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THE RELATIVE VOLUME OR WEIGHT OF CORPUSCLES AND PLASMA IN BLOOD.[1]

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Two chief methods have been hitherto employed for the estimation of the relative volume or weight of corpuscles and plasma in blood, the method of Hoppe-Seyler[1], and the method of Bunge[2]. In Hoppe-Seyler's method the following quantities are estimated: (1) the proteids and hæmoglobin in a portion of the defibrinated blood; (2) the proteids and hæmoglobin in the corpuscles belonging to another portion of the blood, after the serum has been completely got rid of by repeated admixture with salt solution and subsequent centrifugalisation or sedimentation; (3) the proteids in a portion of serum separated from the clotted blood and entirely free from corpuscles. The method when carried out in all the necessary details is fairly accurate but exceedingly laborious.

Bunge's method is only applicable in the case of animals whose blood-corpuscles contain no sodium, the pig, for example. The sodium is estimated in a portion of the blood and in a portion of the clear serum, and a simple calculation gives the relative amount of corpuscles and serum.

In this paper I shall describe two new methods, both applicable to any kind of blood, easily carried out, and requiring only a comparatively short time. I shall speak of them as the colorimetric method and the electrical method respectively.

The colorimetric method.

The principle of this method is very simple. A pigment which is soluble in serum, and neither penetrates the corpuscles nor sensibly

[1] A preliminary account of the methods employed in this research was given in the *Centralb. f. Physiologie*, xi. 1897.

[2] *Hdb. d. physiol. u. pathol. chem. Analyse*, 6te Ausg. p. 274. 1893.

[3] *Physiol. Chemie*, 4te Ausg. p. 228. 1898.

alters the osmotic pressure of the serum, is added in known amount to a definite volume or weight of defibrinated blood. The blood is agitated till the pigment is uniformly distributed through it, and is then centrifugalised. From the amount of pigment contained in a given quantity of the tinged serum the quantity of serum in the blood can easily be calculated.

The pigment employed by me was oxyhæmoglobin. I selected this for two reasons, first, because it was unlikely that it would pass into the corpuscles; and, second, because with a high intensity of colouring power it unites a high molecular weight, and could therefore be used in such quantity that accurate colorimetric comparison was possible, while the osmotic pressure was only slightly changed. The fact that this pigment does not, under normal conditions, pass out of the corpuscles into the plasma, does not of itself justify the assumption that hæmoglobin artificially added to the serum will not pass at all into the corpuscles. For it has never been definitely shown that the hæmoglobin in the corpuscles is in solution as such. I have, however, proved by comparison of the colorimetric method with that of Hoppe-Seyler and with the electrical method in specimens of the same blood (Exp. VIII) that, as a matter of fact, no portion of the hæmoglobin added to the serum penetrates the corpuscles. The proof consists in the agreement between the results of the colorimetric and the other methods, an agreement which could not exist if any appreciable amount of the hæmoglobin added to the blood disappeared from the serum. That the addition of hæmoglobin in amount sufficient for the determination can cause at most only such an increase in the osmotic pressure, and therefore in the volume of the serum as lies within the limits of error of the method, was shown as follows.

Preparation and testing of the hæmoglobin.

Oxyhæmoglobin was prepared by Hoppe-Seyler's method[1] from as much blood as could be collected from the carotid of a large dog. The weather was steadily cold, the external temperature about 0°F. The crystals were dissolved in water and recrystallised three times. A portion of the oxyhæmoglobin was crystallised four times. Finally the crystals were well dried between folds of blotting-paper, and then dried for two days over sulphuric acid *in vacuo*. They were then powdered

[1] *op. cit.* p. 274.

finely in a mortar and again dried *in vacuo* for three days. The greater portion of the thoroughly dry hæmoglobin was sealed up in glass tubes.

Exp. I. Of the thrice crystallised hæmoglobin 0·3976 grm. was thoroughly rubbed up in a mortar with 9·929 grm. of distilled water, and then filtered. 0·0336 grm. of undissolved pigment (hæmatin ?) remained on the filter. In 100 c.c. of the solution there would be 3·53 grm. hæmoglobin. The freezing-point and electrical conductivity of the solution were determined with the following results.

Freezing points (by Beckmann's apparatus).

Distilled water (mean of 5 readings)			4·589°
Hb solution	4·581°		
	4·580°	mean	4·580°
	4·580°		
		$\Delta =$	·009°

This is about $\frac{1}{65}$th of the value of Δ for blood-serum.

The addition to the serum of defibrinated blood of a quantity of this specimen of hæmoglobin amounting to 2 per cent. of the serum would only cause an alteration of 1 per cent. in the osmotic pressure of the serum.

To get the osmotic pressure due to the hæmoglobin alone we must deduct the portion due to the salts that cling to it even after three crystallisations. The most important of these salts will certainly be sodium chloride, since sodium chloride solution is mixed with the blood in the preliminary sedimentation. We may, without serious error, assume that the conductivity of the hæmoglobin solution in distilled water, apart from the small portion of it that depends on the impurities in the distilled water, is entirely due to sodium chloride, and thus arrive at the amount of sodium chloride present. Thus

For the Hb solution $\lambda(5^\circ) \times 10^8 = 0{\cdot}90$.

