The Blood Volume of Mammals as Determined by Experiments upon Rabbits, Guinea-Pigs, and Mice; and Its Relationship to the Body Weight and to the Surface Area Expressed in a Formula

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III. *The Blood Volume of Mammals as determined by Experiments upon Rabbits, Guinea-pigs, and Mice; and its Relationship to the Body Weight and to the Surface Area expressed in a Formula.*

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The question of the blood volume in man and animals has for more than 70 years been the subject of numerous investigations. This is but natural, considering its great practical and theoretical importance in the study of diseases and their treatment.

Although so much work has been done upon this subject, it is remarkable to see the great differences of opinion expressed by the many observers as to the amount of blood contained by various animals: thus we may give, as examples, the ratio of blood weight to body weight in the following animals:—

Man	1 : 13·1	Welcker.
	1 : 13·5	Bischoff.
	1 : 20·5	Haldane and Lorrain Smith.
Cat	1 : 12	Steinberg.
	1 : 15·1	Welcker.
	1 : 21	Ranke.
Rabbit . . .	1 : 13	Steinberg.
	1 : 13·4 to 1 : 15·7	Sherrington and Copeman.
	1 : 18	Jolyet and Laffont.
	1 : 18·1	Welcker.
	1 : 18·4 (doe) . .	Boycott and Douglas.
	1 : 18·1 (buck) . .	,, ,,
	1 : 18·8 (doe) . .	Douglas.
	1 : 20·6 (buck) . .	,,
	1 : 24·2	Ranke.
Guinea-pig . .	1 : 12	Steinberg.
	1 : 18	Jolyet and Laffont.
	1 : 17 to 1 : 22 .	Gscheidlen.

Dog	1 : 12 to 1 : 13 .	Jolyet and Laffont.
	1 : 12 to 1 : 15 .	Panum.
	1 : 12 to 1 : 18 .	Heidenhain.
	1 : 15·1	Ranke.

These great variations are in some cases partly due to the different methods employed, to the individual accuracy of the workers, to the varying condition and size of the animals employed, etc., whilst in others the differences are more apparent than real, as will be shown later on.

In spite of the great divergence of opinion as to the actual quantity of blood which the various animals contain, yet the greater number of observers agree that small animals contain relatively more blood than larger animals of the same species; but Ranke, in his classical investigation on the amount and distribution of blood in various animals, is really the only observer who has undertaken experiments with the special purpose of ascertaining the percentage amount of blood in rabbits, over a long range of weight. He found that rabbits ("Rohgewicht") under 300 grammes contained 7·1 per cent. of their body weight of blood, 400–600 grammes 6 per cent., and 1000–1500 grammes 5·4 per cent. The main result of his experiments, as expressed by himself on p. 42 of his memoir, is:

"Die Grösse des Blutgehaltes steht in directem Verhältniss zur Grösse des Stoffwechsels."

"Jüngere, kleinere Thiere derselben Thierspecies haben wie einen relativ grösseren Stoffwechsel so auch eine relativ grössere Blutmenge als ausgewachsene. Es nimmt sonach die Blutmenge und der Stoffwechsel von dem Jugendzustande an, d. h. mit dem steigenden Körpergewichte, ab."

This fact, that small animals contain a relatively greater amount of blood than larger ones of the same species, is in accordance with the results obtained by the greater number of observers (Welcker, Ranke, Malassez, Sherrington and Copeman, etc., although, in expressing their view, they put it in the form that *young* animals contain relatively more blood), but it is in absolute disagreement with Boycott and Douglas' results, as obtained by using Haldane and Lorrain Smith's modification of Grehant and Quinquaud's CO method.

In a preliminary communication, dealing with the blood volume and the total amount of agglutinin which could be recovered from immunised animals, we expressed the view *that the blood volume of normal healthy rabbits was a function of the surface and not of the body weight*, that is to say that with an increase of the body weight there is a decrease in the percentage amount of blood per kilo, and expressed it in the formula

$$B = W^{\frac{2}{3}}/k,$$

where B is the blood volume in cubic centimetres, W the weight of the animal in grammes, and k a constant calculated from the experiments.

The present series of experiments were undertaken to prove that this statement is a general law as regards mammals, and to give accurate figures for the blood volumes of those animals which are mostly used in experimental work. The further discussion of previous workers' experiments will be undertaken later on in this paper, after having given the results of our own experiments. These deal with the determination of the blood volumes of rabbits, guinea-pigs, and mice.

All the animals used were strong and healthy, in absolutely good and fit condition, and of course never having been used previously for any kind of experiment whatever. They had been kept under as equal conditions as possible as regards food, etc.

Technique.—This varied with the animals used. For the *rabbits* we have made use of two separate and distinct methods, one involving the death of the animal (washing out), the other not interfering with the animal's life (bleeding).

(1) *By washing out:* A sample of blood was obtained from the marginal ear vein into a paraffined watch glass (about 0·4 c.c. of blood) and dilutions immediately made with freshly filtered distilled water, using an accurately calibrated 0·1 c.c. pipette divided into 100 parts. The diluted blood was at once placed in a cold chamber and in the dark.

