocytes per c.mm.	PER CENT.				
												L.	M.	H.	E.	B.
			Mths.	*Days.*												
18	Female.	15/ 3/36	4	—	34·3	1,400,000	0	0	24·50	11,256	16,973	26·2	3·0	54·0	13·7	3·0
		2/ 4/36	4	17	29·4	1,173,000	0	0	25·12	4,650	30,033	7·2	2·7	89·7	0·2	0
19	,,	16/ 3/36	4	—	29·5	1,330,000	0	0	22·18	14,112	22,561	45·7	2·0	48·2	1·5	2·5
20	Male...	16/ 3/36	4	—	38·0	1,573,000	0	0	24·20	7,392	11,204	26·0	3·5	68·2	1·0	1·2
21	,,	20/ 3/36	4	—	25·3	806,000	0	43	31·23	15,394	8,697	27·5	6·2	47·5	8·2	0·5
22	,,	16/12/36	2	—	41·8	1,707,000	1	204	24·44	14,008	13,793	15·5	9·5	70·7	2·2	2·0

* Abbreviations :—

R.P. for percentage volume of the erythrocytes.
R.C. for number of erythrocytes per c.mm. of blood—expressed in millions.
L. for lymphocytes.
M. for monocytes.
H. for heterophiles.
E. for eosinophiles.
B. for basophiles.

TABLE 11.

Statistical Analysis of Counts of Immature Erythrocytes.

Bird Number.*	Sex.	Number of Counts.	Minimum Percentage.	Maximum Percentage.	Mean Percentage.	Standard Error of the Mean.	Standard Deviation.	Coefficient of Variability.
1	Male	15	1·0	4·7	2·4	0·2	1·0	41·6
2	Male	15	1·0	4·0	2·1	0·2	1·1	50·0
3	Male	12	1·2	6·0	2·9	0·3	1·2	41·3
4	Female	15	0	4·2	2·7	0·1	0·6	22·2
5	Female	13	1·0	7·0	2·5	0·4	1·6	64·0
1, 2 and 3	Males	42	1·0	6·0	2·5	0·1	1·1	44·0
4 and 5	Females	28	0	7·0	2·6	0·2	1·1	42·3
1 to 5	Males and Females	70	0	7·0	2·5	0·1	1·1	44·0
6	Female	14	1·2	3·0	2·1	0·1	0·6	28·5
7 to 11	Males	5	0	3·2	1·5	0·5	1·3	86·6
12 to 17	Females	6	0·7	4·0	2·1	0·4	1·0	47·6
7 to 17	Males and Females	11	0	4·0	1·8	0·3	1·0	55·5
18 to 22	Males and Females	—	0	1·0	0·1	—	—	—

* For particulars of birds, see pages 428–429.

The number of erythroblasts observed in smears per 200 leucocytes counted vary for the normal ostriches from 0 to 61 averaging 2·6, and the calculated total counts, which are listed in Tables 3–10, range from 0 to 6283, averaging 264 per c.mm. These cells constitute from 0 to 0·33 per cent. of the erythrocytes, averaging 0·013 per cent.

The maximum count of 6283 is exceptionally high, considering that it is over three times as high as the count nearest to it. It is not clear why bird No. 5 showed such a high count on 24/11/36 as no cause for unusual erythrocyte regeneration was evident. The bird always seemed healthy and only 5 c.c. of blood were drawn from it about two months previously. However, the counts from this bird during the period 28/9/36–4/2/37, are suggestive of unusual erythropoiesis.

The blood of ostriches Nos. 7–17 contained comparatively few basophil erythroblasts.

Thrombocytes. (Figure 5 on Plate.)

These cells, which in fowl blood have been named hematoblasts by Hayem (1879) and by Goodall (1910), are usually oval, nucleated and 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 8·8μ in length and 5·3μ 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 basichromatin 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 by 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 lymphocytes 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 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. (Figs. 6 and 7 on plate.)

These cells are spherical and vary in size from 5·3μ to 14·1μ. 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 scarcely 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 does not appear to surround the nucleus completely. A varying number of azurophil granules sometimes occur in the cytoplasm and occasionally lingulate processes extend from the cytoplasmic rim. As already stated it 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 irregular shapes in smears on account of being wedged in between the erythrocytes. [1])

Monocytes. (Figs. 8 and 9 on plate.)

The cells, which may be considered homologous with the mammalian monocytes, vary in form from round to slightly oval and measure from 8·8μ to 12·4μ in diameter but in the oval forms the long axis may measure up to 21·2μ.

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. 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 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.

(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.

The cytoplasm has a greyish-blue colour and is more granular than that of the lymphocyte, having the ground-glass appearance usually seen in the monocytes of man. Sometimes the cytoplasm is dusted with innumerable minute azurophil granules. Small lingulate runners sometimes 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. (Figs. 10 and 11 on plate.)

These cells are spherical and measure from 8·9μ to 17·7μ in diameter, but like the large lymphocytes they are often irregularly shaped through being wedged between the erythrocytes. The nucleus which stains a reddish violet is polymorphous with varying degrees of lobulation. 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 which is colourless is filled with large spindle and rod-shaped granules which measure about 1·75μ in length and stain a dull red. 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 figure 12 on the plate, whereas the granules of the eosinophiles looked the same as usual. In other smears prepared at the same time from the same bird, the granules were as ordinarily seen. Many of the heterophilic granules were also roundish 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.

Olson (1937) apparently observed similar results for in referring to the heterophilic granules of the fowl he remarks: " Frequently in routinely stained smears these cytoplasmic bodies are distorted and they may then be variable in shape ".

These phenomena are of especial interest, for 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. According to these writers the heterophiles and the cells with the round acidophil granules (eosinophiles) are identical—the eosinophiles being the forms in which the granules retain their round shape.

Ellerman (1921) considers the two cell types distinct from each other as the heterophiles do not react to Graham's oxidase test, whereas the eosinophiles do. The writer can confirm this observation by Ellerman and has ascertained that in the blood of the fowl and the ostrich the eosinophiles are the only cells that are stained by Graham's benzidine stain.

Jackson (1936) in discussing myeloid leucosis and myelocytomas of fowls states that it seems usually to be assumed that the myelocytes present are the neoplastic counterparts of true eosinophile myelocytes because their granules are rounded, but that they are the precursors of the heterophiles for which he uses the name "pseudoeosinophiles". He further remarks: "The confusion arises because of the failure to realise that in the earlier myelocytic stages the pseudoeosinophilic granules are rounded, only becoming rod-shaped as the cell matures. Such rounded pseudoeosiniphilic granulations can be distinquished because the former (i) are coarser (ii) some of them are actually basophilic in reaction, developing eosinophilia at a later stage, and (iii) because on close examination a tendency can often already be seen to a slight elongation of the granules. For those who have difficulty with this conception it is essential to undertake a study of normal avian red marrow, when the above features will readily be determined; it must be borne in mind, however, that in the marrow true eosinophile myelocytes for comparison are often difficult to find and must be searched for".

The transformation of haemocytoblasts into heterophilic and eosinophilic myelocytes may be favourably studied in the ostrich for in its red marrow [1]) both types of myelocytes are found without difficulty.

A few heterophile myelocytes were once seen in a smear prepared from the blood of a normal ostrich—No. 5. The cells were round and measured about 17μ in 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 either round or oval. They are eccentric and stain purple. The acidophilic granules of the heterophile myelocytes stain a dull red and neither the acidophilic nor the basophilic granules of these cells stain with Graham's benzidine stain.

Eosinophiles. (Fig. 13 on plate.)

These cells are also round and measure from 8·7μ to 15·7μ. They are, therefore, of about the same size as the heterophiles.

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 bilobed. The cytoplasm which is colourless contains discrete round granules whose size and staining are uniform. They stain salmon red in marked contrast to the dull red colour of the granules of the heterophiles and they are also much smaller than the granules of the heterophiles. These granules stain a greenish yellow with Graham's benzidine stain.

Basophiles. (Figs. 14 to 15 on plate.)

The basophiles measure from 8·8μ to 10·6μ in diameter. The cell has a simple round nucleus which stains purple and shows a

[1]) (*a*) The marrow examined was taken from the upper extremity of the tibia.
(*b*) It was observed that the femur of the ostrich is devoid of marrow.

diffuse chromatin arrangement. 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, 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 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. (Fig. 16 on plate.) The granules of the basophile do not react to Graham's oxydase test.

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 in 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 a 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.

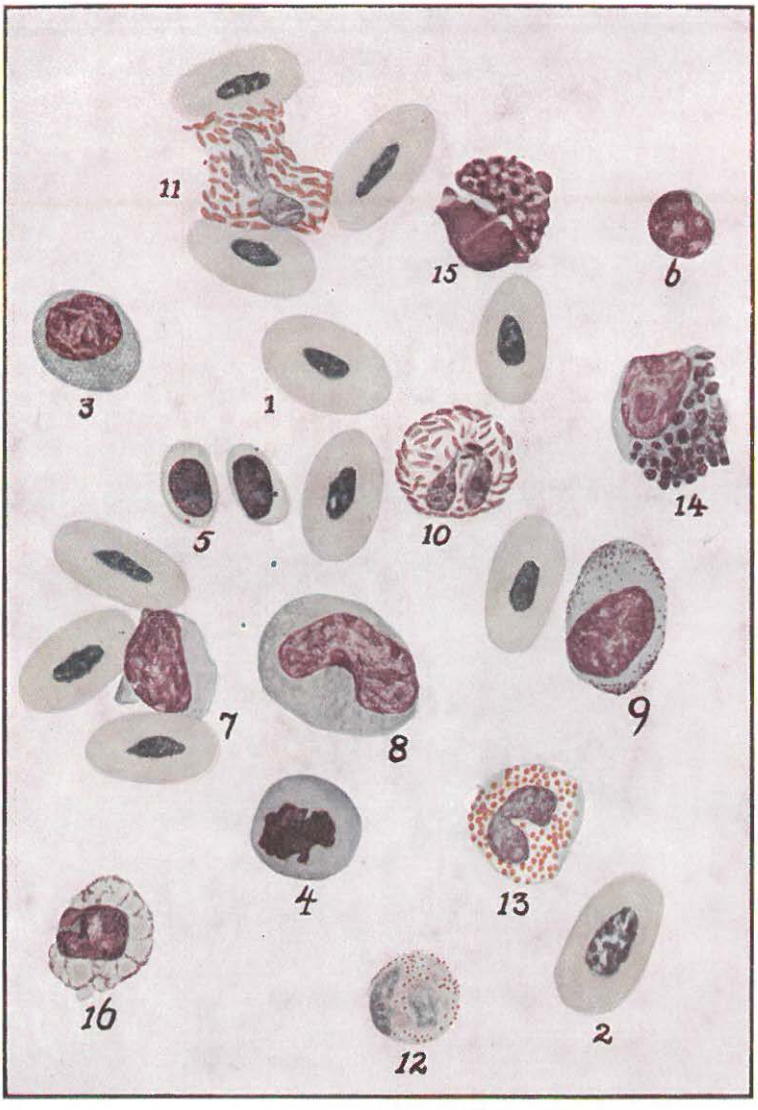
Detailed data pertaining to the erythrocyte counts of twenty-two ostriches are shown in Tables 3–10, and in Table 12 a statistical analysis of the erythrocyte counts is given.

As stated on page 429 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 $1{,}894{,}000 \pm 14{,}939$ (Standard deviation 124,000; Coefficient of variability 6·5 per cent.). The range was 1,653,000 to 2,266,000. The counts 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

(1) Personal communication from Dr. T. B. Magath.

BLOOD CELLS.



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 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 these contained many worm eggs. The low counts may, therefore, be possibly correlated with verminosis as in the case of the worm-infested birds, 18–22, 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.

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 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 with 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 45 minutes and the results read. 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 blood and 4, 5 and 6 heparinised blood.

TABLE 12.

Statistical Analysis of Erythrocyte counts.

Bird Number.*	Sex.	Number 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	Male	15	1,903,000	1,653,000	1,790,000	22,435	86,827	4·8
3	Male	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	Female	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 and 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 and Females	11	1,790,000	1,403,000	1,583,000	37,575	124,000	7·8
18 to 22	Males and Females	6	1,707,000	806,000	1,331,000	132,500	318,000	23·8

* For particulars of birds, see pages 428–429.

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
1,700.....	53·6	49	48·5	48·9	53·5	47·5
2,800.....	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 3,000 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 4,350 r.p.m. obtained a final reading of 39·0. At 5,700 r.p.m. the final result was 37·1 and at 11,600 r.p.m. 35·0. Ponder (1934), using rabbit's blood, obtained a final result of 31·5 at 1,700 r.p.m. and at 14,000 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 sought. The speed of 4,000 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 2,800 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 1,700 to 2,800 r.p.m.