The molecular conductivity of sodium chloride[1] in very dilute solutions at 18° C., expressed in mercury units, may be taken as 86, or expressed in reciprocal ohms at 5° C.[2], say, 66.

If $\mu =$ the molecular conductivity, and $v =$ the number of litres in which a gram-molecule of a substance is dissolved, and $\lambda =$ the specific conductivity, then $v = \dfrac{\mu}{\lambda \times 10^7}$.

[1] Ostwald's *Lehrbuch d. allgemeinen Chemie*, 2te Ausg. II. Th. I. p. 646.

[2] In this paper electrical conductivities are always expressed in reciprocal ohms at 5° C. $\times 10^8$.

Substituting for μ its value 66, and for $\lambda \times 10^7$ its value ·09, we get $v = \frac{66}{\cdot 09} = 730$. A gram-molecule of NaCl, or 58·36 grm., would therefore be dissolved in 730 litres of a solution containing the same proportion of NaCl as the hæmoglobin solution. But when a gram-molecule of a substance not susceptible of electrolytic dissociation is present in a litre of its aqueous solution, the freezing-point is lowered by 1·85° C. Therefore, the quantity of NaCl present in the hæmoglobin solution, if it were non-dissociable, would cause a lowering of

$$\frac{1 \cdot 85^\circ}{730} = \cdot 0025^\circ.$$

But in such dilute solutions we may assume that all the NaCl is dissociated, and therefore the true diminution of the freezing-point would be twice as much, say, ·005°. Deducting this from ·009° we get for the hæmoglobin in the solution $\Delta = \cdot 004^\circ$.

If m is the molecular weight of hæmoglobin we get from the formula $\frac{\Delta}{1 \cdot 85} = \frac{p}{m}$, (where Δ is the diminution of freezing-point when p grammes of hæmoglobin are present in 1 litre of solution,)

$$\frac{\cdot 004}{1 \cdot 85} = \frac{35 \cdot 3}{m}, \text{ and } m = 16300.$$

The molecular weight obtained by Jaquet from his analytical results is 16,669. It will be seen that the molecular weight deduced from the freezing-point observations is of the same order of magnitude; and this is of interest, although no stress is to be laid on the apparent closeness of the agreement, as the next experiment will show. The mere taking into account of the conductivity of the distilled water would cause an alteration in the second figure, and an error of 0·001° in the reading of the freezing-point might make the first figure 2 instead of 1.

Exp. II. 1·192 grm. hæmoglobin (4 times crystallised) was completely dissolved in 14·87 grm. distilled water. (1·3072 grm. Hb was taken, but 0·1152 grm. of pigment remained on the filter.) The solution contained 7·45 grm. Hb in 100 c.c.

For the Hb solution $\Delta = \cdot 011^\circ$ C.

$$\lambda(5^\circ) \times 10^8 = \cdot 53.$$

The addition to defibrinated blood of a quantity of this specimen of hæmoglobin amounting to 2 per cent. of the serum would only alter the osmotic pressure of the serum by 0·5 per cent.

From the formula $v = \frac{\mu}{\lambda \times 10^7}$, we get

$$v = \frac{66}{\cdot 053} = 1245.$$

$\frac{1\cdot 85^\circ}{1245} = \cdot 0015^\circ$ = amount by which the freezing-point would be diminished by the sodium chloride present if it were non-dissociable. Subtracting twice this amount, or ·003°, from ·011°, we get for the hæmoglobin alone

$$\Delta = \cdot 008^\circ \text{ C.}$$

Taking m as the molecular weight of hæmoglobin

$$\frac{\cdot 008}{1\cdot 85} m = 74\cdot 5$$

$$m = 17200.$$

For comparison hæmoglobin was obtained from the blood of a second dog, also by Hoppe-Seyler's method; but instead of being re-crystallised, it was filtered off from the mother liquid, washed repeatedly on the filter with 20 per cent. alcohol cooled to 0° C., then dried with blotting-paper, and finally dried *in vacuo* as before. It was completely soluble and contained no greater amount of electrolytes than the hæmoglobin made in the other way. It was used in several of the experiments on the relative volume of corpuscles and plasma, but hæmoglobin of the first sample was used in the greater number. From the two dogs enough was obtained for a far greater number of experiments than were actually carried out, indeed for hundreds. The preparation of the hæmoglobin is not, therefore, a serious addition to the labour of making the observations.

The details of the method.