The animal was then anæsthetised with ether, and a cannula inserted into the central end of the right jugular vein and another cannula into the central end of the left carotid. The operation was carried out through a single median incision involving the skin only, working after this with blunt instruments, thus avoiding even the slightest amount of hæmorrhage.

Through the cannula in the jugular was delivered oxygenated Locke's fluid at 37–38° C., care being taken to provide an air lock, to prevent bubbles of gas entering the animal's circulatory system.

After the insertion of the cannulæ, and of course before letting any fluid flow in, a second sample of blood was taken either from the ear vein or the carotid, and diluted in precisely the same manner and to the same extent as the first sample.

The blood was then allowed to escape into sterile flasks containing enough "Hirudin" (dry leech extract) to entirely prevent the slightest clotting. This is of the greatest importance, as even a small coagulum will remove a considerable amount of hæmoglobin from the blood.

The apparatus was so arranged that the inflow was at the same rate as the outflow, whilst every minute both inflow and outflow were cut off for from 10–15 seconds.

In this way it was found that the animal continued to respire until only about 2-4 per cent. of the hæmoglobin was left, and that the heart continued to beat until the washings became colourless or nearly so. We are disposed to think that by using Locke's fluid in this way the blood is more completely washed out than would be possible by any artificial circulation.

The washing was continued for from one to six hours after the outflow had become practically colourless. This last part of the washing became so very dilute that it did not represent more than a fraction of a cubic centimetre of original blood. After the washings were complete, the animals were subject to a careful *post-mortem* examination and were found in most cases to be totally devoid of intravascular hæmoglobin. In some cases the inferior vena cava contained a very dilute solution of blood, but upon removing this it was always found to contain less than 0·1 per cent. of the hæmoglobin of the original blood. For the sake of control, we have in some cases chopped up the carcase after washing (and of course after having removed the skin and alimentary canal) and extracted it with distilled water. The hæmoglobin thus recovered (which may contain some muscle hæmoglobin) represented barely 1 per cent. of the total blood recovered by washing out, and in some cases considerably less. The volume found by the washing out conducted in this manner, therefore, represents as nearly as possible a true estimate of the total quantity of blood originally present in the circulation. The amount of hæmoglobin recovered from the circulation, divided by the amount of hæmoglobin per cubic centimetre of the original blood, gives the blood volume in cubic centimetres.

(2) *By bleeding.*—This method has been previously used by a number of investigators (Hühnerfauth, Lyon, Bunsen, Pembrey and Gürber, Hall and Eubank, Koeppe, and Dawson). The results obtained by these workers vary greatly, and it was therefore of interest to see if it were possible, by minimising the many sources of error, to obtain accurate and reliable results. That this is possible is clearly seen by the results obtained by the two methods.

We proceeded as follows :—After having collected two samples of blood, 0·2 c.c. each, from the marginal ear vein the animal was bled an amount of blood averaging 20–25 per cent. of its total blood volume. The blood was received into "Hirudin" so that the total amount of hæmoglobin which it contained could be estimated, and thus it could be calculated, by comparison with the samples taken from the ear, how many cubic centimetres of original blood were actually removed. This is absolutely essential, as the animal during the bleeding draws upon its tissue fluids to a considerable extent, thus diluting the blood, so that if the above correction be not made, the quantity of blood removed would be considerably overestimated. The hæmoglobin content of the blood is determined 12, 24, 48, 72, and 96 hours after bleeding. These hours were chosen after we had ascertained by a number of preliminary experiments that the percentage hæmoglobin content of the blood reaches an equilibrium at 24–48 hours after bleeding, and usually remains at that point for another 24 hours. During this period no appreciable regeneration of hæmoglobin seems to take place.

From the corrected amount of blood originally removed, and from the fall in the hæmoglobin of the blood sample during the period of equilibrium, the blood volume is easily calculated, on the assumption, which is amply justified by the results, that the equilibrium volume is identical with the original blood volume.

In employing this method it is absolutely essential to use animals that have not been bled before.

Guinea-pigs and Mice.—The Welcker method was employed. The sample of original blood was taken from the heart after the animal had been anæsthetised, and diluted in the usual way. In the mice, the original sample contained, however, only 0·01 c.c. of blood. The animal was then bled to death into distilled water which contained some "Hirudin." The alimentary canal and skin were then carefully removed, and the muscles, heart, liver, spleen, etc., were finely clipped and soaked in distilled water for about a quarter of an hour. The first and second washings thus obtained were decanted off and were quite clear. Fresh distilled water was then added and the remains of the animal were cut into fine pieces and gently squeezed in a porcelain mortar with porcelain pestle. The tissues were *not* ground.

The fluid was then decanted off through fine linen. This third washing was always quite turbid, and was always kept distinct from the clear washings. As it was found impossible to render this turbid washing clear without losing a considerable amount of hæmoglobin, and as it is impossible accurately to compare clear and turbid solutions as regards colour strength, the standard used for estimating the amount of hæmoglobin in these turbid washings was always brought to the same degree of turbidity by the addition of a small quantity of pure olive oil and shaking.