Special care was always taken to shake the blood in the bottle 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 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 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 heparinsed 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 6·7. 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 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

TABLE 13. *Pecentage Volume Increases.*

	Sample No.	Temperature.	Anticoagulant. added.	Period which elapsed from time that sample was collected till percentage volume was obtained.														
				2 Hours.	3½ Hours.	5 Hours.	6½ Hours.	8 Hours.	9½ Hours.	11 Hours.	24 Hours.	36 Hours.	48 Hours.	56 Hours.	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	—	—	—
Average Increase..	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	9·6	—	—	—
	15	Kept in ice chest	Heparin	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	—
Average 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	15° C.–25° C.....	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	—	45·8	—	—	—	—	—	—	—	—	—	—	—	—	—
	51	,, ...	,, ,,	46·8	—	—	—	—	—	—	47·4	—	—	—	—	—	—	—	—
	52	Kept in ice chest	—	48·1	—	—	—	—	48·9	—	49	—	—	—	—	—	—	—	—
	53	,, ,,	—	48	—	—	—	—	—	—	49	—	—	—	—	—	—	—	—
	54	,, ,,	—	50	—	—	—	—	51	—	51	—	—	—	—	—	—	—	—

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 14.

Number of Sample.	Percentage Volume (Whole Blood).	Percentage Volume (Citrated Blood).	Difference.
1	47	45	2
2	48	46·5	1·5
3	49·3	48	1·3
4	50	49·2	0·8
5	47·8	46	1·8
6	49	48	1
7	46·9	45	1·9
8	51	49·5	1·5
9	48	46	2
10	45·2	44·3	0·9
11	45·7	44·3	1·4
12	50	48	2
13	49	47·2	1·8
14	46	46	0
15	49·2	47·9	1·3
16	49·3	47·6	1·7
17	50·2	48·4	1·8
18	50	48·5	1·5
19	49·2	48	1·2
20	51	49	2
21	54	52·5	1·5
22	53	52	1
23	48	46·3	1·7
24	47·5	45·5	2
25	40·6	39·5	1·1
26	50	48	2
27	50	48·2	1·8
28	53·8	52	1·8
29	52·5	52	0·5
30	34·3	33	1·3
31	29·5	29·3	0·2
32	38	36	2
33	25·3	25	0·3
35	50·2	48·9	1·3
AVERAGE DIFFERENCE			1·36

Tables 3-10 show the centrifuge readings and in Table 15 a statistical analysis of these is given.

TABLE 15.

Statistical Analysis of Percentange Volume Readings.

Bird Number.*	Sex.	Number of Readings.	Minimum Reading.	Maximum Reading.	Average Reading.	Standard Error of the Mean.	Standard Deviation.	Coefficient. of Variability.
1	Male	15	45·8	54·0	50·0	0·7	2·7	5·4
2	Male	15	43·8	50·0	46·3	0·54	2·0	4·3
3	Male	12	46·5	52·0	49·1	0·54	1·9	3·8
4	Female	15	45·4	51·0	49·1	0·4	1·6	3·2
5	Female	13	40·6	54·0	50·4	1·11	4·0	7·9
1, 2 and 3	Males	42	43·8	54·0	48·4	0·9	2·8	5·8
4 and 5	Females	28	40·6	51·0	49·7	0·56	3·0	6·2
1 to 5	Males and Females	70	40·6	54·0	48·9	0·34	2·9	5·9
6	Female	14	43·0	52·8	49·0	0·66	2·5	5·1
7 to 11	Males	5	38·4	47·4	42·0	1·50	3·3	7·8
12 to 17	Females	6	41·4	49·4	43·0	1·29	3·1	7·2
7 to 17	Males and Females	11	38·4	49·4	42·6	0·93	3·1	7·2
18 to 22	Males and Females	6	25·3	41·8	33·0	2·54	6·1	18·5

* For particulars of birds, see pages 428–429.

The average percentage volume reading for the normal birds (1-5) was $48 \cdot 9 \pm 0 \cdot 3$ ranging from 40·6 to 54·0 (standard deviation 2·9; coefficient of variability 5·9 per cent.).

It will be seen that the coefficient of variability ranged from 3·2 to 7·9 and that the exceptionally low percentage volume reading shown by bird No. 5 on 21/1/36 is correlated with the very low erythrocyte count shown by this bird on that date.

The results shown by No. 6 closely approximate those from the normal birds, but the readings obtained with the blood of most of the clinically healthy ostriches (7 to 17) are, as in the case of the worm-infested birds (18 to22), comparatively low.

Perusal of the literature did not reveal percentage volume results for birds. The writer, however, has made a number of percentage volume determinations also on fowl blood and these are appreciably lower than the results from ostrich blood.

Relation between Percentage Volume of Red Cells and Their Number.

Like Neser (1923), the writer determined the ratio of the volume of red cells to their number and in calculating the ratio the count was considered to the nearest 10,000.

The results are listed in Tables 3-10 and a statistical analysis is shown in Table 16. With a few exceptions, the ratios shown by the normal birds (1-5) closely approximate one another, averaging $25 \cdot 75 \pm 0 \cdot 12$ (standard deviation 1·02; coefficient of variability 3·9 per cent.).

Neser (1923), in discussing osmotic pressure of the blood, remarks: "In view of the above statements it is thought unlikely that the osmotic pressure of horse blood can vary much between individuals or in the same individual from time to time. If this is accepted then it must equally be accepted that the volume of the red cells cannot be influenced to any marked extent owing to changes in the osmotic pressure of the blood."

The ostrich does not usually drink much and as the skin of birds does not contain sweat glands (Bradley 1915 and Kaupp 1929) it may be presumed that in the ostrich there is even less change in osmotic pressure of the blood than there is in the blood of an animal like the horse which has a large fluid intake and perspires freely.

As the centrifuge reading can be relied upon to reflect accurately the true volume under the current circumstances the extreme variations are, no doubt, largely due to errors in the count.

Take, for example, the minimum ratio 23·23—obtained with the results shown by bird No. 2 on 26/9/36—and the maximum ratio 28·91—obtained with the results shown by bird No. 5 on 24/11/'36. The respective counts are 1,983,000 and 1,853,000. In discussing the erythrocyte counts, it is shown that they are subject to an error of 10 per cent. and if it is presumed that the above counts were respectively too high and too low by 10 per cent. then the corrected

TABLE 16.

Statistical Analysis of Ratios between Centrifuge Readings and the Red Corpuscular Counts.

Bird Number.*	Sex.	Number of Determinations.	Minimum Ratio.	Maximum Ratio.	Average Ratio.	Standard Error of the Mean.	Standard Deviation.	Coefficient of Variability.
1	Male	15	23·60	26·94	25·33	0·28	1·09	4·3
2	Male	15	23·23	27·13	25·91	0·22	0·88	3·39
3	Male	12	24·27	27·08	25·52	0·22	0·78	3·05
4	Female	15	24·56	27·47	26·13	0·21	0·83	3·17
5	Female	13	23·34	28·91	25·81	0·38	1·39	5·38
1, 2 and 3	Males	42	23·23	24·27	25·60	0·27	0·94	3·67
4 and 5	Females	28	23·34	28·91	25·98	0·21	1·12	4·31
1 to 5	Males and Females	70	23·23	28·91	25·75	0·12	1·02	3·96
6	Female	14	22·92	26·23	25·11	0·08	0·30	1·19
7 to 11	Males	5	25·56	27·61	26·87	0·38	0·85	3·16
12 to 17	Females	6	25·24	29·65	27·04	0·61	1·51	5·58
7 to 17	Males and Females	11	25·24	29·65	26·96	0·35	1·18	4·37
18 to 22	Males and Females	6	22·18	31·23	25·27	1·28	3·08	12·18

* For particulars of birds, see pages 428–429.

figures would be about 1,785,000 and 2,038,000. The ratios now become 25·84 and 26·35, thus differing only slightly from the average ratio of 25·75. It is, therefore, concluded that also in normal ostrich blood the red cells do not vary appreciably in volume, and that the number of red cells can be as accurately obtained by dividing the percentage volume reading by the figure 25·75 as by counting, provided the samples are centrifuged at the speed at which the centrifuge was run by the writer, and the centrifuge tubes have the same diameter.

The ratios obtained with the results from birds 7-17 are too few to permit of any conclusions being drawn, but the indications are that the red cells of some of them were slightly larger than those of birds 1-5.

The results from the worm-infested birds (18-22) show a comparatively high coefficient of variability.

Minimum and Maximum Resistance of the Red Cells.

The points of minimum and maximum resistance of the red cells of ostrich blood were determined in the following way: Half a c.c. of blood was added to each of a series of test tubes, each containing 10 c.c. sodium chloride solution, but each solution being 0·01 per cent. stronger than the last. Fresh solutions were always made up and the salt was oven-dried before use. After the addition of the blood, the tubes were gently shaken and then allowed to stand for 10 minutes. They were then centrifuged for 10 minutes at a speed of 2,800 revolutions per minute. Within the period specified complete haemolysis and the settling of unhaemolysed erythrocytes and haemoglobin-free stromata took place. It made little or no difference whether the tubes stood for 10 minutes or for 2 hours before being centrifuged and the readings were either the same or differed very slightly, whether the samples were centrifuged or allowed to stand until spontaneous settling of the cells had occurred. It appears that haemolysis is completed within a few minutes, but 10 minutes were allowed to make quite sure. These observations are in agreement with those made on sheep blood by Rossouw (1930).

Kolmer and Boerner (1931) state: "Normally human erythrocytes, carefully collected against injury, can remain for two hours at room temperature in solutions containing 0·42 to 0·44 per cent. sodium chloride before haemolysis begins, whilst under these conditions hemolysis is complete in 0·36 to 0·32 per cent. solutions". From this statement by Kolmer and Boerner we must conclude that the passage of water into the cells occurs much sooner in the red cells of the ostrich and the sheep than in those of man.

If, according to the method of Rossouw (1930), 1 c.c. ostrich blood be added to 20 c.c. distilled water—and the solution is considered as representing 100 per cent. haemolysis—then a one per cent. haemolysis can usually just be observed with the naked eye, for the solution is only very slightly tinged. When the first evidence of haemolysis was observed, lysis of at least one per cent. of the red

cells had therefore already occurred, for the samples were not examined spectroscopically, and the points of minimal resistance recorded indicate haemolysis of at least one per cent. of the cells. Tinging due to the plasma itself can be ignored, for, even when 1 c.c. of plasma is added to 10 c.c. of saline, no colouring of the solution can be detected. Whole blood was used and it was always added to the solutions within half an hour of bleeding. When haemolysis tests were conducted the temperature ranged from 15° C. to 20° C.

Workers in determining the fragility of the red cells used different concentrations of blood in saline. Isaacs (1929), for instance, added 1 drop (a variable quantity) of blood to 2 c.c. saline whereas Rossouw (1930) added 1 c.c. blood to 20 c.c. saline (approximately 1 drop to 1 c.c. saline) and Pepper and Farley (1933) added 1 drop blood to 4 c.c. saline. It is unfortunate that there should be no standard method of performing the test, as accurate comparison of results is often impossible. For example, from the graph given by Rossouw (1930) it will be observed that when 1 c.c. sheep blood was added to 20 c.c. of a 0·72 per cent. sodium chloride solution 3 per cent. haemolysis resulted, and in a 0·67 per cent. sodium chloride solution approximately 12 per cent. haemolysis occurred. The same author states that a 2 per cent. haemolysis gives almost a clear soloution, and a 3 per cent. haemolysis was found to be the lowest practical margin to work with. As the first evidence of haemolysis was observed in the 0·72 per cent. sodium chloride solution, 0·72 might have been considered the point of minimum resistance.

If 0·25 c.c. blood had been added to 20 c.c. of a 0·67 per cent. sodium chloride solution, as had been done by Pepper and Farley (1933), also 12 per cent. of the cells in the 0·25 c.c. blood would have haemolysed, but the solution would have shown a tint similar to that of a 3 per cent. haemolysis in the aforementioned concentration, viz., a colour which can just be observed, and in the latter concentration of blood and saline the point of minimum resistance would have been determined as 0·67.

Wiseman and Bierbaum (1932) have devised a fragility test in which the patient's cells are tested in his own plasma. Centrifugation of the blood is required, but it is not necessary to make up fresh saline each time the test is carried out, or to set up a control test with a known normal blood.

From the results given in Table 17 it will be noted that the point of minimum resistance of the red cells of the ostrich varies from 0·44 to 0·52 with an average of 0·47, and that the point of maximum resistance ranged from 0·25 to 0·3, with an average of 0·26.

Wirth (1931), citing Friedl (1931), gives the points of minimum and maximum resistance of fowl blood as 0·59 and 0·37 respectively, but according to Kleineberger and Carl (1927) the point of minimum resistance of fowl blood varies from 0·4 to 0·47.

TABLE 17.

Resistance of Erythrocytes to Haemolysis. Nos. 1 to 5—Ostriches clinically healthy and found free from disease on Post-mortem Examination. No. 6—Ostrich with club-foot. *See pages* 428-429.