Blood was obtained from the carotid artery of dogs, which, as a rule, had fasted for 24 hours. The animals were always anæsthetised, usually with morphia and the A.C.E. mixture, sometimes with the A.C.E. mixture alone. The blood was defibrinated, and strained through muslin. An exactly measured or weighed quantity of the blood (usually 75 c.c.) was then centrifugalised till a large quantity of clear

serum had separated. As much as possible of the serum was removed, placed in a weighed dish, and weighed, or accurately measured; or the amount removed was determined by weighing the centrifuge tube or tubes. Only serum perfectly free from corpuscles was taken, or if a few corpuscles were present they were removed by further centrifugalisation in a separate tube[1]. When a few corpuscles are suspended in a large quantity of serum they sink much more rapidly than they do in ordinary defibrinated blood. A weighed or measured amount of the clear serum (say, 5 to 15 c.c.) was rubbed up carefully in a small mortar with a weighed amount of hæmoglobin. Usually so much hæmoglobin was taken as would yield a 1 to 2 per cent. solution in the serum. A known amount of the hæmoglobin solution was added to the sediment of the centrifugalised blood. If two centrifuge tubes had been used the sediments were thoroughly mixed with the hæmoglobin solution and with each other. The sediment was now again centrifugalised till a layer of transparent reddish or blackish red serum which I speak of as the "tinged serum" had separated. A few c.c. of this were removed, and a drop examined under the microscope. If any corpuscles were present the tinged serum was again centrifugalised in a small tube by itself. When it was free from corpuscles it was compared with the original solution of hæmoglobin in serum as regards the amount of hæmoglobin in it, the original solution being diluted with clear serum till the depth of tint was the same in both solutions when viewed side by side in two small hæmatinometers of similar dimensions. Sometimes the hæmoglobin was converted into carbonic oxide hæmoglobin in the two solutions before comparison. From the amount of serum which it is necessary to add to the original hæmoglobin solution, the amount of serum with which each grm. or c.c. of it added to the sediment must have been mixed, and therefore the amount of serum left in the sediment after the removal of the first portion of serum, can be immediately deduced. The specific gravity of the defibrinated blood and serum was always determined, so that the quantity of serum in the blood could be expressed either in c.c. per 100 c.c., or in grm. per 100 grm. of blood.

If V is the volume of blood taken; v, the volume of the first portion of serum; v', the volume of the hæmoglobin solution added to the

[1] In one experiment, although the animal had fasted 24 hours, the serum was turbid with granules and globules (of fat?) which could not be separated either by the centrifuge or by filtration in the cold. The experiment was, therefore, abandoned.

sediment, and a, the volume of serum which must be added to each c.c. of v' to make its tint the same as that of the tinged serum, then

$$\frac{100\,(v + av')}{V} = \text{volume of serum in 100 c.c of blood.}$$

The hæmoglobin determination is the only measurement which cannot be made with great accuracy, but the removal and estimation of the greater portion of the serum before the addition of the hæmoglobin solution reduces the error arising from this source. When a spectrophotometer is available the amount of hæmoglobin in the hæmoglobin solution and in the tinged serum can be directly estimated, and the error materially lessened.

In some experiments a portion of the blood obtained from the animal was allowed to clot, the rest being defibrinated. The clear serum was pipetted off (in 16 to 24 hours) from the clot, hæmoglobin dissolved in a portion of it, and a measured quantity of the filtered hæmoglobin solution added to a portion of the defibrinated blood. The tinged serum obtained by centrifugalising the mixture was then compared as before with the hæmoglobin solution. This method, however, while it requires only a single centrifugalisation, labours under the defect that unless the blood is kept at a temperature not much above 0° C. some hæmoglobin is apt to pass into the serum of the defibrinated blood from the corpuscles. The consequence is that the tinged serum separated by the centrifuge after the addition of the hæmoglobin solution has a higher percentage of hæmoglobin than it ought to have, and therefore the proportion of serum in the defibrinated blood comes out too low. An example of this is seen in Exp. III.

Exp. III. 185 c.c. of blood was drawn from the carotid of a dog, and allowed to clot. At the same time a quantity of defibrinated blood was obtained. Both were allowed to stand for 19 hours. The clear serum was then pipetted off the clot, and 0·475 grm. hæmoglobin (4 times crystallised) was dissolved in 30 c.c. of the serum. The defibrinated blood was well shaken, and to 70 c.c. of it was added 20 c.c. of the filtered hæmoglobin solution. The mixture was well stirred, and then centrifugalised till a considerable quantity of transparent reddish serum had separated. A few c.c. of this was removed from the top of the centrifugal tube, and compared with the original hæmoglobin solution. 1 c.c. of the solution + 1·5 c.c. of distilled water was slightly weaker in tint than the tinged serum.

1 c.c. of the Hb solution + 1·4 c.c. water was equal in tint to the tinged serum. The 70 c.c. of blood, therefore, contained $20 \times 1{\cdot}4 = 28$ c.c. of serum, or 37·3 c.c. in 100 c.c. of blood.

There is no doubt that this result is too low. For 96 c.c. of serum was actually separated from the 185 c.c. of clotted blood, and this is equivalent to 51·9 c.c. in 100 c.c. of blood, so that the real percentage of serum must certainly be more than 51·9. Further the electrical method gave by the formula

$$p = \frac{\lambda_{(b)}}{\lambda_{(s)}}(180 - \lambda_{(b)} - \sqrt{\lambda_{(b)}}) \text{ (see p. 364),}$$

$\frac{41{\cdot}7}{80{\cdot}99}(180 - 41{\cdot}7 - \sqrt{41{\cdot}7})$ as the percentage of serum, or 67·9 per cent. The main cause of the large error is doubtless the presence of hæmoglobin in the serum of the defibrinated blood before the addition of the hæmoglobin solution. It could be seen indeed that the layer of separated serum was red. It would of course be possible to eliminate this error by separately estimating the hæmoglobin in a portion of the serum of the defibrinated blood, or by using this serum for the dilution of the hæmoglobin solution in the colorimetric comparison. A small error may be caused by the use of water for dilution instead of hæmoglobin-free serum, and this error would also be in the direction of too small a percentage of serum.