We made preliminary control experiments to prove that this method would give reliable results. In all of these various methods, the accurate determination of the hæmoglobin is essential, and for this purpose we have invariably used a Meisling hæmoglobinometer. The extreme delicacy and accuracy of this instrument, described by a number of observers, we have amply confirmed.

We will first deal with the experiments upon rabbits made by ourselves and other observers, and finally give our results upon guinea-pigs and mice. In all tables the body weight of the animals is "Rohgewicht" in grammes, that is to say that the weight of the contents of the alimentary canal is not deducted. The blood volume is given in cubic centimetres. The hæmoglobin is always expressed as percentages of the amount normal in man (man = 100 per cent.).

In Table I are given the blood volumes found in 22 rabbits, varying in weight from 670 to 3250 grammes. In the experiments marked by an asterisk the blood volumes were estimated by following the fall in hæmoglobin after bleeding, in the others by washing out.

From this table it is evident that the ratio of blood volume to body weight decreases as the body weight of the animal increases. If we attempt to find the best mathematical expression for these experiments, it will be seen that the body weight to the nth power, divided by the blood volume, gives a constant, and it will be further seen that the power n is approximately two-thirds. As MEEH has found that the surface area of an animal is equal to a constant multiplied by the body weight to the two-third power, our formula actually shows that the ratio between the blood volume and the surface area is a constant or nearly so.

TABLE I.

No.	Sex.	Body weight. ("Rohgewicht.")	Hæmoglobin per cent. (Man = 100.)	Blood volume observed.	Blood constant, $k\ (= W^{\frac{2}{3}}/B)$.	Blood volume as percentage of body weight.
		grammes.		c.c.		
* 1	♀	670	53·5	47·3	1·62	7·06
* 2	♀	1230	52·9	65·0	1·77	5·28
* 3	♀	1450	53·4	86·8	1·48	5·99
4	♀	1510	52·7	74·0	1·78	4·90
5	♀	1700	60·7	90·5	1·57	5·32
6	♀	1840	52·7	93·7	1·61	5·09
7	♂	1900	50·3	89·0	1·72	4·68
8	♀	1920	48·3	97·4	1·59	5·07
9	♂	2240	59·7	98·0	1·75	4·38
*10	♀	2370	72·9	111·0	1·60	4·68
*11	♂	2400	76·4	123·5	1·45	5·14
12	♂	2400	74·1	128·0	1·40	5·33
*13	♂	2630	73·8	119·0	1·60	4·52
14	♀	2650	63·7	122·0	1·57	4·60
15	♂	2770	54·6	136·0	1·45	4·91
16	♂	2900	70·5	129·0	1·58	4·45
*17	♀	2900	73·9	130·0	1·56	4·48
*18	♀	2920	63·1	130·0	1·57	4·45
*19	♀	3050	68·0	133·0	1·58	4·36
20	♀	3100	55·9	138·7	1·53	4·47
21	♂	3150	54·9	142·0	1·51	4·51
*22	♀	3250	68·8	148·0	1·48	4·55
		Average . . .	61·6	—	1·58	4·92

Further, in Table I is calculated the "blood constant" $k = W^{\frac{2}{3}}/B$, which is the body weight in grammes to the two-third power divided by the blood volume in cubic centimetres; and in the next column the blood volume in cubic centimetres as percentage of the body weight in grammes. Comparing these two columns it is obvious that, while there is a more or less regular decrease in the ratio of blood volume to body weight, as the animals increase in weight, no such periodical change in the blood constant, k, takes place; the variations being only such as are caused by differences in the individual experiments, depending upon individual differences, experimental errors, the difficulty of getting an absolutely correct body weight, etc.

It is thus found that the average blood constant, k, is 1·58 for males and females taken together, and the average figure representing the blood as percentage of the body weight is 4·92. Whilst the greatest variation from the average value of k is 12·6 per cent., the greatest individual variation from the average percentage is 43·5 per cent.

If we consider the males and females separately, we find that the average value of

k for the 14 females is 1·59, and for the eight males 1·56, which of course means that in these experiments the males have slightly more blood than the females (about 2 per cent.). The average percentage of blood to body weight for the females is 5·02 per cent., and for the males 4·74 per cent., which is to say that the females should have about 6 per cent. more blood than the males. But that no stress can be laid upon this is proved by the fact that, as the average weight of the females is smaller than that of the males (females, 2183 grammes; males, 2549), the females should have according to our view, just as they appear to have when the blood volume is calculated as percentage of body weight, a greater percentage of blood.

Whether it is justifiable to draw from the limited number of experiments any definite conclusion as to differences of blood volume in males and females is doubtful, but if there be a difference it is certainly not a marked one, and accordingly at the present moment we prefer to treat males and females together. In saying this, we naturally exclude animals which are pregnant. It will also be seen that the individual difference from the average blood constant k is smaller than the individual difference in the percentage amount of hæmoglobin in animals of the same sex and very nearly of the same weight.

In Table II is calculated the blood volume from the average blood constant 1·58, and from the average blood percentage 4·92, and the percentage differences between the observed figures and those calculated in the two different ways.

