TABLE 14.

No. 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
Av. Difference	----------	----------	1.36

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

The average percentage volume reading for the normal birds (1 - 5) was 48.9 ± 0.3 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 per cent, 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 ~~21/1/36~~ 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 to 22), 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. It will be seen that, with a few exceptions, the ratios shown by the normal birds (1 - 5) closely approximate one another, averaging 25.75 ± 0.12 (standard deviation 1.02; coefficient of variability 3.9 per cent).

Neser...../-

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/and 1915 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 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.../

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.

TABLE 15.

Statistical analysis of percentage volume readings.

Bird No. [1]	Sex	No. 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	"	15	43.8	50.0	46.3	0.54	2.0	4.3
3	"	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	"	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 & 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 & Females	11	38.4	49.4	42.6	0.93	3.1	7.2
18 to 22	Males & Females	6	25.3	41.8	33.0	2.54	6.1	18.5

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

TABLE 16.

Statistical analysis of ratios between centrifuge readings and the red corpuscular counts.

Bird No. [1]	Sex	No. 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	4.30
2	"	15	23.23	27.13	25.91	0.22	0.88	3.39
3	"	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	"	13	23.34	28.91	25.81	0.38	1.39	5.38
1, 2 & 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

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

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 2800 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 of 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 occured. 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 hemolysis 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 ..

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 spectroscoptically, 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 fortunate 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 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 solution, and a 3 per cent. haemolysis was found to be the lowest

practical/.....

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 0.27 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 9 - 10.

Bird No.	Sex	Date	Age	Minimum resistance.	Maximum resistance.
1	Male	1/10/35	11 mths. 1 day	0.47	0.3
		8/10/35	11 " 8 days	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 day	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
Av. 1,2 & 3	Males -			0.47	0.27
Av. 4 & 5	Females			0.48	0.28
Av. 1 to 5	Males & Females			0.47	0.28
6	Female	28/9/35	10 mths. 28 days	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 ~~with~~ 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 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. NaCl	35
1 c.c. 0.9 " " "	34
1 c.c. 1 " " "	33
1 c.c. Ringer solution	35
1 c.c. Ringer-Locke 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 2800 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 then 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 the 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.

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 9-10).
No.11 - Unthrifty ostrich chick which on post-mortem examination showed marked verminosis.

Bird No.	Sex	Date	Age	Gm. per 100 c.c. Blood.
1	Male	4/2/37	27 mths, 4 days	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
Av. 1, 2 and 3	Males	... ...		15.6
Av. 4 and 5	Females	... ...		16.5
Av. 1 to 5	Males & Females	... ...		16.0
6	Female	4/2/37	27mths 4days	14.5
		20/2/37	27 " 20 "	14.4
		17/5/37	30 " 17 "	14.5
Av.		... ...		14.4
11	Male	5/2/37	4 mths,	11.0
		8/2/37	4 " 3 days	11.5
		11/2/37	4 " 6 "	11.0
		19/2/37	4 " 14 "	11.8
		23/2/37	4 " 18 "	11.8

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.

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.

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), 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.

/Kleinberger .....

(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.