Exp. IV. is an example of the colorimetric method when the errors of Exp. III. are eliminated.

Exp. IV. Obtained defibrinated blood from the carotid of a dog weighing 3·6 kilos. Also allowed some of the blood to clot. Immediately centrifugalised 75 c.c. of the defibrinated blood. Took off 43·5 c.c. of clear serum. Again centrifugalised the serum to separate the few corpuscles in it. Dissolved 0·384 grm. hæmoglobin (4 times crystallised) in 35 c.c. of the serum. Filtered. Added 15 c.c. of the filtrate to the sediment of the defibrinated blood after centrifugalisation. Mixed well, and centrifugalised again, till transparent blackish red serum, free from corpuscles, had separated. The sediment was bright red. Took off 21·5 c.c. of the tinged serum.

2 c.c. of the Hb solution + 1·5 c.c. of serum from clot is slightly stronger than the tinged serum.

2 c.c. of the Hb solution + 1·6 c.c. of serum is exactly equal in tint to the tinged serum.

Accordingly, 0·8 c.c. of serum must have been mixed in the sediment with each c.c. of the Hb solution added to it; and, therefore, after the removal of 43·5 c.c. of serum, $15 \times 0{\cdot}8 = 12$ c.c. must still have remained in the sediment. This gives in all 55·5 c.c. of serum in the 75 c.c. of blood = 74·0 c.c. in 100 c.c. of blood.

By the electrical method from the formula

$$p = \frac{\lambda_{(b)}}{\lambda_{(s)}}(180 - \lambda_{(b)} - \sqrt{\lambda_{(b)}}),$$

we get

$$p = \frac{52{\cdot}59}{85{\cdot}35}(180 - 52{\cdot}59 - \sqrt{52{\cdot}59})$$

$$= 74{\cdot}03.$$

For a comparison of the colorimetric method with Hoppe-Seyler's method see p. 369, Exp. VIII.

The electrical method.

The fact that in comparison with the serum the red blood corpuscles are exceedingly bad conductors[1] suggests that there must be a relation between the conductivity of the blood and serum and the relative volume of corpuscles and serum. I have already shown[2] that the simple proportion $\frac{V_{(s)}}{V_{(b)}} = \frac{\lambda_{(b)}}{\lambda_{(s)}}$, (where $V_{(s)}$, $V_{(b)}$ are respectively the volume of the serum and of the entire blood, and $\lambda_{(s)}$ and $\lambda_{(b)}$ the conductivity of the serum and blood) does not hold, although in blood very rich in serum it is more nearly correct than in blood rich in corpuscles. Bugarsky and Tangl[3] on the basis of a very small number of experiments, give the formula $p = 92\frac{\lambda_{(b)}}{\lambda_{(s)}} + 13$, where p is the percentage volume of serum, $\lambda_{(b)}$, the conductivity of the blood, and $\lambda_{(s)}$, the conductivity of the serum. They state that in all probability if they had made a larger number of observations they would have had to modify their formula. They only compared their results with those given by the hæmatocrite, which, of course only gives the limit below which the percentage of plasma cannot lie, and not the actual amount of plasma. They assumed that after long centrifugalisation the amount of serum in the sediment of defibrinated blood might be neglected.

I find that the formula (cited in what follows as formula (1))

$$p = \frac{\lambda_{(b)}}{\lambda_{(s)}}(180 - \lambda_{(b)} - \sqrt{\lambda_{(b)}})$$

[1] G. N. Stewart, *Journ. of the Boston Society for Med. Sciences*, June, 1897; *Centralbl. f. Physiol.* xi. p. 332, 1897. W. Roth, *ibid.* p. 271, 1897. St Bugarsky and F. Tangl, *ibid.* p. 297, 1897.

[2] *loc. cit.*

[3] *loc. cit.*

is approximately correct for a wide range in the relative proportion of corpuscles and serum in dog's blood. It gives results which agree most closely with the results of the colorimetric method and of Hoppe-Seyler's method when the percentage of serum is such as is found in normal blood. The agreement is less close for artificial mixtures of corpuscles and serum in which the percentage of serum is higher than the maximum or lower than the minimum percentage found in normal blood, the formula giving too low a value in the latter case, and, in general, somewhat too high a value in the former.