It will be seen that if we consider the figures calculated from the formula $B = W^{\frac{2}{3}}/k$, the greatest individual deviations between the calculated and observed figures are $+$ 12·87 per cent. and $-$ 10·59 per cent.; and the average deviation for the individual experiments calculated in this way is 4·61 per cent. If, on the other hand, the experiments are calculated in the way which has been made use of by all previous observers, namely, that the blood volume equals the weight multiplied by the percentage of blood, the greatest individual deviations are $+$ 43·3 per cent. and $-$ 11·39 per cent., and the average deviation for the individual experiments calculated in this way is 9·11 per cent. From this there is no doubt that if the blood volumes are calculated according to our view, a much better representation of the experimental facts is given than if calculated as percentage of body weight, as has invariably been done hitherto.

Since, however, the difference in the percentage deviation between the values observed and the values calculated by the two methods will depend to a very large extent upon the size of the animals, there is really only one way to make clear the difference in the percentage amount of blood in animals of the same species of varying weight, and that is to group the animals of about the same weights together. If this be done, the individual variations will to a great extent disappear, and the actual differences between the various groups of weights will not be obscured.

TABLE II.

No.	Body weight. ("Rohgewicht.")	Blood volume observed.	Blood volume calculated. $B = W^{\frac{2}{3}}/k$. (k = 1·58.)	Difference between blood volume calculated and observed.	Blood volume calculated as per cent. (4·92) of body weight.	Difference between blood volume calculated and observed.
	grammes.	c.c.	c.c.	per cent.	c.c.	per cent.
* 1	670	47·3	48·4	2·27	33·0	43·33
* 2	1230	65·0	72·7	10·59	60·5	7·44
* 3	1450	86·8	81·0	7·16	71·3	21·74
4	1510	74·0	83·3	11·16	74·3	0·40
5	1700	90·5	90·1	0·44	83·6	8·25
6	1840	93·7	95·0	1·37	90·5	3·54
7	1900	89·0	97·1	8·34	93·5	4·81
8	1920	97·4	97·7	0·31	94·5	3·07
9	2240	98·0	108·3	9·51	110·2	11·07
*10	2370	111·0	112·5	1·33	116·6	4·80
*11	2400	123·5	113·4	8·91	118·1	4·57
12	2400	128·0	113·4	12·87	118·1	8·38
*13	2630	119·0	120·6	1·33	129·4	8·04
14	2650	122·0	121·2	0·66	130·4	6·44
15	2770	136·0	124·8	8·97	136·3	0·22
16	2900	129·0	128·7	0·23	142·7	9·60
*17	2900	130·0	128·7	1·01	142·7	8·90
*18	2920	130·0	129·2	0·62	143·7	9·53
*19	3050	133·0	133·1	0·08	150·1	11·39
20	3100	138·7	134·5	3·12	152·5	9·05
21	3150	142·0	136·0	4·41	155·0	8·39
*22	3250	148·0	138·8	6·63	159·9	7·44
			Average . . .	4·61	—	9·11

With this object Table III has been made. The animals are here divided into seven groups, varying in weight from 670 grammes in Group A to 3039 grammes in Group G, by averaging the weights and blood volumes of the animals in each group. The blood constant and the blood as percentage of the body weight are then calculated from these figures.

It is at once seen that, while the average blood constant for the groups is nearly the same as the average k for the individual experiments in Table I, viz., 1·58 and 1·59 (a difference of 0·63 per cent.), the average percentage varies considerably: 4·92 and 5·26 (a difference of 6·9 per cent.).

From Table III it is further seen that there is no marked periodical variation in the blood constant for each group, and that the largest individual variation from the average k is 5 per cent.; if, on the other hand, we consider the blood as percentage of body weight, we see that there is an absolutely regular decrease in the percentage (from 7·06 to 4·47) as the average weight of the group increases from 670 grammes to 3039 grammes, and that the greatest individual variation from the average is 34 per cent.

TABLE III.

Group.	Rabbits from Table I included in group.	Average body weight. ("Rohgewicht.")	Average blood volume observed.	Blood constant,* k (= $W^{\frac{2}{3}}/B$).	Blood volume* expressed as percentage of body weight.
		grammes.	c.c.		
A	1	670	47·3	1·62	7·06
B	2—3	1340	75·9	1·60	5·66
C	4—5	1605	82·3	1·67	5·13
D	6—8	1887	93·4	1·64	4·95
E	9—12	2353	115·1	1·54	4·89
F	13—15	2683	125·7	1·54	4·69
G	16—22	3039	135·8	1·55	4·47
			Average . . .	1·59	5·26

* The figures in these columns are calculated from the average body weight and the average blood volume of the group.

In Table IV is calculated the blood volume from the average blood constant k for the groups (= 1·59) and from the average percentage of the groups (= 5·26), and also the percentage differences between the observed figures and those calculated in the two different ways :—

TABLE IV.