Bird Number.*	Sex.	Date.	Age.		Minimum Resistance.	Maximum Resistance.
			Months.	Days.		
1	Male	1/10/35	11	1	0·47	0·3
		8/10/35	11	8	0·45	0·29
		30/11/35	13	—	0·44	—
2	Male	26/ 9/35	10	26	0·5	—
		4/10/35	11	4	0·48	0·27
		5/10/35	11	5	0·44	0·26
		19/10/35	11	19	0·5	0·27
		26/10/35	11	26	0·5	—
		11/11/35	12	11	0·5	—
		30/11/35	13	—	0·48	—
3	Male	26/ 9/35	10	26	0·45	0·27
		20/11/35	12	20	0·47	0·27
		7/12/35	13	7	0·46	—
4	Female	24/ 9/35	10	24	0·5	0·3
		1/10/35	11	1	0·47	0·3
		5/10/35	11	5	0·49	0·28
		11/11/35	12	11	0·5	—
		29/11/35	12	29	0·47	—
5	Female	25/ 9/35	10	25	0·49	0·3
		28/ 9/35	10	28	0·52	0·25
		20/11/35	12	20	0·44	0·27
Average 1, 2 and 3	Males	—	—	—	0·47	0·27
Average 4 and 5	Females	—	—	—	0·48	0·28
Average 1 to 5	Males and Females	—	—	—	0·47	0·28
6	Female	28/ 9/35	10	28	0·45	0·27
		20/11/35	12	20	0·44	0·27
		29/11/35	12	29	0·45	—

Osmotic Pressure on the Red Cells.

The effects of osmotic pressure on the red cells were determined by the method used by Neser (1923), and all the observations were made on whole blood. Repeated observations were made on the blood of six ostriches, and it was found that the cells in the 0·9 per cent. sodium chloride solution always gave either the same reading as those in the plasma, or the reading nearest to those in the plasma; 0·9 per cent. sodium chloride solution can, therefore, for practical purposes be accepted as isotonic for ostrich blood.

As already stated the writer's original intention had been to use isotonic lithium citrate solution as anticoagulant, and it was similarly determined that a 2·8 per cent. solution of this salt is also isotonic for ostrich blood.

Two sets of readings, which also reflect the osmotic effects of certain other solutions on the red cells, are given below:—

(1)

1 c.c. Concentrated blood plus	*Centrifuge readings.*
1 c.c. Plasma	36
1 c.c. 0·7 per cent. sodium chloride solution	39·5
1 c.c. 0·8 ,, ,, ,, ,,	38
1 c.c. 0·9 ,, ,, ,, ,,	36
1 c.c. 1 ,, ,, ,, ,,	35
1 c.c. 1·1 ,, ,, ,, ,,	34·5
1 c.c. 1·2 ,, ,, ,, ,,	34
1 c.c. Ringer's solution	38·5

(2)

1 c.c. Concentrated blood plus	*Centrifuge readings.*
1 c.c. Plasma	33·7
1 c.c. 0·8 per cent. sodium chloride solution	35
1 c.c. 0·9 ,, ,, ,, ,,	34
1 c.c. 1 ,, ,, ,, ,,	33
1 c.c. Ringer's solution	33
1 c.c. Locke's solution	33·5

In order to ascertain if the cells were similarly packed in 0·9 per cent. saline as in plasma, samples of whole blood were centrifuged for one and a half hours at 2,800 r.p.m. The plasma was then pipetted off till near the level of the red cell column and an amount of 0·9 per cent. saline, equal to the amount of plasma drawn off, was added. The tubes were shaken until the saline and the cells were well mixed and then the tubes were recentrifuged at the same speed for the same length of time.

The set of readings given below, which are typical of many other readings, show that the results obtained with whole blood were either the same or differed only slightly from those given by the saline mixtures, and that, in determining the isotonicity of the cells, the percentage volume readings obtained with the cells in the saline are therefore strictly comparable with those given by the cells in the plasma.

Blood.	*Centrifuge Readings.*
Whole blood	51
Cells plus 0·9 per cent. saline	52
Whole blood	48
Cells plus 0·9 per cent. saline	48
Whole blood	36
Cells plus 0·9 per cent. saline	37
Whole blood	50
Cells plus 0·9 per cent. saline	49·5

Haemoglobin Content.

The haemoglobin content was determined by means of a Newcomer Haemoglobinometer (Bausch and Lomb). The improved model is fitted with a complementary blue filter with the use of which a satisfactory colour match is obtained.

Schultze and Elvehjem (1934) state: "The Newcomer method was never as satisfactory for chicken blood as for mammalian blood because the turbidity of the acid solution makes it difficult to compare the unknown solution with the standard."

These authors describe a method in which the haemoglobin content is also determined colorimetrically and a Newcomer disc is used, but the latter requires restandardisation.

The haemoglobin content of the blood of the normal ostriches (1–5) varied from 14·6 to 17·2 grams per 100 c.c. blood with an average of 16 grams per 100 c.c. blood (Table 18).

Bird No. 22 was received from Bredasdorp District. It showed a heavy worm infestation and it will be noted that the haemoglobin determinations on its blood are appreciably lower than those on the blood of the normal birds.

Cook and Dearstyne (1934) appear to be the only investigators who have previously used the recent modification of the Newcomer Haemoglobinometer. They made haemoglobin determinations on the blood of 78 normal fowls and found the figure to vary from 7 to 17 grams with an average of 10·8 grams per 100 c.c. blood.

Leucocyte Counts.

The enumeration of the white cells in ostrich blood is attended with the same difficulties which are encountered in obtaining an accurate count of these cells in the blood of fowls and other birds.

The red cells are nucleated, and when the method ordinarily applied to mammalian blood, of using a diluting fluid containing acetic acid to dissolve the red cells, is used, then, upon the loss of the haemoglobin, the stromata of these cells contract about the nuclei, making it impossible to discriminate between these and the leucocytes.

TABLE 18.

Haemoglobin Content.

Nos. 1 to 5.—Ostriches clinically healthy and found free from disease on post-mortem examination.

No. 6.—Ostrich with club-foot. (See pages 428-429.)

No. 11.—Unthrifty ostrich chick which on post-mortem examination showed marked verminosis.

Bird Number.	Sex.	Date.	Age.		Gm. per 100 c.c. Blood.
			Months.	Days.	
1	Male	4/ 2/37	27	4	16
		20/ 2/37	27	20	17·2
		19/ 5/37	30	19	17·2
2	Male	4/ 2/37	27	4	15·0
		20/ 2/37	27	20	15·3
		19/ 5/37	30	19	15·5
3	Male	4/ 2/37	27	4	15·0
		20/ 2/37	27	20	14·6
		19/ 5/37	30	19	15·2
4	Female	4/ 2/37	27	4	16·0
		20/ 2/37	27	20	16·8
		23/ 3/37	28	23	17·0
		17/ 5/37	30	17	16·0
5	Female	4/ 2/37	27	4	17·0
		20/ 2/37	27	20	16·2
		15/ 5/37	30	15	17·0
Average 1, 2 and 3	Males	—	—	—	15·6
Average 4 and 5	Females	—	—	—	16·5
Average 1 to 5	Males and Females	—	—	—	16·0
6	Female	4/ 2/37	27	4	14·5
		20/ 2/37	27	20	14·4
		17/ 5/37	30	17	14·5
Average	—	—	—	—	14·4
11	Male	5/ 2/37	4	—	11·0
		8/ 2/37	4	3	11·5
		11/ 2/37	4	6	11·0
		19/ 2/37	4	14	11·8
		23/ 2/37	4	18	11·8

On the other hand, if a fluid like Toisson's solution is used, which preserves the red cells, the white cells and the thrombocytes, then again it is impossible to distinguish with certainty between the thrombocytes and the small lymphocytes under the powers of magnification that can be used in conjunction with the counting chamber.

Hayden (1927) used Toisson's solution, but mentions that the method was not satisfactory. Furth (1931), however, remarks that in his experience lymphocytes and thrombocytes can be easily differentiated in Toisson's solution. The solution has the following composition: Distilled water 160 c.c., glycerine (neutral), 30 c.c. sodium sulphate 8 gms., sodium chloride 1 gm., methyl violet 5B. 0·25 gm.

According to Forkner (1929), the thrombocytes of fowl blood may also be confused with red cells in which there is little haemoglobin, but such a difficulty is less likely to occur in ostrich blood, since the disparity in size between the two types is much greater than in the case of the fowl. Various methods have from time to time been advocated to overcome these difficulties, and these are considered below.

According to Shaw (1930),[1] Warthin (1907) and Schmeisser (1915) resorted to an indirect method. The erythrocytes were counted in the ordinary way, and then in films the ratio between them and the leucocytes was determined. Kleineberger and Carl (1912) counted the erythrocytes, leucocytes and thrombocytes in the counting chamber, and then calculated their respective values from the ratios of these cells found in stained smears.

The indirect methods are obviously subject to great error for they presuppose an absolutely even distribution of the cellular elements; this rarely obtains, as Forkner (1929) and others have pointed out.

Blain (1928) was the first to introduce a direct method for the purpose of staining the leucocytes differentially so that they could be counted in the counting chamber. He utilised the vital staining properties of neutral red and prepared two solutions:—Solution 1 contained neutral red (1 : 5,000), made up in Locke's solution and solution 2 contained 12 per cent. formalin, also made up in Locke's solution. Both solutions were adjusted to a pH of 7·4, and kept at a temperature of 39° C. while in use. The blood was first mixed with the neutral red solution, and the other solution was then added—the white cells take up the neutral red, whereas the red cells do not.

According to Shaw (1930), Blain's method when applied to human blood gives results 20 to 30 per cent. below those obtained by the acetic method. Ohlson (1935) also found lower leucocyte counts with this method than with the others which he tried, and it seems that it is chiefly the lymphocytes which are overlooked by this

(1) The writer consulted the publications cited by Shaw. Warthin does not definitely state that he used an indirect method, for he merely remarks: "Toissons solution was found to be the most satisfactory for the blood counting." Schmeisser states that he counted the leucocytes by the indirect method, but he gives no particulars about the method nor any reference.

method. It would also appear that Blain included the thrombocytes either in the red cell or the white cell count, since he does not mention these and, in a further communication (1928) already referred to, he states that he identified no structures in avian blood corresponding to the blood platelets of mammals.

Coates (1928) recommends the solutions which Wright and Kinnicut (1911) used for counting platelets in human blood. Two solutions are employed: Solution 1 contains 1 gm. brilliant cresyl blue in 300 c.c. distilled water, and solution 2 contains 1 gm. potassium cyanide in 1,400 c.c. distilled water. Equal parts of the two solutions are mixed and filtered immediately before use and the resulting solution constitutes the diluting fluid. With subdued illumination, the leucocytes are said to refract the light in such a manner as to render direct counting possible.

Ohlson (1935) found the method unsatisfactory, as the distinction was not well marked between the thrombocytes and the small lymphocytes.

Kyes (1929) recommended a 2 per cent. solution of osmic acid to eliminate the possibility of red cell haemolysis and consequent liberation of nuclei.

Ohlson (1935) states that there is a danger, in this method, of injury to the cornea of the observer, and that no particular advantages were apparent in the trials made.

Forkner (1929) used a diluting fluid, containing 25 mg. neutral red in 100 c.c. of 0·9 per cent. sodium chloride solution, and states that the polymorphonuclear granulocytic cells and the monocytes can easily be differentiated from the other cells; the red cells with little or no haemoglobin, the thrombocytes and the lymphocytes remaining almost entirely unstained. The proportion of granulocytes and monocytes is then determined from a blood smear, and the total leucocyte count is calculated.

Wiseman (1930) states that with Forkner's method the difference in staining intensity of the monocytes frequently occasions inaccuracies through the missing of individual cells, and that diluting fluids, which do not contain a fixative, often fail to preserve the erythrocytes intact. Cook and Dearstyne (1934) used this method, but made no comment on its efficacy.

Kozma (1929) also counted all three types of cells in the counting chamber, using the following solution: Sodium citrate 0·2 gm., ammonium oxalate 0·1 gm., distilled water 100. To this solution is added 8 c.c. of a one per cent. solution of eosin and 4 c.c. of a one per cent. trypanblue solution, and staining of the blood is allowed for 30 minutes before counting is commenced.

In Shaw's (1930) method, 2 solutions are used. Number 1 contains 25 mg. neutral red (Grubler), 0·9 gm. sodium chloride and 100 c.c. distilled water. Number 2 solution contains 12 mg. crystal violet, 3·8 gm. sodium citrate, 0·4 c.c. formaldehyde and 100 c.c. distilled water. Both solutions are filtered and heated to 107° F., the pipette is half filled with No. 1 solution and then filled with

solution No. 2. Shaw's observations were made on Homer pigeons, and all the cellular elements were differentiated by their staining reactions in the diluting fluid.

Palmer and Biely (1935) consider Shaw's method satisfactory for the enumeration of the leucocytes in fowl blood, but Ohlson (1935) is of opinion that it is no easier to distinguish the thrombocytes by this method than in Blain's or Toisson's solution. However, he does not state to what extent he considers the discrimination of the thrombocytes possible by Blain's method or in Toisson's solution, but Shaw himself thinks that the thrombocytes cannot be distinguished with certainty from lymphocytes in Toisson's solution and according to Forkner (1929) the thrombocytes may be confused with the lymphocytes in Blain's method.