A somewhat simpler, but, on the whole, a less accurate formula is

$$p = \frac{174{\cdot}5\lambda_{(b)} - (\lambda_{(b)})^2}{\lambda_s} \text{ (cited as formula (2)).}$$

For a first approximation the factor 174·5 can become 174. When the percentage of serum is low this formula gives somewhat lower values than the other, and somewhat higher values when the percentage of serum is high. In both formulæ the conductivities are to be expressed in reciprocal ohms at 50° C. multiplied by 10^8. Bugarsky and Tangl's formula gives considerably lower results, in general, than either of my formulæ, and results that are doubtless too low. The observations from which formulæ (1) and (2) are deduced consist, (1) of measurements of the conductivity of the serum and entire blood in normal defibrinated blood in which the proportion of corpuscles of serum was also determined by the colorimetric method, or by Hoppe-Seyler's method, or by both. (2) Of measurements of the conductivity of the serum and entire blood in artificial mixtures of corpuscles and serum made by diluting the sediments obtained by centrifugalising defibrinated blood, with known quantities of its own serum. The proportion of corpuscles to serum in the original sediment was determined by the colorimetric method or by Hoppe-Seyler's, or by both.

The following experiments illustrate the relation between the electrical conductivity of the serum and blood in sediments diluted with serum and the quantity of serum found by other methods. In all the experiments a weighed or measured quantity of the sediment was taken, and to it was added a weighed or measured quantity of the serum of the same blood, obtained either from the clot or from the defibrinated blood by centrifugalisation. The pipettes used for measuring the blood were all corrected by determining the weight of the distilled water needed to fill them to the mark. When the serum

was measured by volume the actual weight of the serum which ran out of the pipettes used was separately determined. A definite quantity of serum, having been added to a definite quantity of blood-sediment, say 1 c.c. of serum to 10 or 20 c.c. of sediment, the mixture was thoroughly agitated in a test-tube, and then the pipette used for measuring the sediment was washed out several times with the mixture. The sediments being so thick, this seemed a safer method than to trust to any preliminary estimate of the outflow from the blood-pipettes. Some of the mixture was now poured into the U-tube used for measuring the resistance, which was immersed in a large water-bath, the temperature of which was read by a thermometer with its bulb in immediate contact with the tube. After the resistance measurement the contents of the U-tube were poured back into the test-tube. A measured or weighed quantity of the mixture was placed in another test-tube, a known quantity of serum added, and the whole performance repeated, and so on. When all the measurements were made by weighing, the blood was contained in the same test-tube throughout the experiment. A test-tube was weighed, a quantity of sediment placed in it, and the test-tube again weighed. The difference gave the amount of sediment. Some serum was now added, and the test-tube again weighed to get the amount of serum. After the resistance measurement the contents of the U-tube were poured back into the test-tube, any dried blood on the outside or inside of the test-tube around its mouth carefully removed, and the test-tube weighed. In this way the amount of the first mixture used for making the second was ascertained. A quantity of serum was now added and the test-tube again weighed to ascertain its amount, and so on. The test-tube was kept covered with a small watch-glass to prevent drying of the blood. In quoting the results I have usually translated from weights to volumes.

Exp. V. In the sediment obtained in Exp. IV. after the second centrifugalisation the amount of serum present after the removal of the 21·5 c.c. of tinged serum was 5·5 c.c. The volume of the corpuscles in the sediment was 18·5 c.c. 2·7 c.c. of serum was now removed from the sediment, leaving 2·8 c.c. of serum in 22·3 c.c. of sediment, or 12·5 c.c. of serum in 100 c.c. of sediment. The conductivity of the defibrinated blood, the serum, the sediment, and of 11 dilutions of the sediment was measured. The percentage of serum known to be present in the defibrinated blood, the sediment and its

dilutions is compared in the table with that calculated from the electrical observations. S stands for the original sediment, S (1) for its first dilution, S (2) for its second dilution &c.

	Serum found by colorimetric method c.c. in 100 c.c. of blood	$\lambda\,(5°) \times 10^8$	Serum calculated from formula (1) c.c. in 100 c.c. of blood	Serum calculated from formula (2) c.c. in 100 c.c. of blood
S	12·5	5·86	11·79	11·57
S (1)	36·14	19·72	36·11	35·76
S (2)	46·66	27·14	46·94	46·86
S (3)	55·50	34·49	56·41	56·58
S (4)	62·83	41·29	63·99	64·45
S (5)	69·00	47·21	69·65	70·41
S (6)	74·10	52·27	73·79	74·85
Defib. blood	74·0	52·59	74·03	75·12
S (7)	79·30	57·61	77·48	78·91
S (8)	82·01	61·53	79·75	81·44
S (9)	84·24	65·84	81·80	83·83
S (10)	85·60	68·15	82·72	85·03
S (11)	86·07	69·26	83·10	85·41
Serum		85·35		

Exp. VI. Dog, body-weight 5·45 kilo. Drew off 170 c.c. of defibrinated blood, and allowed 105 c.c. of blood to clot. Sp. gr. of defib. blood 1060·8, sp. gr. of serum from clot, 1023·4.