Group.	Rabbits from Table I included in group.	Average body weight. ("Rohgewicht.")	Average blood volume observed.	Blood volume calculated. $B = W^{\frac{2}{3}}/k$. (Average k for the groups, 1·59.)	Difference between blood volume calculated and observed.	Blood volume calculated as percentage of body weight. (Average for the groups, 5·26.)	Difference between blood volume calculated and observed.
		grammes.	c.c.	c.c.	per cent.	c.c.	per cent.
A	1	670	47·3	48·1	1·66	35·2	34·38
B	2—3	1340	75·9	76·4	0·65	70·5	7·66
C	4—5	1605	82·3	86·2	4·52	84·4	2·49
D	6—8	1887	93·4	96·0	2·71	99·3	5·94
E	9—12	2353	115·1	111·2	3·51	123·7	6·95
F	13—15	2683	125·7	121·5	3·46	141·1	10·91
G	16—22	3039	135·8	132·0	2·88	159·9	15·07
				Average . . .	2·77	—	11·91

It is to be seen from this table that, considering the figures calculated by the formula, and according to our view, the blood volume is a surface function, that the greatest individual deviations are +4·52 per cent. and −2·71 per cent., and that the average deviation between the calculated and observed figures for all groups is 2·77 per cent. If we then consider the figures calculated for the blood volume as an average percentage of the body weight, it will be found that the greatest individual differences are −34·38 per cent. and +15·07 per cent., whilst the average deviation is 11·91 per cent. It is further obvious that, although the individual percentage deviations in the experiments calculated by our formula do not vary greatly, *inter se*, and not periodically, yet, in the figures calculated as percentage of the body weight, in the group of small-weight animals the observed value is much bigger than the calculated one, while in the heavy-weight group the observed figure is much smaller than that calculated. This must obviously be the case if our view holds good.

From what we have said, there is hence no doubt that our formula $B = W^{\frac{2}{3}}/k$ holds good for the blood volume of rabbits within the range of our experiments. That is, as we have said above, *that the blood volume of the rabbit is a function of the surface.*

At the same time, it will be seen from our experiments that it is absolutely unjustifiable to consider the blood volume as a function of the weight of the animal, since the body weight divided by the blood volume is never a constant for animals of different weights.

The importance of this is seen from the fact that, while a rabbit of 1340 grammes contains 5·7 per cent. of blood, that is 1/17·7 of its body weight, a rabbit of 3039 grammes only contains 4·5 per cent. of blood, or 1/22·4 of its body weight. If, now, one would judge from either of these animals the quantity of blood contained in the other, the error would be a very considerable one, as it would be calculated either that the smaller animal contained 60 c.c. instead of 76 c.c. (more than 25 per cent. too little), or that the larger animal contained 174 c.c. instead of 136 c.c. (about 22 per cent. too much).

Having now shown that the body weight to the two-third power, divided by the blood volume, is a constant, it is of interest to see how closely the two methods which we have employed for the determination of the blood volume agree. In Table V we have collected together the rabbits in which the blood volume was determined by washing out, and in Table VI the determinations by noting the fall in hæmoglobin after bleeding. The first group includes 12 experiments and the second 10.

In Table V the average blood constant k is found to be 1·59, and in Table VI 1·57, which is only a difference of 1·26 per cent. between the two methods. It is seen that the two methods, when properly carried out, give practically identical results. On the other hand, the difference present tends to show that the blood volume estimated by noting the fall of hæmoglobin is about 1 per cent. larger than that found by the washing-out method, which is, of course, what may be expected, owing to the fact that small but negligible traces of blood necessarily remain within the body in the latter method. It is clear that, if the bleeding method be carried out, paying due attention to all the minute sources of error, it can yield absolutely reliable results, and it is, moreover, a harmless method, which can be employed upon living animals. But, as we have mentioned previously, it is not permissible to use animals which have been bled before, as the method would then, in the majority of cases, give erroneous results.

TABLE V.

No.	Sex.	Body weight. ("Rohgewicht.")	Blood volume observed.	Blood constant, k (= $W^{\frac{2}{3}}/B$).
		grammes.	c.c.	
4	♀	1510	74·0	1·78
5	♀	1700	90·5	1·57
6	♀	1840	93·7	1·61
7	♂	1900	89·0	1·72
8	♀	1920	97·4	1·59
9	♂	2240	98·0	1·75
12	♂	2400	128·0	1·40
14	♀	2650	122·0	1·57
15	♂	2770	136·0	1·45
16	♂	2900	129·0	1·58
20	♀	3100	138·7	1·53
21	♂	3150	142·0	1·51
			Average . . .	1·59

TABLE VI.

No.	Sex.	Body weight. ("Rohgewicht.")	Blood volume observed.	Blood constant, k (= $W^{\frac{2}{3}}/B$).
		grammes.	c.c.	
1	♀	670	47·3	1·62
2	♀	1230	65·0	1·77
3	♀	1450	86·8	1·48
10	♀	2370	111·0	1·60
11	♂	2400	123·5	1·45
13	♂	2630	119·0	1·60
17	♀	2900	130·0	1·56
18	♀	2920	130·0	1·57
19	♀	3050	133·0	1·58
22	♀	3250	148·0	1·48
			Average . . .	1·57

Having dealt with our own experiments upon rabbits, it is of interest to consider previous workers' experiments upon this animal in the light of our own.