Wiseman (1930) recommends the use of a solution having the following formula: 95 c.c. Ringer's solution, 5 c.c. formalin and 50 mg. phloxine. This solution stains the granulocytes a bright red in contrast with the other cells which stain much less brilliantly.

The total leucocyte count is then obtained by calculation of the percentage of these cells in the differential count. Wiseman states that maximum staining is obtained within one hour, but suggests that where time is not a factor of importance less phloxine should be used as more dilute solutions (3 mg. dye in 100 c.c. solution) stain the granulocytes almost specifically and the contrast is then at its best, but then a time interval of at least 3 hours is required.

Magath and Higgins (1934) consider that in none of the direct methods are the leucocytes stained in such a manner as to be always distinguishable from the erythrocytes.

Emmel (1935) counted the white cells in a 0·85 per cent. sodium chloride solution tinted with gentian violet, but he says: "There is often difficulty in differentiating the lymphoid precursors of erythrocytes, young erythrocytes and occasionally basophilic erythroblasts when counting erythrocytes and leucocytes in the same chamber."

From this review of the methods advocated for avian leucocyte counting, it will be evident that, as there is no unanimity of opinion about them, the writer had to select one most suitable according to his own observations. After trying them all, he reached the following conclusion: By none of the direct methods could the agranulocytes in ostrich blood be satisfactorily distinguished in the counting chamber from basophil erythroblasts, free red cell nuclei or from the thrombocytes.

The methods of Blain and Shaw are also impracticable when counts have to be made from adult ostriches which cannot be brought into or very near a laboratory, for the diluting fluids have to be at body temperature when mixed with the blood and when counts are being made. When the blood has to be collected eight miles away from the laboratory, as had to be done in this case—for there were no facilities for keeping the ostriches nearer—it is very difficult to conform to such conditions.

The writer's observations on the counting of the leucocytes of ostrich blood in Forkner's solution coincide with Wiseman's on fowl blood.

Wiseman's solution is preferred for leucocyte counting in ostrich blood for the following reasons: The granules in the granulocytes stain in such a distinctive manner, that these cells can be readily identified. This counting fluid is particularly suitable for counting white cells in ostrich blood, since ostrich blood contains a high percentage of granulocytes. The cells are stained satisfactorily within a short time and they are preserved for a long time. Counts made weeks later agreed, within limits of counting errors, with those made shortly after mixing. The counts can, therefore, be made at leisure at any convenient time. Since the staining qualities of the solution do not deteriorate, it can be prepared in large quantity if the bottle is kept well corked—to prevent evaporation of the formalin—and shaken every time before use.

Ohlson (1935), who made a comparative study (on fowl blood) of various methods in order to gauge their accuracy for both erythrocyte and leucocyte counts, found that Blain's method gave the most consistent results for leucocytes, Wiseman's method being placed third. He favours Wiseman's solution when dealing with pathological blood, stating that all the direct methods failed with the blood of an erythroleucotic chicken—the immature erythrocytes being confused with leucocytes—whereas in Wiseman's solution all the haemoglobin—bearing cells are stained in a characteristic manner; also that Wiseman's technique gave the lowest coefficient of variation in the erythrocyte counts and that it showed a tendency to give higher red cell counts. He also remarks: "An explanation for the tendency of the Wiseman method to give higher erythrocyte and leucocyte values is lacking. The erythrocytes are fixed and more distinctly stained than with the other methods." (The writer used Wiseman's solution as diluent for erythroleucotic fowl blood but also in this solution haemocytoblasts and basophil erythroblasts could not be differentiated from non-granular leucocytes.)

Wiseman's solution was used in making both the erythrocyte and the leucocyte counts, but the solution was slightly modified by substituting 0·9 per cent. sodium chloride solution containing 20 gm. sodium bicarbonate per 100 c.c. for Ringer's solution. The modified Wiseman diluting fluid, therefore, has the following formula: Phloxine 50 mg., sodium bicarbonate 20 mg., sodium chloride 0·9 mg., formalin 5 c.c., distilled water 95 c.c.

This solution proved as satisfactory as that containing Ringer's solution and its preparation entailed less work. (Locke's solution is isotonic for ostrich blood and in Wiseman's solution it is also a suitable substitute for Ringer's solution). Staining was allowed for at least one hour before the cells were counted. It is possible to recognize unstained granulocytes, but counting is greatly facilitated when the cells are well stained.

Turbidity may appear in an old sample of diluent, but this can be easily removed by adding a small quantity of sodium bicarbonate.

A difficulty with all the methods is that solutions sufficiently concentrated to preclude a large counting error cannot be used. With all the methods the erythrocytes are preserved and when the red cell count is somewhat high it is necessary to use dilutions of 1 in 200.

However, in normal ostrich blood—though the red cells are comparatively large—counts can be made wih ease in dilutions of 1 in 100. Dilutions of 1 in 50 are also suitable but it was customary also to count the red cells in the same chamber and in this concentration accurate counting is difficult and, if prolonged, a severe strain on the observer's eyes as the cells are then close together. But as the numerical error resultant on multiplication is halved by using the 1 in 50 dilution it may be used with advantage when the intention is to count the granulocytes only for the erythrocytes are not so close together as to obscure the granulocytes.

It was customary always to have ready for use a number of bottles each having a capacity of a little over 100 c.c. and containing 99 c.c. of the diluent, for the dilutions were effected by using 1 c.c. blood to 99 diluent. Only pipettes and burettes were used for which certificates of accuracy had been issued by the United States Bureau of Standards.

Another drawback, which the writer has not seen mentioned by previous workers, is the marked tendency which the leucocytes—both in ostrich blood and also in fowl blood—show to clump after the blood has been drawn. The intention at first was to collect the ostrich blood in an anticoagulant and to make the subsequent dilutions when convenient, as Neser (1923) did. But it was found that, unless the blood is mixed with the diluting fluid immediately after it has been drawn, clumping of the white cells and particularly of the heterophiles is so marked as to vitiate the count; as many as 20 or more leucocytes may be together, usually intermixed with thrombocytes. When the mixing takes place immediately after the blood has been drawn, clumping is usually absent or only slight.

The best results were obtained when a red cell pipette was used, for then the interval between the drawing of the blood and the mixing was reduced to a minimum, but using a pipette on the intractable ostrich is usually so difficult that it may be considered impracticable.

It was ascertained that clumping will take place also in fowl blood if there is some delay in filling the pipette, or if the blood is first received into an anti-coagulant. Many diluting fluids besides Wiseman's and Wiseman's (modified) were tried, namely, Hayem's, Toisson's, Tyrode's, Locke's, Ringer's, 0·9 per cent. sodium chloride and also the bird's own plasma, but clumping was no less marked in any of them.

The bottle containing the blood and the diluting fluid was usually shaken for about three minutes before the fluid was introduced into the chamber, but even shaking for longer periods had no effect in reducing the clumping; on the contrary it often increased it. The fluid was also shaken with beads, but this also proved ineffective. Blood, allowed to stand for half an hour, was added to a

1 per cent. solution of acetic acid, but clumping was marked. Human, equine and bovine blood, similarly treated, did not show agglutination, so that this quality seems to be characteristic only of avian blood.

All the anticoagulants mentioned under " Preventing Coagulation of the Blood " were tried but without success in overcoming this disadvantage. The mixing had, therefore, to be always made at the place of bleeding. When only the red cells have to be counted the blood may be diluted hours later if kept cool in the interim.

Forkner (1929) mentions the rapidity with which coagulation takes place in the blood of the fowl as one of the difficulties in securing an accurate count of the white cells. The writer experienced no difficulty in filling the pipettes when making counts of fowl blood, as the blood never coagulated before it could be properly diluted, but it was observed that, when the dilution of fowl blood did not take place immediately, clumping was at times also marked. It may have been this that was responsible for the difficulty which Forkner experienced, rather than that fowl blood coagulates too soon.

At first a Burker counting chamber was used, but later it was replaced by a Levy-Hausser counting chamber, guaranteed to be within the tolerance for accuracy established by the United States Bureau of Standards. This counter, which is recommended by Kolmer and Boerner (1931), has features which make counting much easier and which are not found with some other counters.

The total number of granulocytes in at least four of the entire ruled areas, viz., in 3·6 c.mm. of the mixture, was always counted and so were all the red cells in at least 0·12 c.mm. Errors due to smallness of samples were thus reduced to a minimum. The counting of the red cells was spread over four of the entire ruled areas, the two rectangular areas, each measuring 3 mm. × 0·05 mm. and crossing each other in the centre of the ruled area, being used, i.e., the cells in 0·03 c.mm. of the mixture were counted in each chamber. In this way a better average count check was ensured.

The difference between 2 sets of red cell counts thus made seldom varied by more than 5 per cent. but differences up to 10 per cent. were noted in spite of the utmost care in making the preparations as recommended by Neser (1923) and others to ensure accurate counting. Marked differences were sometimes obtained when smaller samples were counted or when the count was spread over two ruled areas only.

All the diluting fluids mentioned on page 479 were tried to see whether more regular distribution of the red cells could be obtained but none of them proved to be better than the diluent used.

Neser's method of using an ordinary pipette drawn from glass tubing for filling the counting chamber was adopted for ostrich blood. The point should be cut in such a way that the fluid flows readily in between the two surfaces but without flooding the chamber when the forefinger is slightly withdrawn from the upper end of the pipette.

It was found most convenient to use a 6 × ocular and an 8 mm. lens and a strong white light for illumination.

TABLE 19.

Statistical Analysis of Leucocyte Counts.

Bird Number.*	Sex.	Number of Counts.	Minimum Count per c.mm.	Maximum Count per c.mm.	Mean Count per c.mm.	Standard Error of the Mean.	Standard Deviation.	Coefficient of Variability.
1	Male	15	12,326	29,255	21,985	846	3,279	14·9
2	Male	15	13,157	24,751	19,859	829	3,210	16·1
3	Male	12	19,436	31,303	22,708	655	2,267	9·9
4	Female	15	14,157	32,273	20,733	974	3,773	18·1
5	Female	13	15,880	27,495	20,179	798	2,875	14·2
1, 2 and 3	Males	42	12,326	31,303	21,432	489	3,172	14·8
4 and 5	Females	28	14,157	32,273	20,476	736	3,892	19·0
1 to 5	Males and Females	70	12,326	32,273	21,050	419	3,482	16·5
6	Female	14	17,422	45,372	29,562	3,441	9,130	30·8
7 to 11	Males	5	8,409	16,146	13,125	1,439	3,166	24·1
12 to 17	Females	6	7,868	24,570	14,066	2,449	5,878	41·7
7 to 17	Males and Females	11	7,868	24,570	13,638	1,264	4,172	30·5
18 to 22	Males and Females	6	8,697	30,033	17,210	2,803	6,729	39·0

* For particulars *re* birds, see pages 428–429.

The leucocyte cells counts are given in Tables 3–10 and a statistical analysis of the data is shown in Table 19. The results show that the mean leucocyte count of all the results from the normal birds (1–5) was 21,050 ±419 with a range of 12,326 to 32,273 (standard deviation 3,482; coefficient of variability 16·5 per cent.).

It will be seen that the coefficient of variability is much higher than that for the erythrocytes. Palmer and Biely (1935), who made leucocyte counts of 100 normal S.C. white Leghorn hens, reported a coefficient of variability of 20·77, while, according to these writers, the leucocyte counts made by Cook and Dearstyne (1934) on 75 normal Rhode Island Red and Barred Plymouth Rock fowls show a coefficient of variability of 55·09.

The average leucocyte count for bird No. 6 is comparatively high and so is the coefficient of variability of the results from this bird. It would, therefore, appear that the leucocyte count was affected by the defect from which the bird suffered.

The clinically healthy birds (7–17) showed a mean leucocyte count which is significantly lower than that of the normal birds and the coefficient of variability of their counts is comparatively high. The results from the worm-infested birds (18–22) fluctuate considerably between the figures 8,697 and 30,033.

As in the case of the erythrocyte counts, the leucocyte counts cannot be correlated with the age or the sex of the birds.

Hayem (1879) recorded for the ostrich a leucocyte count of 9,000 per c.mm.. (Table 1).

Differential Counts.

In the differential counts at least 400 cells were always counted in each case and the counts were recorded on sheets as were used by Neser (1923).

Table 20 shows sixteen differential counts. These were obtained from the same smear prepared from ostrich blood by counting 100 leucocytes each time. A statistical analysis of these counts which can be regarded as typical gives the following results for example in respect of the lymphocytes and the heterophiles: By counting 100, 200, 300 and 400 leucocytes the chances are 20:1 that the errors associated with the lymphocyte counts are respectively 8·0, 5·1, 4·6 and 4·0; the errors associated with the heterophile counts are respectively 11·2, 8·0, 6·4 and 5·6. It is evident that by counting 400 leucocytes, fairly accurate results may be expected.