Immediately after the blood was shed 76·154 grm. = 71·78 c.c. of defib. blood was centrifugalised for 40 minutes. Took off of clear serum 30·818 grm. = 30·11 c.c. Added to 15 c.c. of the serum 0·235 grm. hæmoglobin (not recrystallised, but purified by repeated washing with cold 20 % alcohol). Added to the sediment 4·85 c.c. of the filtered Hb solution, mixed, and after the second centrifugalisation took off 5·78 c.c. of the tinged serum. Each c.c. of the hæmoglobin solution must be diluted with 1·2 c.c. of serum to make its tint the same as that of the tinged serum. Therefore after the removal of the clear serum after the first centrifugalisation the sediment must still have contained $4{\cdot}85 \times 1{\cdot}2 = 5{\cdot}82$ c.c. serum. After removal of the tinged serum the sediment must contain 4·89 c.c. of serum. The total amount of serum in the 75 c.c. of defibrinated blood is $30{\cdot}11 + 5{\cdot}82$ c.c. = 35·93 c.c. This leaves 35·85 c.c. of corpuscles.

In the sediment accordingly we have 35·85 c.c. of corpuscles and 4·89 c.c. of serum, or 12·00 c.c. of serum in 100 c.c. of sediment. In 100 c.c. of defibrinated blood we have 50·05 c.c. of serum. The table shows the actually

observed percentages of serum and the percentages calculated by the electrical method in the defibrinated blood, the sediment, and 21 dilutions of the sediment.

	c.c. serum in 100 c.c. blood by colorimetric method	$\lambda (5°) \times 10^3$	c.c. serum in 100 c.c. blood calculated from formula (1)	c.c. serum in 100 c.c. blood calculated from formula (2)
S	12·00	5·03	10·48	10·29
S (1)	15·03	6·28	12·98	12·77
S (2)	18·76	7·92	16·18	15·92
S (3)	22·61	9·86	19·88	19·60
S (4)	26·28	11·81	23·48	23·19
S (5)	29·76	13·89	27·21	26·93
S (6)	33·11	15·60	30·22	29·92
S (7)	36·28	17·42	33·30	33·03
S (8)	41·18	21·30	39·62	39·39
S (9)	46·09	25·03	45·26	45·16
S (10)	50·59	28·52	50·31	50·26
Defib. blood	50·05	28·74	50·61	50·57
S (11)	54·68	31·69	54·58	54·63
S (12)	58·36	34·97	58·72	58·91
S (13)	61·72	37·95	62·25	62·55
S (14)	64·77	41·09	65·72	66·17
S (15)	67·54	44·01	68·7	69·32
S (16)	72·17	48·84	73·21	74·09
S (17)	76·08	54·28	77·55	78·77
S (18)	79·33	57·35	79·67	81·09
S (19)	82·00	61·98	82·41	84·18
S (20)	85·85	67·42	84·94	87·14
S (21)	88·57	71·81	85·57	89·02
Serum		82·84		

Exp. VII. Dog, body-weight 15·9 kilos. Anæsthetised with A.C.E. mixture. No morphia. Both thyroids much enlarged. Aortic thyroid also enlarged. Sp. gr. of defib. blood 1065·9, of serum 1023·6.

Centrifugalised 71·4 c.c. of defibrinated blood immediately after it was drawn from the carotid. Took off 28·3 c.c. of clear serum. Found in sediment by colorimetric method 4·7 c.c. Total serum, 33·0 c.c. in 71·4 c.c. of blood = 46·21 c.c. serum in 100 c.c. of blood. Serum in 45·4 c.c. of sediment after second centrifugalisation 6·9 c.c.; or 15·2 c.c. in 100 c.c. of sediment. Determined percentage of serum in defibrinated blood, sediment and 14 dilutions of sediment by electrical method. Thus

	c.c. serum in 100 c.c. blood by colorimetric method	$\lambda(5°) \times 10^8$	c.c. serum in 100 c.c. blood calculated by formula (1)	c.c. serum in 100 c.c. blood calculated by formula (2)
S	15·2	7·46	15·03	14·79
S (1)	22·90	11·24	22·07	21·78
S (2)	29·90	15·22	29·07	28·78
S (3)	36·27	19·02	35·37	35·11
S (4)	42·06	22·88	41·38	41·18
Defib. blood	46·21	26·13	46·15	46·02
S (5)	47·32	26·80	47·10	46·99
S (6)	52·17	30·65	52·34	52·35
S (7)	56·57	34·07	56·67	56·81
S (8)	60·56	37·84	61·10	61·42
S (9)	64·18	41·29	64·85	65·31
S (10)	67·46	44·55	66·93	68·74
S (11)	70·43	47·65	71·03	71·65
S (12)	73·13	50·40	73·30	74·26
S (13)	77·44	55·11	76·86	78·12
S (14)	81·06	59·23	81·10	81·07

EXP. VIII. *Comparison of colorimetric method, electrical method, and Hoppe-Seyler's method.* Dog, body-weight 11·3 kilos. Anæsthetised with A.C.E. mixture. No morphia. Obtained blood from carotid. Defibrinated a portion, and let the rest clot. Sp. gr. of the defibrinated blood 1055·3; sp. gr. of serum 1023·3.

Colorimetric method. Duplicate determinations made.