As we have mentioned before, the greater number of previous observers (WELCKER, Ranke, Malassez, Sherrington and Copeman, etc.) have found, as we have also done, that young and lighter animals have a relatively bigger blood volume than older and heavier animals of the same species, independently of the various methods used. Contrary to this are the results obtained by Douglas and Boycott and Douglas using the CO method. Although there are a great number of publications, it is only the quite classical experiments of Ranke, the experiments of Sherrington and Copeman, and the extensive researches of Douglas and Boycott and Douglas which are sufficiently numerous and cover a wide enough range of body weight to enable one to make use of them for calculation purposes, in accordance with our view.

We will first deal with the experiments of Ranke, then with those of Sherrington and Copeman, and finally with those of Douglas and Boycott and Douglas.

In Table VII are given the figures which Ranke arrived at in the determination of the blood volume of 15 rabbits by the Welcker method. The individual animals vary in weight from 300 to 1700 grammes. The blood volume is given in cubic centimetres, which we have arrived at by taking Ranke's figures in grammes and using 1·05 as the specific gravity of the blood. We do this to make the results readily comparable with our own. In the same table are calculated the blood constant *k* and the blood as percentage of body weight.

Table VII.

No.	Body weight. ("Rohgewicht.")	Blood volume observed.	Blood constant, k (= $W^{\frac{2}{3}}/B.$)	Blood volume expressed as percentage of body weight.
	grammes.	c.c.		
1	307·5	17·2	2·64	5·60
2	399·0	18·8	2·88	4·71
3	569·0	24·3	2·83	4·26
4	631·0	28·7	2·57	4·54
5	743·0	31·5	2·60	4·24
6	773·0	43·3	1·95	5·60
7	799·0	40·8	2·11	5·11
8	851·0	39·0	2·30	4·58
9	1198·0	37·6	3·00	3·14
10	1582·0	46·5	2·92	2·94
11	1596·0	52·4	2·61	3·28
12	1664·0	69·3	2·03	4·16
13	1672·0	77·6	1·82	4·64
14	1719·0	49·8	2·88	2·90
15	1743·0	40·5	3·58	2·32
		Average . . .	2·58	4·13

It is at once evident that the individual blood constants show no periodical deviation from the average *k*, and that the ratio of blood to body weight is more or less regularly decreasing as the body weight increases, which is quite in conformity with what has been seen from our own experiments. On the other hand, the average blood constant, 2·58, is very much bigger than our own average k, 1·58, which is to say that the *absolute* blood volume found for rabbits by RANKE is about 40 per cent. less than what it actually should be. What this difference is due to in these otherwise accurate experiments is difficult to say, but it is not unlikely that a great amount of hæmoglobin has been removed by filtering the turbid fluids in attempting to make them clear.

Control experiments of our own have shown us that a turbid fluid cleared by centrifugalisation could lose even up to 40–50 per cent. of its original hæmoglobin, this being absorbed by the suspended matter.

In Table VIII we have divided RANKE'S experiments into four groups by averaging the weights and blood volumes of animals of somewhat the same weight, covering a range from about 350 to 1700 grammes.

TABLE VIII.

Group.	Rabbits from Table VII included in group.	Average body weight. (" Rohgewicht.")	Average blood volume observed.	Blood constant,* k (= $W^{\frac{2}{3}}/B$).	Blood volume* expressed as percentage of body weight.
		grammes.	c.c.		
A	1—2	353·3	18·0	2·78	5·09
B	3—6	679·0	31·9	2·42	4·70
C	7—9	949·0	39·1	2·47	4·12
D	10—15	1663·0	56·0	2·51	3·37
			Average . . .	2·55	4·32

* The figures in these columns are calculated from the average body weight and the average blood volume of the group.

The average blood constant of the groups is here 2·55, and the average blood percentage is 4·32. Just as in our own experiments, there is no periodical deviation in the size of the blood constants of the individual groups from the average blood constant of the groups, but there is a regular fall in the blood as percentage of body weight from about 5·1 to 3·4 as the animals increase in size, thus proving that RANKE was absolutely justified in his conclusion that animals of smaller weight contained relatively more blood than heavier animals of the same species.

In Table IX is calculated the blood volume from the average blood constant k for the groups (2·55), and from the average blood percentage of the groups (4·32 per cent.). The percentage deviations from the observed figures are given in each case, as with our own experiments.

TABLE IX.

Group.	Rabbits from Table VII included in group.	Average body weight. ("Rohgewicht.")	Average blood volume observed.	Blood volume calculated. $B = W^{\frac{2}{3}}/k$. (Average k for the groups, 2·55.)	Difference between blood volume calculated and observed.	Blood volume calculated as percentage of body weight. (Average for the groups, 4·32.)	Difference between blood volume calculated and observed.
		grammes.	c.c.	c.c.	per cent.	c.c.	per cent.
A	1—2	353·3	18·0	19·6	8·89	15·3	17·65
B	3—6	679·0	31·9	30·3	5·28	29·3	8·87
C	7—9	949·0	39·1	37·9	3·17	41·0	4·63
D	10—15	1663·0	56·0	55·0	1·82	71·8	22·01
				Average . . .	4·79	—	13·29

From this table it is seen, in as convincing a manner as from our own results, that our formula, $B = W^{\frac{2}{3}}/k$, represents the experimental facts in a very satisfactory manner, while the same facts are quite distorted if we look upon the blood volume as a function of the body weight. In the one case the average deviation between the observed and calculated figures is 4·79 per cent., and in the other about 13·3 per cent.