The differential counts were always made vertically across the smear, and an Ehrlich eye-piece was used for limiting the field, thus rendering critical observation of the cells at the edges of the field possible.

The differential counts are tabulated in Tables 3-10 and statistical analyses are shown in Tables 21-25. Examination of the results shows that the lymphocyte counts of the normal birds (1-5) varied from 19·2 to 37·0 per cent., averaging 26·8±0·5 per cent.

(standard deviation 4·1; coefficient of variability 15·3 per cent.). The average lymphocyte count of birds 1-5 is significantly higher than that of birds 7-17.

TABLE 20.

Differential Counts from same Smear prepared from Ostrich Blood.

Lymphocytes.	Monocytes.	Heterophiles.	Eosinophiles.	Basophiles.
%	%	%	%	%
31	5	46	7	11
38	7	41	4	10
32	5	47	3	13
26	1	52	7	14
28	3	52	6	11
26	5	56	6	7
28	1	59	5	7
27	5	55	5	8
20	4	61	4	11
30	4	49	8	9
26	3	56	6	9
22	5	59	11	3
30	6	45	8	11
29	5	48	1	17
32	2	46	5	15
25	5	53	7	10
Av. 28·1	4·1	51·5	5·7	10·3

The monocyte counts of all the results from birds 1-5 varied from 0·5 to 8·5 per cent., averaging $3·0 \pm 0·3$ per cent. (standard deviation 1·2; coefficient of variability 40·0 per cent.). Though the counts show high variability the averages do not differ much.

The heterophile counts shown by birds 1-5 varied from 40·5 to 78·5 per cent., averaging $59·1 \pm 0·9$ per cent. (standard deviation 7·7; coefficient of variability 13·0 per cent.). This average differs appreciably from that obtained with the results from bird No. 6 and from the average shown by birds 7 to 17.

The eosinophile counts of birds 1-5 varied from 0 to 19·5 per cent., averaging $6·3 \pm 0·5$ per cent. (standard deviation 4·0; coefficient of variability 63·5 per cent.). These cells show the highest variability.

The averages for birds 5 and 6 are exceptionally low while the average figure for birds 7-17 is exceptionally high compared with the mean count shown by birds 1-5. As stated on page 429 it is most likely that all the birds Nos. 7-17 were worm-infested and the high eosinophile counts of these birds may possibly be correlated with verminosis; however, some of the worm-infested birds (18–22) showed comparatively low eosinophile counts.

The basophile counts of birds 1-5 varied from 1·0 to 10·5 per cent., averaging $4·7 \pm 0·3$ per cent. (standard deviation 2·5; coefficient of variability 53·2 per cent.). This average does not differ appreciably from that shown by birds 7-17. The few differential counts on the worm-infested birds (18-22) were most erratic.

TABLE 21.

Statistical Analysis of Lymphocyte Counts.

Bird Number.*	Sex.	Number of Counts.	Minimum Percentage.	Maximum Percentage.	Mean Percentage.	Standard Error of the Mean.	Standard Deviation.	Coefficient of Variability.
1	Male	15	21·0	32·5	26·3	1·0	4·0	15·2
2	Male	15	22·0	37·0	28·3	0·9	3·5	12·4
3	Male	12	23·0	36·2	27·6	0·9	3·4	12·3
4	Female	15	19·2	35·5	28·2	0·9	3·7	13·1
5	Female	13	17·0	33·5	23·1	1·2	4·4	19·0
1, 2 and 3	Males	42	21·0	37·0	27·4	0·5	3·6	13·1
4 and 5	Females	28	19·2	35·5	25·9	1·0	4·6	17·8
1 to 5	Males and Females	70	19·2	37·0	26·8	0·5	4·1	15·3
6	Female	14	14·2	28·2	21·4	1·4	5·4	25·2
7 to 11	Males	5	8·7	15·7	12·8	1·2	2·7	21·0
12 to 17	Females	6	4·0	13·7	8·3	1·6	3·8	45·8
7 to 17	Males and Females	11	4·0	15·7	10·3	1·2	4·0	38·8
18 to 22	Males and Females	6	7·2	45·7	26·3	0·6	14·0	53·2

* For particulars of birds, see pages 428–429.

TABLE 22.

Statistical Analysis of Monocyte Counts.

Bird Number.*	Sex.	Number of Counts.	Minimum Percentage.	Maximum Percentage.	Mean Percentage.	Standard Error of the Mean.	Standard Deviation.	Coefficient of Variability.
1	Male	15	1·7	8·5	3·5	0·4	1·5	42·8
2	Male	15	1·7	6·0	3·5	0·3	1·3	37·1
3	Male	12	1·2	4·2	2·8	0·2	0·8	28·5
4	Female	15	0·5	5·5	2·5	0·3	1·2	48·0
5	Female	13	1·5	4·2	2·7	0·2	0·9	33·3
1, 2 and 3	Males	42	1·2	8·5	3·3	0·2	1·3	39·3
4 and 5	Females	28	0·5	5·5	2·6	0·2	1·1	42·3
1 to 5	Males and Females	70	0·5	8·5	3·0	0·3	1·2	40·0
6	Female	14	0·7	6·2	3·4	0·4	1·6	47·0
7 to 11	Males	5	1·0	4·5	3·1	0·6	1·3	41·9
12 to 17	Females	6	2·0	4·5	3·2	0·4	1·1	34·3
7 to 17	Males and Females	11	1·0	6·2	3·2	0·3	1·1	34·3
18 to 22	Males and Females	6	2·0	9·5	4·4	1·2	2·8	63·6

* For particulars of birds, see pages 428–429.

TABLE 23.

Statistical Analysis of Heterophile Counts.

Bird Number.*	Sex.	Number of Counts.	Minimum Percentage.	Maximum Percentage.	Mean Percentage.	Standard Error of the Mean.	Standard Deviation.	Coefficient of Variability.
1	Male	15	41·5	64·0	54·2	1·5	6·0	11·1
2	Male	15	46·3	63·2	55·1	1·4	5·6	10·0
3	Male	12	54·2	65·5	60·4	1·1	3·7	6·1
4	Female	15	40·5	71·5	58·0	2·0	7·9	13·6
5	Female	13	53·0	78·5	68·1	1·8	6·6	9·7
1, 2 and 3	Males	42	41·5	64·0	56·6	0·9	5·8	10·2
4 and 5	Females	28	40·5	78·5	62·7	1·7	8·9	14·2
1 to 5	Males and Females	70	40·5	78·5	59·1	0·9	7·7	13·0
6	Female	14	61·0	81·0	71·1	1·8	6·7	9·4
7 to 11	Males	5	62·0	73·0	68·3	1·8	4·0	5·8
12 to 17	Females	6	68·7	83·2	74·4	2·4	5·8	7·8
7 to 17	Males and Females	11	62·0	83·2	71·6	1·7	5·7	8·0
18 to 22	Males and Females	6	47·5	89·7	63·0	6·8	16·4	26·0

* For particulars of birds, see pages 428–429.

TABLE 24.

Statistical Analysis of Eosinophile Counts.

Bird Number.*	Sex.	Number of Counts.	Minimum Percentage.	Maximum Percentage.	Mean Percentage.	Standard Error of the Mean.	Standard Deviation.	Coefficient of Variability.
1	Male	15	2·7	19·7	7·8	1·3	5·0	64·1
2	Male	15	3·5	14·7	7·6	0·8	3·0	39·4
3	Male	12	3·0	8·7	6·4	0·5	1·7	26·6
4	Female	15	2·0	16·5	7·3	1·2	4·6	63·0
5	Female	13	0	3·7	1·9	0·3	1·2	63·1
1, 2 and 3	Males	42	2·7	19·7	7·3	0·5	3·5	47·9
4 and 5	Females	28	0	16·5	4·8	0·8	4·4	91·7
1 to 5	Males and Females	70	0	19·5	6·3	0·5	4·0	63·5
6	Female	14	0·2	4·5	1·7	0·4	1·6	94·1
7 to 11	Males	5	6·0	18·0	12·4	2·2	4·8	38·7
12 to 17	Females	6	3·2	18·7	10·1	2·2	5·2	51·5
7 to 17	Males and Females	11	3·2	18·7	11·1	1·5	4·9	44·1
18 to 22	Males and Females	6	0·2	13·7	4·4	1·5	5·3	120·4

* For particulars of birds, see pages 428–429.

TABLE 25.

Statistical Analysis of Basophile Counts.

Bird Number.*	Sex.	Number of Counts.	Minimum Percentage.	Maximum Percentage.	Mean Percentage.	Standard Error of the Mean.	Standard Deviation.	Coefficient of Variability.
1	Male	15	3·5	10·5	8·1	0·5	1·8	22·2
2	Male	15	1·7	8·5	4·4	0·5	2·0	45·4
3	Male	12	1·0	4·0	2·7	0·3	1·0	37·0
4	Female	15	1·2	6·5	4·0	0·1	1·7	42·5
5	Female	13	1·0	6·5	4·0	0·5	1·8	45·0
1, 2 and 3	Males	42	1·0	10·5	5·2	0·4	2·8	53·8
4 and 5	Females	28	1·0	6·5	4·0	0·3	1·7	42·5
1 to 5	Males and Females	70	1·0	10·5	4·7	0·3	2·5	53·2
6	Female	14	1·0	4·5	2·3	0·3	1·1	47·8
7 to 11	Males	5	1·2	4·0	3·0	0·5	1·2	40·0
12 to 17	Females	6	0·5	10·0	3·7	1·4	3·4	91·9
7 to 17	Males and Females	11	0·5	10·0	3·4	0·8	2·6	76·5
18 to 22	Males and Females	6	0	3·0	1·5	0·4	1·1	73·3

* For particulars of birds, see pages 428–429.

Thrombocyte Counts.

The thrombocytes were counted by the method employed by Magath and Higgins (1934). The ratio between these and the leucocytes was computed from the number of thrombocytes enumerated each time 200 leucocytes were counted in the smear.

As the thrombocytes are very unevenly distributed the ratio obtained is only approximate, even when 200 leucocytes have been counted. The method is far from satisfactory but there is no better method of obtaining total thrombocyte counts of ostrich blood.

The counts are listed in Tables 3–10 and statistical determinations are given in Table 26. The results from the normal birds (1–5) ranged from 2,478 to 20,086, averaging 10,521 ± 427 (standard deviation 3,545; coefficient of variability 33·6 per cent.). The numbers of thrombocytes and leucocytes stand in a ratio of about 1 to 2.

Kleineberger and Carl (1912) record a thrombocyte count of 22,000 to 130,000 per c.mm. for the fowl while according to Fritsch (1920), cited by Wirth (1931), fowl blood contains very few thrombocytes, and Blain (1928), as already stated, apparently did not see any. Magath and Higgins (1934) reported for the duck an average thrombocyte count of 30,706 ± 703 with a coefficient of variability of 32 per cent. Hayem (1879 and 1889) recorded counts of 11,500 and 11,600 for the ostrich. It will be seen that these figures come very near to the averages obtained by the writer.

Viscosity.

Hess's viscosimeter was used. Naegeli (1921) and others give a detailed description of the apparatus. The readings listed in Table (27) are those of citrated blood but tests carried out with whole, citrated, and heparinised blood showed no significant differences. The necessary corrections were made when tests were conducted outside the temperature range of 17° C.–23° C. in which the apparatus gives correct readings.

The viscosity of the blood of the normal ostriches (1–5) varied from 4·0 to 5·0, with an average of 4·5, the corresponding figures for the plasma being 1·5 to 1·8 and 1·6.

The results from the worm-infested birds (18–22) are comparatively low. These birds showed also low erythrocyte counts (Table 10) and on post-mortem examination were found to be severely infested with worm parasites.

Wirth (1931), quoting Kruger (1925), states that the viscosity of fowl serum is 1·4 and that of duck serum 1·24, but figures for avian blood or plasma are not given.

Specific Gravity.

The specific gravity was determined at a temperature of 15° C. with the use of a pycnometer. The blood and plasma were brought to the desired temperature in a thermostat on occasions when the

TABLE 26.

Statistical Analysis of Thrombocyte Counts.

Bird Number.*	Sex.	Number of Counts.	Minimum Count per c.mm.	Maximum Count per c.mm.	Mean Count per c.mm.	Standard Error of the Mean.	Standard Deviation.	Coefficient of Variability.
1	Male	15	6,014	14,076	10,073	725	2,807	27·8
2	Male	15	2,478	19,623	10,456	960	3,718	35·5
3	Male	12	5,562	14,803	10,802	792	2,741	25·3
4	Female	15	4,752	20,086	11,758	1,156	4,476	38·0
5	Female	13	2,686	15,958	9,428	1,103	3,971	42·1
1, 2 and 3	Males	42	2,478	19,623	10,418	476	3,084	29·6
4 and 5	Females	28	2,686	20,086	10,677	793	4,198	39·4
1 to 5	Males and Females	70	2,478	20,086	10,521	427	3,545	33·6
6	Female	14	4,061	26,650	12,439	1,667	6,235	50·1
7 to 11	Males	5	8,946	17,415	13,091	1,375	3,026	23·1
12 to 17	Females	6	7,720	15,336	12,693	1,250	3,002	23·6
7 to 17	Males and Females	11	7,720	17,415	12,890	867	2,864	22·2
18 to 22	Males and Females	6	4,650	15,394	11,135	1,780	4,274	38·3

* For particulars of birds, see pages 428–429.

atmospheric temperature in the room was not exactly 15° C. It will be noted from the determinations given in Table 28 that the specific gravity of the blood of the ostrich varies from 1·060 to 1·065, the average being 1·063. The corresponding figures for the plasma are 1·021 to 1·024 and 1·022.