Tube A. Quantity of blood taken 36·0158 grm. = 34·128 c.c. Centrifugalised 45 minutes. Took off 19·169 grm. serum = 18·732 c.c.; 0·150 grm. of the sediment was thrown away. Dissolved 0·24 grm. hæmoglobin (not recrystallized, but repeatedly washed with cold 20 per cent. alcohol before drying) in 20 c.c. of the clear serum. (The serum was obtained by mixing clear serum from tubes *A* and *B*.) Added 4·97 grm. of the filtrate to the sediment remaining in tube *A*. Mixed, and again centrifugalised. Took off from *A* 4·9877 grm. of tinged serum free from corpuscles. Found by colorimetric comparison that the sediment of *A* must have contained 1·35 c.c. serum after removal of the first portion of serum. The total serum in 34·128 c.c. of blood is therefore 20·082 c.c. or 58·843 c.c. in 100 cc. of blood.

Tube B. Quantity of blood taken, 40·5223 grm. = 38·304 c.c. Centrifugalised at the same time as *A* and for the same length of time. Took off 21·2744 grm. serum. Added to the sediment 4·97 grm. of the same Hb solution as was added to tube *A*. Mixed and centrifugalised again at the same time as *A* and for the same length of time. Took off 4·9197 grm. of

tinged serum free from corpuscles. Found by colorimetric comparison that the sediment of *B* must have contained 2·131 grm. of serum after removal of the first portion of serum. The total serum in 40·5223 grm. blood is, therefore, 23·4054 grm., or 22·872 c.c. serum in 38·304 c.c. blood, *i.e.* 59·711 c.c. in 100 c.c. of blood.

Mean of *A* and *B*, 59·277 c.c. serum in 100 c.c. blood.

Hoppe-Seyler's method. Weighed portions of blood and serum, and the serum-free sediment of a weighed portion of blood, were precipitated with 4 times their volume of 95 per cent. alcohol containing a little acetic acid. After standing 15 hours in the cold, the precipitates were collected on weighed ash-free filters, washed on the filters first with 95 per cent. alcohol, then repeatedly with separate portions of hot absolute alcohol, then repeatedly with several portions of ether, then with a little 95 per cent. alcohol to remove the ether, then repeatedly with hot water. They were then dried at 110° C. to constant weight, and finally incinerated and the ash estimated. The first filtrate was evaporated to dryness on the water-bath, the residue stirred up with the absolute alcohol and ether washings, and then collected on small, weighed, ash-free filters. The aqueous washings after being heated to boiling were passed through the filters, which were then weighed and incinerated along with the corresponding main precipitate. Duplicate experiments were made.

A. *Blood.* Quantity taken 10·0372 grm.

Proteids and hæmoglobin 1·7425 grm. = 17·360 grm. in 100 grm. blood.

Corpuscles. Quantity of blood taken 8·5068 grm.

Proteids and Hb 1·2126 = 14·254 grm. in 100 grm. blood.

Difference between blood and corpuscles 3·106 grm. in 100 grm.

Serum. Quantity taken 20·2342 grm.

Proteids 1·0976 = 5·424 grm. in 100 grm.

$$\text{Serum in 100 grm. blood} = \frac{3{\cdot}106}{5{\cdot}424} \times 100 = 57{\cdot}26 \text{ grm.}$$

Serum in 100 c.c. blood = 59·051 c.c.

B. *Blood.* Quantity taken 9·6678 grm.

Proteids and Hb 1·7043 = 17·627 grm. in 100 grm. blood.

Corpuscles. Quantity of blood taken 10·9225 grm.

Proteids and Hb 1·5848 grm. = 14·509 grm. in 100 grm. blood.

Difference between proteids and Hb of blood and corpuscles 3·118 grm. in 100 grm.

Serum. Quantity taken 12·1845 grm.

Proteids 0·6700 grm. = 5·498 grm. in 100 grm.

$$\text{Serum in 100 grm. blood} = \frac{3{\cdot}118}{5{\cdot}498} \times 100 = 56{\cdot}711 \text{ grm. in 100 grm., } i.e.$$

58·480 c.c. in 100 c.c. blood.

Mean of *A* and *B* 56·987 grm. serum in 100 grm. blood, or 58·765 c.c. in 100 c.c. blood.

Mean of the results of Hoppe-Seyler's and the colorimetric method 59·021 c.c. serum in 100 c.c. blood.

Electrical Method. For serum $\lambda\,(5°) \times 10^8 = 81{\cdot}25$. For defibrinated blood = 34·58.

Substituting in formula (1) we get $p = \frac{34{\cdot}58}{81{\cdot}25}\,(180 - 34{\cdot}58 - \sqrt{34{\cdot}58})$ = 59·388 c.c. of serum in 100 c.c. of blood.

In the blood in tube *A* which was used for the colorimetric determination there must have been altogether (calculating from the mean of the Hoppe-Seyler and the colorimetric results) 20·147 c.c. of serum in 34·128 c.c. of blood. Deducting what was taken off from *A*, the sediment of *A* after the removal of the tinged serum must have contained 1·42 c.c. of serum in 15·25 c.c. of sediment or 9·31 c.c. in 100 c.c.