In Tables X and XI we give the results obtained by SHERRINGTON and COPEMAN on rabbits, by intravenous injection of a known quantity of normal saline solution, and determining the change produced in the specific gravity of the blood.

TABLE X.

No.	Body weight. ("Rohgewicht.")	Blood volume observed.	Blood constant, k $(= W^{\frac{2}{3}}/B)$.	Blood volume expressed as percentage of body weight.
	grammes.	c.c.		
1	1061	166·6	0·62	15·70
2	1871	135·0	1·12	7·22
3	2041	186·0	0·86	9·11
4	2289	150·0	1·16	6·55
5	2319	139·0	1·26	5·99
6	2884	180·0	1·13	6·24
7	3478	218·0	1·05	6·26
8	3572	261·0	0·89	7·31
		Average . . .	1·01	8·05

Here again it is seen that as the animals increase in size the ratio of blood to body weight decreases, while the blood constant only varies irregularly, depending upon the experimental errors, etc. The average *k* found in these experiments, calculated after our formula, is 1·01, and the average percentage 8·05. As the variation between the individual experiments is rather great, we have divided the experiments into only two groups, as shown in Table XI.

TABLE XI.

Group.	Rabbits from Table X included in group.	Average body weight. ("Rohgewicht.")	Average blood volume observed.	Blood constant,* k ($= W^{\frac{2}{3}}/B$).	Blood volume* expressed as percentage of body weight.
		grammes.	c.c.		
A	1—5	1916	155·3	0·99	8·11
B	6—8	3311	219·7	1·01	6·64
			Average . . .	1·00	7·38

* The figures in these columns are calculated from the average body weight and the average blood volume of the group.

Group A contains five animals of an average weight of 1916 grammes and Group B three animals of an average weight of 3311. The calculated average *k* of the groups is 1, while the average percentage is 7·38.

Here, again, it is seen that the group of animals of smaller weight, compared with the group of animals of heavier weight, gives practically the same constant *k*, whilst the ratio of blood to body weight decreases from 8·11 to 6·64 per cent. as the weight of the group increases. If calculated according to our formula the average deviation between calculated and observed figures is under 1 per cent., but the average deviation is nearly 10 per cent. if the blood volume is calculated as percentage of the body weight (see Table XII).

TABLE XII.

Group.	Rabbits from Table X included in group.	Average body weight. ("Rohgewicht.")	Average blood volume observed.	Blood volume calculated. $B = W^{\frac{2}{3}}/k$. (Average k for the groups, 1·00.)	Difference between blood volume calculated and observed.	Blood volume calculated as percentage of body weight. (Average for the groups, 7·38.)	Difference between blood volume calculated and observed.
		grammes.	c.c.	c.c.	per cent.	c.c.	per cent.
A	1—5	1916	155·3	154·2	0·71	141·4	9·83
B	6—8	3311	219·7	222·0	1·04	244·4	10·11
				Average . . .	0·88	—	9·97

It is thus seen that although SHERRINGTON and COPEMAN find a much greater absolute amount of blood for a given weight of rabbit, their results show clearly that small animals have relatively more blood than larger ones of the same species. It is hence seen that although the absolute blood volume obtained by these observers is *greater* than it should be for a given weight of animal, our formula holds good here as well as in the case of RANKE'S experiments, in which the absolute blood volume found was much *smaller* than it should have been.

It is quite natural, and *a priori* to be expected, that SHERRINGTON and COPEMAN should find blood volumes that are too large, considering the method they have employed. We know that even an isotonic solution of NaCl injected intravenously will disappear extremely rapidly, and this would clearly lead to an over-estimation of the blood volume. Further, as the rate of disappearance of the injected fluid varies in different animals, especially at the beginning of the injection, large individual variations are to be expected.

We will now pass on to the experiments of DOUGLAS and of BOYCOTT and DOUGLAS.

TABLE XIII.

No.	Sex.	Body weight. ("Rohgewicht.")	Blood volume observed.	Blood constant, k ($= W^{\frac{2}{3}}/B$).	Blood volume expressed as percentage of body weight.
		grammes.	c.c.		
* 1	♀	1608	92·4	1·48	5·75
* 2	♂	1704	71·6	1·99	4·20
* 3	♀	1815	68·5	2·17	3·77
4	♀	2166	99·0	1·69	4·57
5	♂	2184	116·0	1·45	5·31
* 6	♂	2210	103·1	1·65	4·67
* 7	♂	2320	141·2	1·24	6·09
8	♀	2453	112·0	1·62	4·57
9	♂	2470	171·0	1·07	6·92
10	♂	2625	147·5	1·29	5·62
11	♂	2665	149·5	1·29	5·61
*12	♂	2760	125·8	1·49	4·56
*13	♂	2760	128·2	1·46	4·64
*14	♂	2780	125·2	1·58	4·50
*15	♀	2860	156·8	1·28	5·48
16	♀	2940	177·0	1·16	6·02
17	♀	3172	183·0	1·18	5·77
18	♀	3187	131·0	1·65	4·11
19	♀	3210	176·0	1·24	5·48
*20	♂	3330	176·7	1·26	5·31
21	♀	3350	161·0	1·39	4·81
*22	♀	3380	212·2	1·06	6·28
23	♀	3515	175·0	1·32	4·98
24	♀	3600	212·0	1·11	5·89
25	♀	3626	187·0	1·26	5·16
26	♀	3666	258·0	0·92	7·04
			Average . . .	1·40	5·27