According to Kruger, cited by Wirth (1931), the specific gravity of the blood of the fowl is 1·0545 and that of its serum 1·0232. The corresponding figures as given by him for the blood and the serum of the goose are 1·0549 and 1·0202—for the duck 1·0563 and 1·0202 and for the guinea-fowl 1·0577 and 1·0214.

Wirth (1931) gives a number of specific gravity determinations—by various workers—of the blood and the serum of a number of animals. The average figure obtained for the blood of the ostrich exceeds the average figures listed by Wirth, the nearest to the average being that given by Augsburger (1919), viz., 1·062 for the horse. Other workers, however, give lower values for the horse.

TABLE 27.

Viscosity Determinations.—Nos. 1-5—*Ostriches clinically healthy and found free from disease on Post-mortem Examination. No.* 6—*Ostrich with club-foot.* (*See pages* 428-429.) *Nos.* 18-22—*Unthrifty Ostrich Chicks which on Post-mortem Examination showed marked Verminosis.*

Bird Number.	Sex.	Date.	Age.		Viscosity Blood.	Viscosity Plasma.
			Months.	Days.		
1	Male	30/11/35	13	—	4·6	—
		12/12/35	13	12	4·8	—
		24/ 3/36	16	24	4·7	1·7
		9/ 5/37	18	9	4·8	1·7
2	Male	7/12/35	13	7	4·1	1·7
		12/12/35	13	12	4·4	1·6
		9/ 5/36	18	9	4·6	1·7
		26/ 8/36	21	26	4·3	—
3	Male	7/12/35	13	7	4·5	—
		9/12/35	13	9	4·2	1·6
		12/12/35	13	12	4·3	1·6
		7/ 9/36	22	7	4·9	1·7
4	Female	29/11/35	12	29	4·1	—
		12/12/35	13	12	4·3	1·6
		24/ 3/36	16	24	4·6	1·5
		9/ 5/36	18	9	4·6	1·6
5	Female	15/11/35	12	15	4·0	1·7
		20/11/35	12	20	4·7	1·8
		12/12/35	13	12	4·7	1·8
		7/ 9/36	22	7	5·0	—

TABLE 27—(*continued*).

Bird Number.	Sex.	Date.	Age.		Viscosity Blood.	Viscosity Plasma.
			Months.	Days.		
Average 1, 2 and 3.....	Males.....	—	—	—	4·5	1·6
Average 4 and 5.....	Females...	—	—	—	4·5	1·6
Average 1 to 5.....	—	—	—	—	4·5	1·6
6.....	Females...	20/11/35	12	20	5·5	—
		29/11/35	12	29	4·1	1·7
		7/ 9/36	22	7	5·4	1·8
		1/12/36	25	1	4·8	1·7
18.....	—	15/ 3/36	4	—	3·2	—
19.....	—	19/ 3/36	4	—	2·3	—
20.....	—	20/ 3/36	4	—	2·3	—
21.....	—	22/ 3/36	4	—	2·0	—
22.....	—	8/ 2/37	4	—	3·5	—

TABLE 28.

Specific Gravity Determinations.—Ostriches clinically healthy and found free from Disease on Post-Mortem Examination.

Bird Number and Sex.	Date.	Age.		Temperature at which Specific Gravity Determined.	Specific Gravity Blood.	Specific Gravity Plasma.
		Months.	Days.			
1 Male.....	24/ 3/36	16	24	15° C.	1·064	—
	5/ 6/36	19	5	,,	1·065	1·023
2 Male.....	9/ 5/36	18	9	15° C.	1·060	1·022
3 Male.....	9/ 5/36	18	9	15° C.	1·064	1·023
4 Female.....	24/ 3/36	16	24	15° C.	1·064	—
	31/ 1/36	19	3	,,	1·066	1·021
5 Female.....	9/ 5/36	18	9	15° C.	1·062	1·022
	27/ 5/36	19	—	15° C.	1·062	1·024
Average Males.....	—	—	—	—	1·063	1·022
Average Females.....	—	—	—	—	1·063	1·022
Average 1 to 5 Males and Females	—	—	—	—	1·063	1·022

Inorganic Phosphorus, Calcium, Sodium, Potassium and Magnesium Content.

Theiler, Green, du Toit, and others in the series of articles "Studies in Mineral Metabolism" 1927, et. seq., have shown that in many parts of South Africa cattle and sheep suffer from marked aphosphorosis resultant on phosphorus deficiency in the pastures and that phosphorus feeding is an essential factor in successful cattle and sheep farming. It seems, therefore, that phosphorus feeding to ostriches grazing over areas where other animals show aphosphorosis might profitably be investigated. Malan (1930) has shown that phosphorus deficiency, even in the earliest stages may be diagnosed by determining the inorganic phosphorus content of the blood.

As the ostriches Nos. 1 to 5 could be regarded as entirely free from disease and as they always received a liberal supply of bones and other necessary foodstuffs, there was a good opportunity for obtaining data which might contribute toward establishing what levels of phosphorus and of the other mineral elements mentioned above may be considered normal in the blood of the ostrich. Blood analyses were, therefore, made from these birds and—for the purpose of comparison—from bird No. 6, and also from birds (Nos. 7-17) grazing on natural pasture in the Bredasdorp district.

The calcium content was determined particularly with the object of ascertaining whether the prolonged coagulation time could be associated with a low calcium content of the blood, but, as has been shown under "Coagulation of the Blood", this would not appear to be so.

The analyses were carried out under the supervision of Dr. A. I. Malan, Head of the Department of Bio-chemistry, Onderstepoort Laboratories, Pretoria, by his staff. The methods advocated by Malan and van der Lingen (1931) were employed. The writer collected the blood, precipitated the proteins and forwarded the trichloracetic acid filtrate to Onderstepoort.

The results are tabulated in Table 29 and the following are the average values per 100 c.c. blood of all the results obtained from the normal birds (1-5). Inorganic phosphorus $9{\cdot}1 \pm 0{\cdot}3$ mgm., calcium $10{\cdot}1 \pm 0{\cdot}4$ mgm., magnesium $7{\cdot}6 \pm 0{\cdot}4$ mgm., sodium $273{\cdot}7 \pm 13{\cdot}0$ mgm. and potassium $196{\cdot}6 \pm 6{\cdot}0$ mgm.

The inorganic phosphorus content of the blood of all five birds was comparatively low on 29/7/35, and it is not apparent why they showed relatively low values on that date. At no time was any alteration made in their food and it does not seem as if the values can be associated with their age, for bird No. 6 at the age of six months and sixteen days—before it had sustained the injury—showed a higher value than it did at the age of nine months and two days. The results are, however, suggestive of a seasonal variation.

It will be noted that the average values of all the above-named elements obtained from bird No. 6 and those of inorganic phosphorus and calcium shown by the results from the clinically healthy birds (7-17) do not differ appreciably from the mean values of all the results from birds 1–5.

Table 29.

Analytical Results.

Nos. 1 to 5.—Ostriches clinically healthy and found free from disease on post-mortem examination. Kept on farm Mariendahl, Stellenbosch District. (See pages 428-429.)

No. 6.—Ostrich with club-foot. Kept on farm Mariendahl, Stellenbosch District. (See pages 428-429.)

Nos. 7 to 13.—Clinically healthy semi-mild ostriches on which post-mortem examinations were not conducted. From farm Negwag, Bredasdorp District. (See page 429.)

	Bird No.	Sex.	Date.	Age.		Mgm. per 100 c.c. Blood.				
						Inorg. P.	Ca.	Mg.	Na.	K.
				Months.	Days.					
	1	Male.....	29/ 7/35	9	2	5·8	10·0	—	—	—
			12/12/35	13	17	10·2	8·2	7·8	250·0	211·5
			17/ 4/36	17	24	8·9	8·5	—	—	—
			20/ 2/37	28	4	10·2	8·1	—	—	—
	2	Male.....	29/ 7/35	9	2	6·7	11·0	—	—	—
			12/12/35	13	17	10·2	10·3	8·4	270·0	177·5
			17/ 4/36	17	24	9·2	9·4	—	—	—
			20/ 2/37	28	4	10·1	8·9	—	—	—
	3	Male.....	29/ 7/35	9	2	7·6	10·7	—	—	—
			12/12/35	13	17	11·4	13·3	7·1	256·2	202·0
			17/ 4/36	17	24	9·3	10·0	—	—	—
			20/ 2/37	28	4	10·4	8·3	—	—	—
	4	Female.....	29/ 7/35	9	2	6·7	10·1	—	—	—
			12/12/35	13	17	10·2	11·6	7·7	270·0	189·0
			17/ 4/36	17	24	9·2	10·3	—	—	—
			20/ 2/37	28	4	10·3	8·6	—	—	—
	5	Female.....	29/ 7/35	9	2	6·9	10·0	—	—	—
			12/12/35	13	17	10·2	14·0	7·4	322·5	203·0
			17/ 4/36	17	24	9·0	14·2	—	—	—
			20/ 2/37	28	4	10·1	8·3	—	—	—
Averages.....	1, 2, 3	Males.....	—	9	2	6·7	10·5	—	—	—
,, ...	,,	,, ...	—	13	17	10·6	10·6	7·7	258·7	197·0
,, ...	,,	,, ...	—	17	24	9·1	9·3	—	—	—
,, ...	,,	,, ...	—	28	4	10·2	8·4	—	—	—
Averages.....	4, 5	Females.....	—	9	2	6·8	10·5	—	—	—
,, ...	,,	,, ...	—	13	17	10·2	12·8	7·5	296·2	196·0
,, ...	,,	,, ...	—	17	24	9·1	12·2	—	—	—
,, ...	,,	,, ...	—	28	4	10·2	8·4	—	—	—

TABLE 29 (*continued*).

	Bird No.	Sex.	Date.	Age.		Mgm. per 100 c.c. Blood.				
						Inorg. P.	Ca.	Mg.	Na.	K.
				Months.	Days.					
Averages.....	1 to 5	Males and Females..	—	9	2	6·7	10·3	—	—	—
,, ...	,,	,, ,, ..	—	13	17	10·4	11·4	7·6	273·7	196·6
,, ...	,,	,, ,, ..	—	17	24	9·1	10·4	—	—	—
,, ...	,,	,, ,, ..	—	28	4	10·2	8·4	—	—	—
Averages, all Results.....	1 to 5	—	—	—	—	9·1	10·1	7·6	273·7	196·6
Standard Error.....	—	—	—	—	—	0·3	0·4	0·4	13·0	6·0
Standard Deviation.....	—	—	—	—	—	1·5	1·6	0·9	28·6	13·3
Coefficiency of Variability	—	—	—	—	—	16·4	15·8	11·8	10·4	6·7
	6	Female.....	15/ 5/35	6	16	8·2	10·5	8·2	238·0	181·5
			29/ 7/35	9	2	6·9	10·1	—	—	—
			12/12/35	13	17	11·6	13·1	7·7	270·0	212·0
			17/ 4/36	17	24	9·9	10·2	—	—	—
			20/ 2/37	28	4	10·4	8·1	—	—	—
Averages.....	—	—	—	—	—	9·4	12·4	7·9	254·0	196·7
Standard Error.....	—	—	—	—	—	0·6	0·7	—	—	—
Standard Deviation.....	—	—	—	—	—	1·5	1·7	—	—	—
Coefficiency of Variability	—	—	—	—	—	15·9	13·7	—	—	—
	7	Male.....	1/ 5/36	Over 3	Years.	10·5	10·2	—	—	—
	8	,, ...	1/ 5/36	,,	,,	7·3	8·6	—	—	—
	9	,, ...	1/ 5/36	,,	,,	9·7	9·8	—	—	—
	10	,, ...	1/ 5/36	,,	,,	8·6	8·7	—	—	—
	11	Female.....	1/ 5/36	,,	,,	8·1	9·3	—	—	—
	12	,, ...	1/ 5/36	,,	,,	7·9	8·7	—	—	—
	13	,, ...	1/ 5/36	,,	,,	10·0	9·3	—	—	—
Averages.....	7 to 13	—	—	—	—	8·9	9·2	—	—	—
Standard Error.....	—	—	—	—	—	0·4	0·2	—	—	—
Standard Deviation.....	—	—	—	—	—	1·2	0·6	—	—	—
Coefficient of Variability	—	—	—	—	—	13·4	6·0	—	—	—

Malan (1930) recorded for the ostrich a value of 5·5 mgm. inorganic phosphorus per 100 c.c. blood (Table 1) and, according to this writer, fowl blood contains 2 mgm. and pigeon blood 1·6 mgm. inorganic phosphorus per 100 c.c. blood. Under the heading "Preventing Coagulation of the Blood" the calcium value obtained for the ostrich has already been referred to.