A series of dilutions of the sediment of *A* was made with the following results.

	Quantity of serum actually present. c.c. in 100 c.c. of blood	$\lambda\,(5°) \times 10^8$	c.c. of serum in 100 c.c. blood calculated from formula (1)	c.c. of serum in 100 c.c. blood calculated from formula (2)	c.c. of serum in 100 c.c. blood calculated from the formula of Bugarsky and Tangl
S	9·31	5·20	11·04	10·87	18·88
S (1)	17·86	8·94	18·49	18·27	23·12
S (2)	25·45	12·38	25·00	24·78	27·01
S (3)	32·18	16·07	31·50	31·43	31·19
S (4)	38·36	19·62	37·65	37·51	35·21
S (5)	43·90	23·17	43·36	43·29	39·23
S (6)	49·00	26·49	48·37	48·41	42·99
S (7)	53·63	29·79	53·07	53·23	46·73
S (8)	57·81	33·36	57·83	58·14	50·77
Defib. blood	59·02	34·58	59·39	59·73	52·15
S (9)	61·63	36·48	61·72	62·16	53·07
S (10)	68·15	42·68	68·70	69·47	61·32
S (11)	73·65	47·92	73·90	74·89	67·26
S (12)	78·20	52·81	77·95	79·36	72·79
S (13)	81·90	56·97	80·96	82·66	77·50
S (14)	84·74	60·50	83·19	85·15	81·50
S (15)	88·60	65·84	85·93	88·34	87·55
Serum		81·25			

The following table shows the extent of the variations in the percentage of serum in the blood of 30 dogs. The results are taken at random from among observations which I possess on more than 100 dogs. A few observations are added on the rabbit, hen and goose.

Dog.

No. of animal	Serum $\lambda(5°) \times 10^8$	Defib. blood $\lambda(5°) \times 10^8$	c.c. of serum in 100 c.c. blood calculated by formula (1)	
1	83·66	33·67	56·56	Sp. gr. blood 1057·6; sp. gr. serum 1024·3
2	80·99	41·69	67·86	
3	80·73	34·70	59·92	
4	85·35	52·59	74·03	
5	84·78	31·97	53·68	
6	81·25	31·97	56·02	
7	83·66	32·58	55·19	
8	81·51	28·04	50·44	Sp. gr. blood 1060·7
9	80·99	33·76	58·54	„ „ 1057·0
10	82·04	39·86	65·01	„ „ 1050·3; sp. gr. serum 1022·8
11	79·72	25·10	47·19	„ „ 1063·4; „ „ 1023·8
12	80·48	30·04	53·92	„ „ 1061·6; „ „ 1024·7
13	82·84	28·74	50·61	„ „ 1060·8; „ „ 1023·4
14	84·22	26·13	46·15	„ „ 1065·9; „ „ 1023·6
15	80·99	25·55	47·13	„ „ 1066·7; , „ 1025·1
16	80·99	34·58	59·56	„ „ 1055·3; „ „ 1023·3
17	82·57	33·80	57·46	
18	86·82	47·74	68·91	
19	82·04	39·18	64·03	
20	80·22	28·91	52·49	
21	86·52	42·97	64·79	
22	93·89	45·11	61·57	
23	85·35	22·78	40·68	
24	83·12	23·13	42·34	
25	84·22	35·96	58·93	
26	85·64	22·17	39·63	
27	80·22	25·10	46·89	
28	78·73	38·70	66·39	
29	79·22	29·01	53·32	
30	79·47	24·61	46·58	

	No. of animal	Serum $\lambda\,(5°) \times 10^8$	Defib. blood $\lambda\,(5°) \times 10^8$	c.c. of serum in 100 c.c. blood calculated from formula (1)
Hen	1	102·63	48·84	59·08
	2	87·72	49·32	69·51
Goose	1	80·73	26·43	48·58
Rabbit	1	80·22	41·69	68·51
	2	75·90	38·24	67·91

SUMMARY.

1. The relative quantity of corpuscles and serum in defibrinated blood can be determined by adding to a definite quantity of the blood a known amount of a solution of hæmoglobin in the serum of the blood, centrifugalising and comparing the concentration of the hæmoglobin in the serum and in the original solution.

2. The relative quantity of corpuscles and serum in defibrinated blood can be determined by measuring the electrical conductivity of the blood and serum, and substituting the values in the formula

$$p = \frac{\lambda_{(b)}}{\lambda_{(s)}}(180 - \lambda_{(b)} - \sqrt{\lambda_{(b)}}),$$

or in the formula

$$p = \frac{174\lambda_{(b)} - (\lambda_{(b)})^2}{\lambda_{(s)}},$$

where $\lambda_{(b)}$ is the conductivity of the blood at $5°$ C. $\times 10^8$, $\lambda_{(s)}$ the conductivity of the serum at $5°$ C. $\times 10^8$, and p, the number of c.c. of serum in 100 c.c. of blood.

3. In the dog the quantity of serum has been found to vary in different individuals from 40 to 74 per cent. of the total volume of the blood.