Magnesium, sodium and potassium values from other birds were not available, but it is interesting to note that the potassium content of ostrich blood is high when compared with that of the blood of some mammals. Groenewald (1935) recorded an average figure of 58·7 mgm. potassium per 100 c.c. bovine blood, and the following—given by Dukes (1934)—are potassium values per 1000 parts by weight of blood: Cow 0·407, Sheep 0·405, Goat 0·396.

Total Blood Volume.

For determining the approximate amount of blood in the ostrich five adult birds were used. Narcosis was induced by the administration of chloroform of which 60-80 c.c. were poured on cotton wool placed in a jar. After the right jugular vein had been severed, bleeding continued for about 45 minutes before death resulted.

The blood remaining in the heart and large vessels—about 500 c.c.—was also collected.

The average total blood volume was 5,466 c.c. and its weight constituted 5·8 per cent. of the average body weight: (Table 30).

Barlow and Biskund (1928), cited by Dukes (1937), found the weight of the blood volume of pigeons to be 6·5 per cent of the body weight.

Table 30.

Total Blood Volume.

Bird No. and Sex.	Age.	Live Weight in Kilograms.	Weight of Blood in Grams.	Calculated Blood Volume in Cubic Centimetres (Specific Gravity, 1·063).	Weight of Blood expressed as Percentage of Body Weight.
3 M.....	33 Months.....	111·659	5,901	5,551	5·2
4 M.....	3 Years.....	108·936	5,787	5,444	5·3
23 M.....	Over 3 Years.....	84·879	5,787	5,444	6·8
5 F.....	33 Months.....	108·936	6,355	5,978	5·8
24 F.....	Over 3 years.....	82·156	5,220	4,911	6·3
Average.....		99·313	5,810	5,466	5·8

GENERAL DISCUSSION.

The erythrocyte count of the ostrich appears to be the lowest of the counts recorded for birds and mammals, but the ostrich erythrocyte is exceptionally large and, according to available results, it is exceeded in size only by that of the emu (*Casuarius emu*). Ponder (1919) remarks: "In general the larger the bird the larger the cell." In the moist state the cells are larger than in the dried state.

Although horse blood contains four times as many erythrocytes per c.mm. as ostrich blood, the relative volume of plasma and corpuscles of horse blood is considerably less than that of ostrich blood, and the haemoglobin content of ostrich blood is more than that of horse blood. The ostrich erythrocyte, therefore, is about four times the size of that of the horse and contains about four times as much haemoglobin. It weighs about four times as much as that of the horse, for the specific gravity of ostrich blood is about the same as that of horse blood. The points of minimum and maximum resistance of the ostrich erythrocyte are about the same as those of the erythrocyte of the fowl. The erythrocyte count of the fowl is much higher than that of the ostrich, but the haemoglobin content of fowl blood is much lower.

It is of particular interest that regeneration of erythrocytes is most clearly marked in ostrich blood. The percentage which in Romanowsky stained preparations show polychromasia is considerably higher than that observed in the blood of other animals. In vitally stained smears many reticulocytes are seen, and it is significant that in normal ostrich blood even basophil erythroblasts may constitute a fairly high percentage of the erythrocyte count.

Contrary to what would be expected, the blood of the group of unthrifty ostrich chicks, which showed marked oligocythemia as the result of severe worm-infestation, contained very few immature erythrocytes. Apparently the marrow exhausted by the long continued attempt to keep the blood up to normal eventually failed. The erythrocyte counts of the blood of the normal ostriches are fairly uniform. The coefficient of variability and the standard error of the mean can be regarded as low, but the range of 1,653,000 to 2,266,000 erythrocytes per c.mm. for normal birds is rather wide. Counts reported for the fowl show greater variation.

The leucocyte and the thrombocyte counts show considerable variation and the coefficient of variability of the thrombocyte counts is exceptionally high. Wide divergence is found also in the leucocyte and the thrombocyte counts, reported for the fowl; this may probably be largely attributed to differences in technique. It is difficult to compare results, as there is no uniform method of making total leucocyte counts of avian blood. A large number of average leucocyte counts reported for the fowl closely agree with the average count obtained for the ostrich, but the average thrombocyte count obtained for the ostrich is much lower than most counts reported for the fowl and other birds.

It is noteworthy that most of the erythrocyte and the leucocyte counts from the group of clinically healthy, semi-wild ostriches reared in the Bredasdorp District were appreciably lower than those from the five normal birds reared at Mariendahl, Stellenbosch District; also that the lymphocyte counts from the " Bredasdorp " birds were much lower than those from the " Mariendahl " birds, and the heterophile counts from the former much higher than those from the latter. As previously stated, all the " Bredasdorp " birds were probably worm-infested, but their blood gave no indication of unusual erythropoiesis, and it, therefore, appears that environmental and genetic factors might also have been partly responsible for the differences.

The percentage of lymphocytes in ostrich blood is much lower than that recorded for the fowl by most investigators, but the heterophiles constitute a much higher percentage of the leucocyte count than they do in fowl blood. On the whole, the percentages recorded for the other types of leucocytes in fowl blood do not differ greatly from those obtained for the ostrich, in which the heterophile counts showed the least variation and the eosinophile counts the greatest. The basophile of the ostrich is distinct from that of the fowl.

The inorganic phosphorus and calcium values for the group of birds grazing on natural pasture in the Bredasdorp District compare very favourably with the results from the normal birds which received a liberal supply of bones and other foodstuffs. As previously stated, the inorganic phosphorus values shown by the normal birds are suggestive of a seasonal variation.

The prolonged coagulation time of ostrich blood, which apparently cannot be associated with a low calcium content, the higher percentage volume readings obtained with old samples of ostrich blood and the marked tendency of the leucocytes to clump immediately after the blood is drawn are interesting features which offer fertile ground for further investigation.

The writer hopes that this work, which may be defined as a preliminary study of the blood of the ostrich, will provide a basis for future research.

SUMMARY.

1. Methods are described for collecting blood and preparing smears from the ostrich.

2. Ostrich blood usually has a very prolonged coagulation time and often it fails to coagulate if drawn directly from a blood vessel without coming in contact with the tissues. Heparin (1 mgm. to 5 c.c. blood) invariably prevented coagulation and it had no deleterious effect on the blood.

3. Various methods recommended for counting leucocytes in avian blood were tried on ostrich blood. Wiseman's method was found to be the best.

4. Morphological and biochemical studies of the blood of twenty-two ostriches were made, and the following are the average values and ranges obtained for the blood of normal ostriches:—

Erythrocyte count per c.mm. = 1,894,000 ± 14,939. Range = 1,653,000 to 2,266,000.

Relative volume of corpuscles and of plasma = 48·9 ± 0·34. Range = 40·6 to 54·0.

Minimum resistance point of red cells in sodium chloride solution = 0·47. Range = 0·44 to 0·52.

Maximum resistance point of red cells in sodium chloride solution = 0·27. Range = 0·3 to 0·25.

Sodium chloride solution which is isotonic for the erythrocytes = 0·9 per cent.

Haemoglobin content per 100 c.c. blood = 16·0 gms. Range = 14·6 to 17·2 gms.

Leucocyte count per c.mm. = 21,050 ± 419. Range = 12,326 to 32,273.

Lymphocyte count = 26·8 ± 0·5 per cent. Range = 19·2 to 37·0 per cent.

Monocyte count = 3·0 ± 0·3 per cent. Range = 0·5 to 8·5 per cent.

Heterophile count = 59·1 ± 0·9 per cent. Range = 40·5 to 78·5 per cent.

Eosinophile count = 6·3 ± 0·5 per cent. Range = 0 to 19·5 per cent.

Basophile count = 4·7 ± 0·3 per cent. Range = 1·0 to 10·5 per cent.

Thrombocyte count per c.mm. = 10,521 ± 427. Range = 2,478 to 20,086.

Viscosity: Blood = 4·5. Range = 4·0 to 5·0. Plasma 1·6. Range = 1·5 to 1·8.

Specific gravity: Blood = 1·063. Range = 1·060 to 1·065 Plasma 1·022 Range = 1·021 to 1·024

Inorganic phosphorus content per 100 c.c. blood = 9·1 ± 0·3 mgm. Range = 5·8 to 11·4 mgm.

Calcium content per 100 c.c. = 10·1 ± 0·4 mgm. Range = 8·1 to 14·2 mgm.

Magnesium content per 100 c.c. = 7·6 ± 0·4 mgm. Range = 7·1 to 8·4 mgm.

Sodium content per 100 c.c. = 273·7 ± 13·0 mgm. Range = 250·0 to 322·5 mgm.

Potassium content per 100 c.c. = 196·6 ± 6·0 mgm. Range = 177·5 to 211·5 mgm.

5. The average total blood volume was 5,466 c.c. and its weight expressed as a percentage of the average body weight was 5·8. The total blood volume ranged from 5,220 to 6,355 c.c. and its weight constituted 5·2 to 6·3 per cent. of the body weight.

6. Cell types corresponding to those seen in fowl blood occur in ostrich blood. These have been described.

ACKNOWLEDGMENTS.

I am greatly indebted to Dr. P. A. van der Byl, Principal, Stellenbosch-Elsenburg College of Agriculture, for the facilities granted for this work, and to Dr. P. J. du Toit, Director of Veterinary Services, for his willingness at all times to discuss various aspects of the problem and for reviewing the manuscript.

I am also grateful to Dr. C. Jackson, Professor of Anatomy, University of Pretoria, Dr. W. P. Mulligan, Consulting Pathologist, Cape Town, and Mr. W. H. Fouche, Assistant Farm Manager, Stellenbosch-Elsenburg College of Agriculture, for assistance rendered in the course of this work.

For the colour drawings my thanks are due to Mr. C. G. Walker, Artist at Onderstepoort Laboratories.

LITERATURE CITED.

AUGSBURGER, E. (1919). Lungendampf, *Pferd Schweiz. Arch. Tierheilk.* Bd. 61, S. 199-228.

BAINBRIDGE, F. A., AND MENZIES, J. A. (1919). Essentials of Physiology, 3rd Ed., p. 187. Longman's, Green & Co., London.

BARLOW, O. W., AND BISKUND, M. S. (1928). *Amer. Journ. Physiol.*, Vol. 86, p. 587.

BAYON, H. P. (1930). The comparative pathology of anaemia and leucocythemia in fowls. *Jour. Comp. Path. and Therap.*, Vol. 43, pp. 188-204.

BERKSON, J., MAGATH,, T. B., AND HURN, M. (1935). Laboratory standard in relation to chance fluctuations of the erythrocyte count as estimated with the haemocytometer. *Journal American Statistical Association*, Vol. 30, pp. 414-426.

BIELY, J., AND PALMER, E. I. (1935). Studies of total erythrocyte and leucocyte counts of fowls. III Variations in number of blood cells of normal fowl. *Canadian Journal of Research*, Vol. 13, Sec. D, pp. 61-71.

BLAIN, D. A. (1928). A direct method of making total white blood counts on avian blood. *Proc. Soc. Exp. Biol. and Med.*, Vol. 25, pp. 594-596.

BLAKEMORE, F. (1934). The leucocytes of fowl blood with special reference to fowl paralysis. *Vet. Record.*, Vol. 14, pp. 418-422.

BRADLEY, O. C. (1915). The structure of the fowl, p. 104, Baillière, Tindall & Cox, London.

BREUSCH, E. (1928). Beitrage zur Blutmorphologie des Huhnes, *Z. Infectionskrankh.*, Bd. 33, S. 219.

BUCHBINDER (1924). A new heterophile antigen common to avian erythrocytes and some varieties of genus Pasturella; its significance in the classification of birds. *J. Immun.*, Vol. 26, pp. 215-231.

BURCKHARDT, J. L. (1912). Hühner Tuberkulose, hühnerleukämie, normales Hühnerblut. *Z. Immunforschg.*, 14, S. 544, bis 604.

BURNETT, S. A. (1917). Clinical pathology of the blood of domesticated animals, 2nd Ed., pp. 32, 54 and 55. The Macmillan C., New York.

CARLETON, H. M. (1926). Histological technique, p. 115. Humphrey Milford, Oxford University Press, London.

COATES, I. (1928). A method of counting white cells in the blood of the fowl. *Report Ontario Vet. Coll.*, p. 63.

COOK, F. W., AND DEARSTYNE, R. S. (1934). Haematology of the fowl.. *Tech. Bull.* 44, *pp.* 10 *and* 14. *North Carolina State College of Agric. and Engineering.*

CRONWRIGHT SCHREINER, S. C. (1898). The Angora goat and a paper on the ostrich, p. 260. Longmans, Green & Co., 39 Paternoster Row, London.

DE KOCK, G. v. d. W. (1931). Studies on the blood of mice. *17th Report Dir. Vet. Services and Animal Industry.* Union of South Africa, pp. 574-575.

DUKES, H. H. (1937). The physiology of domestic animals, 4th Revised Ed., pp. 45, 53, and 54. Baillière, Tindall & Cox, London.

EMMEL, M. W. (1935). The etiology of fowl paralysis; leucaemia and allied conditions in animals. *Bulletin* 284. *University of Florida, Agric. Experiment Station, Gainsville, Florida,* p. 25.

ELLERMAN, V., AND BANG, O. (1908). Experimentelle Leukämie bei Hühnern. *Centralbl. f. Bakt.*, 1 abt., Originale Bd. 46, S. 597.

ELLERMAN, V. E. (1921). The Leucosis of fowls and leucemia problems. Gyldendal, London.

ELSDON-DEW, R. (1937). An apparatus for the performance of blood counts. *South African Journal of Science*, Vol. 33, pp. 850-851.

FERNSTERMACHER, R. (1932). Studies of leucamia of fowls. *Journal Amer. Vet. Med. Assoc.*, Vol. 80 (N.S. 33), No. 5, pp. 791-799.

FINK, O. (1922). Über die Beeinflüssing des Blutbildes gesunder Hühner durch die Injection von Blutserum Kranker Pferde. *B.T.W.* 40, S. 18.

FORKNER, C. E. (1929). Blood and bone marrow cells of the domestic fowl. *Jour. Exp. Med.*, Vol. 50, pp. 121-141.

FRIEDL, A. (1931). Ez. Resistenz, Huhn, Splenektomie. Dissert. Wien.

FRITSCH, G. (1919). Kaninchen- Tauben- und Hühnerblut. Dissert. Giessen. *Phlügers Arch.*, Bd. 175, S. 263.

FURTH, J. (1931). Erythroleucosis and the anaemias of the fowl. *Arch. Path.*, Vol. 12, pp. 1-30.

FURTH, J. (1931). Observations with a new transmissable strain of leucosis (leucamia) in fowls. *Jour. Exp. Med.*, Vol. 53, pp. 243-267.

GIBBS, C. S. (1934). Preliminary studies on neurolymphomatosis and some more or less related diseases. *Bull. No.* 308, *pp.* 26-29. *Massachusetts Agric. Exp. Station, Amherst, Mass.*

GOHS, W. (1928). Uber die Zahl und die Zahlung der Leukozyten und Thrombozyten bei Hühnern. *Fol. Haemat.*, Bd. 36, S. 337-341.

GOODALL, A. (1910). The numbers, proportions and characters of the red and white corpuscles in certain animals. *Journ. of Path. and Bact.*, Vol. 14, 195.

GRAHAM, G. S. (1918). *Jour. Med. Research*, 39 : 15.

GROENEWALD, J. W. (1935). The influence of rations low in certain minerals on the composition of the blood and milk of cows, and on the blood of their progeny. *Onderstepoort Jour. of Vet. Science and Animal Industry*, Vol. 4, No. 1, p. 158.

GULLIVER, G. (1875). Observations on the size and shape of the red corpuscles of the blood of vertebrata with drawings of them to a uniform scale and extended and revised tables of measurements. *Proc. Zool. Soc. of London*, pp. 474-495.

HAYEM, G. (1879). Recherches sur l'evolution des hematies. *Arch. Physiol.*, 2nd series, Vol. 6, pp. 201-261.

HAYEM, G. (1889). Du sang et de ses alterations anatomiques, p. 1035. Masson, Paris.

HAYDEN, C. E. (1927). The physiology of the blood of chickens. *State Rep. of the New York State Vet. Col. Cornell Univ.*, pp. 212-220.

HEDFELD, E. (1911). Untersuchingen über die koperlichen Elementen des Blutes gesunden und kranken Hühner und Tauben. *D.T.W.* 21, S. 266-267.

ISAACS, R. (1929). McClungs Microscopical Technique, p. 231. Paul B. Hoeber, New York.

JACKSON, C. (1936). The incidence and pathology of tumours of domesticated animals in South Africa. *Onderstepoort Jl.*, Vol. 6, No. 1, p. 343.

JOHNSON, E. P., AND CONNER, B. V. (1933). Blood studies of fowls with various forms of lymphomatosis (fowl paralysis). *Journ. Amer. Vet. Med. Assoc.*, Vol. 83 (N.S., Vol. 36), pp. 325-343.

KAUPP, B. S. (1929). Poultry diseases, 5th Ed., pp. 36 and 45. Alexander Eger, Chicago.

KLEINEBERGER, C., AND CARL, W. (1912). Die Blutmorphologie der Laboratoriums Tiere. Johann Ambrosius Barth, Leipzig.

KLEINEBERGER, C., AND CARL, W. (1927). Die Blutmorphologie der Laboratoriums Tiere, 2. Aufl. Johann Ambrosius Barth, Leipzig.

KNOWLES, H. R. (1934). The variation in the calcium level of the blood of the domestic fowl. *Poultry Science*, Vol. 14, No. 2, p. 89.

KOLMER AND BOERNER (1931). Approved laboratory technique, pp. 60, 93 and 107. D. Appleton—Century Company, New York.

KOZMA, J. (1929). Differentialzählung Vogelblut. *D.T.W.*, Bd. 38, 1930, S. 281.

KRÜGER, F. v. (1925). Die Chemie des Blutes. Die Fermente des Blutes. Anhang: Blutesverwandtschaft. In H. Wintersteins Hdb. d. vergl. Physiol. 1, Bd. 1, Hälfte G. Fischer, Jena.

KYES, P. (1929). Normal leucocyte content of birds' blood. *Anat. Rec.*, Vol. 43, p. 197-198.

LAITE (1915). Laite's commercial Blue Book for South Africa, p. 302, South African Publishers, Ltd., Capetown.

LUCIA, S. P., AND LUCIA, E. L. (1928). The differential blood count of the normal guinea-pig. *Archives of pathology*, Vol. 5, p. 616.

LUNDQUIST, C. W., AND BIRGER, H. (1925). Über die verschieden Granulaformen der sog. Pseudoesinophilen Leukozyten im Hühnerblut. *Fol. haemat*, Bd. 31, S. 253-264.

MAGATH, T. B., AND HIGGINS, G. M. (1934). The blood of the normal duck. *Folia Haemat.*, Bd. 51, heft 2, pp. 234-236 and 238.

MALAN, A. I. (1930). Mineral metabolism and blood analysis. *16th Report Dir. Vet. Services and Animal Industry, Union of South Africa*, pp. 307-311.

MALAN, A. I. (1930). Studies in mineral metabolism (13)—The phosphorus partition of the blood of some animals. *16th Report Dir. Vet. Services and Animal Industry, Union of South Africa*, p. 328.

MALAN, A. I., AND VAN DER LINGEN, G. W. B. (1931). Studies in mineral metabolism—16. The micro-determination of some inorganic elements in blood and vegetation. *17th Report Dir. Vet. Services and Animal Industry, Union of South Africa*, pp. 443-452.

MALASSEZ, L. 1872). De la numeration des globules rouges du sang chez les mammiferes les oiseaux et les poissons, Vol. 75, pp. 1528-1531, *C.R. Acad. Sci., Poris.*

MAXIMOW, A., AND BLOOM, W. (1931). A text-book of histology, p. 64. W. B. Saunders Co., Philadelphia and London.

McJUNKIN, F. A. (1920). A benzidin-polychrome stain for blood. *Jour. Amer. Med. Assn.*, Vol. 74, p. 17.

MILLAR, W. G. (1925). Observations on the haematocrite method of measuring the volume of erythrocytes. *Quart. Jour. Exp. Physiol.*, Vol. 15, p. 189.

MÖNNIG, H. O. (1929). The " guinea-worm " of the ostrich, Contortospiculum Spicularia. *15th Annual Report Dir. Vet. Services, Union of South Africa*, pp. 299-301.

MÖNNIG, H. O. (1934). Veterinary helminthology and entomology, pp. 17, 96, 160 and 194. Bailliére, Tindall and Cox. London.

MORGAN, V. E., AND CHICHESTER, D. F. (1935). Properties of the blood of the domestic fowl. *Jour. Biol. Cchem.* 110, pp. 285-298.

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

NESER, C. P. (1923). The blood of equines. *9th and 10th Reports Dir. Vet. Education and Research, Union of South Africa*, pp. 479-555.

Official Year Book of the Union of South Africa, 1910-1917, p. 429.

OHLSON, C. (1935). Available methods for examination of the blood of the fowl. *Jour. Amer. Vet. Med. Ass.*, Vol. 86 (N.S., Vol. 39), pp. 474-487).

OLSON, C. (1937). Variations in the cells and hemoglobin content in the blood of the normal domestic chicken. *Cornell Veterinarian*, Vol. 27, No. 3, pp. 235-263.

PALMER, E. J., AND BIELY, J. (1935). Studies of total erythrocyte and leucocyte counts of fowls. *Fol. Haemat.*, Vol. 53, pp. 143-154.

PEPPER, O. H. P., AND FARLEY, D. L. (1933). Practical haematological diagnosis, pp. 51, 73 and 149. W. B. Saunders Co., Philadelphia and London.

PONDER, E. (1924). Erythrocyte and the action of simple haemolysins. Oliver & Boyd, Paternoster Row, London.

PONDER, E. (1934). The mammalian red cell and the properties of haemolytic systems, pp. 7 and 48. Verlag von Gebruder Brontraeger, W.35 Schoneberger, Ufer 12, a Berlin.

PYPER, A. (1919). " Diffraction—Phenomena in films of blood cells." *The Medical Journal of S.A.*, Vol. 14, pp. 472-488.

RÖMER, K. (1921). Über das Blutbild bei gesunden und tuberkulösen Hühnern. *Cent. f. Bakt.* 73, S. 333.

ROUSSOUW, S. D. (1930). A short study of the isotonicity of sheep blood. *16th Report Dir. Vet. Services and Animal Industry, Union of South Africa*, pp. 525-529.

SOLOMON, W. (1920). Normales Hühnerblut. Dissert Giessen.

SCARBOROUGH, R. A. (1931). *Yale J. Bil. Medic.* 4, pp. 202-206 and 323-324.

SCHILLING, V. (1933). Das Blutbild und seine Klinische Verwertung, p. 11. Gustav Fisher, Jena.

SCHMEISSER, H. C. (1915). Spontaneous and experimental leukemia of the fowl. *Jour. Exp. Med.*, 22, pp. 820-838.

SCHULTZE, M. O., AND ELVEHJEM, C. A. (1934). An improved method for the determination of hemoglobin in chicken blood. *Jour. Biol. Chemistry*, Vol. 105, pp. 253-257.

SEAGAR, E. A. (1933). Cellular reactions in the blood in neurolymphomatosis gallinarum (fowl paralysis). *Vet. J.*, Vol. 89, pp. 557-561.

SHAW, A. F. B. (1930). A direct method for counting the leukocytes, thrombocytes and erythrocytes of birds' blood. *Jour. Path. and Bact.*, 33, pp. 833-835.

SLIDER AND DOWNEY (1929). McClungs microscopical technique, p. 245. Paul B. Hoeber, New York.

SLOAN, H. J., AND WILGUS JR., H. S. (1930). Heartprobe—A method for obtaining blood samples from chickens. *Poultry Science*, 10, pp. 10-16.

STEEN, G. (1913). Blutbild Huhn. Dresden. Hochschulnachr. (N.F. 8), S. 186-188.

SUGIYAMA (undated). Origin of thrombocytes and of the different types of blood cells as seen in the living chick blastoderm. Contributions to Embryology, No. 97.

THEILER, A., GREEN, H. H., AND DU TOIT, P. J. (1927). Minimum mineral requirements in cattle. *Jour, Agric. Science*, Vol. 17, pp. 291-314.

VENZLAFF, W. (1911). Über Genesis und Morphologie der rothen blutkörperchen der Vögel. *Arch. f. Mikr. Anat.*, Vol. 77, Abt. 1, pp. 377-431.

WARTHIN, A. S. (1907). Leucamia of the common fowl. *Jour. of Infect. Diseases*, Vol. 4, p. 369.

WIRTH, D. (1931). Grundlagen einer Klinischen Haematologie der Haustiere. Urban und Schwarzenberg. Berlin N.24.

WISEMAN, B. K. (1930). An improved direct method for obtaining total white cell counts in avian blood. *Proc. Soc. Exp. Biol. and Med.*, Vol. 28, pp. 1030-1033.

WISEMAN, B. K., AND BIERBAUM, O. S. (1932). A new method for determining the fragility of red blood cells. *Proc. Soc. Exp. Biol. and Med.*, Vol. 29, p. 835.

WORMSER, M. F. (1930). The ostrich industry in South Africa. M.Sc. Thesis, submitted to University of South Africa, Pretoria.

WRIGHT AND KINNICUT (1911). *Jour. Amer. Med. Assoc.*, Vol. 46, p. 1457.