In Table XIII we have arranged the experiments collected from their papers according to the weights of the animals. The experiments of DOUGLAS are marked with an asterisk and are taken from Table III, p. 503, of his paper.* The other experiments are taken from the table in BOYCOTT and DOUGLAS's joint paper, p. 269.*

If we first consider the question of the sex it will be seen that no definite conclusions can be drawn as to the differences in the relative blood volume of males and females.

TABLE XIV.

	Sex.	Number of rabbits of each sex.	Average body weight. ("Rohgewicht.")	Average blood volume observed.	Average blood constant k for each sex.	Average blood volume expressed as percentage of body weight for each sex.
			grammes.	c.c.		
Douglas	♀	4	2416	132·5	1·50	5·32
	♂	7	2552	126·0	1·52	4·85
Boycott and Douglas .	♂	3	2491	137·7	1·34	5·51
	♀	12	3113	170·1	1·30	5·44

As in DOUGLAS's experiments taken alone, there is no appreciable difference, if the experiments are calculated according to our view—females 1 per cent. more (see Table XIV)—although as percentage of body weight the females have 9 per cent. more. If we look at BOYCOTT and DOUGLAS's experiments (Table XIV) there is a slightly greater difference when calculated in accordance with our view—females 3 per cent. more than males—but as percentage of body weight the females contain about 1 per cent. less blood than the males, which is just the reverse of what is to be seen from DOUGLAS's experiments. Calculated according to our view, however, both sets of experiments agree in that females if anything contain a slightly greater blood volume than males—1-3 per cent. But as our own experiments calculated according to our formula show, if any difference, a slightly larger blood volume for the males (see Table I)—under 2 per cent.—it seems fairly obvious that we are justified in disregarding for the present, as we have done in the case of our own experiments, the difference due to sex.

From Table XIII it will be seen that as the weight of the animal increases the ratio of blood to body weight increases also, more or less regularly. To make this obvious, Table XV has been made, where the 26 animals are divided into three groups of as nearly as possible the same number of experiments in each (9, 9 and 8), giving average weights with nearly the same absolute differences.

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* *Vide* References at end.

TABLE XV.

Group.	Rabbits from Table XIII in each group.	Average body weight. ("Rohgewicht.")	Average blood volume observed.	Average blood constant k for each group.	Average blood volume expressed as percentage of body weight for each group.
		grammes.	c.c.		
A	1—9	2103	108·3	1·60	5·09
B	10—18	2861	147·1	1·38	5·15
C	19—26	3460	194·7	1·20	5·62

From this table it is obvious that as the animals increase in weight they have a greater and greater percentage of blood. This difference is so regular and marked that it cannot be disregarded, whether calculated according to our formula or as percentage of body weight. If calculated as indicated by us the Group B, weighing 2861 grammes, will contain relatively 16 per cent. more blood than Group A, the average weight of which is 2103 grammes, and Group C, of an average weight of 3460 grammes, about 33 per cent. more blood than Group A. If calculated on the other hand according to percentage of weight, the differences are as regular and increase with increase of body weight. Group B, weighing 2861 grammes, contains over 1 per cent. more blood than Group A, of 2103 grammes, whilst Group C, weighing 3460 grammes, contains more than 10 per cent. more than Group A.

These results are of the greatest importance and interest, as they are so absolutely contrary to the results obtained by RANKE, WELCKER, SHERRINGTON and COPEMAN, etc., and ourselves, and are therefore of much value in throwing light upon the CO method of determining the blood volume.

If we turn our attention to Group A in Table XV, containing nine animals, four males and five females, whose average weight is 2103 grammes, it will be seen that the average blood constant is 1·60, which falls as near as possible to our own average blood constant 1·58, giving a blood content less than 1·5 per cent. smaller than we have given, and very much the same result as DOUGLAS finds by the Welcker method, where the average k is 1·66, for the four out of the five experiments for which one is able to trace the weights and blood volumes of the animals.

When calculated as percentage of body weight our experiments give 4·9 per cent. and the CO method 5·09, that is, the latter gives less than 3 per cent. more blood than is found in our own experiments. For animals up to this weight, therefore, the CO method, taking a large number of experiments, seems to give results that fall very close to the results as obtained by us.

If we then, on the other hand, consider Group B in Table XV, containing four females and five males, whose average weight is 2861, it will be seen that the blood constant here is considerably smaller than our own—1·38, as against 1·58—which is to say that the average blood volume of this group is more than 14 per cent. larger than the corresponding one found by us for animals of the same weight if calculated according to our formula. If we finally consider Group C in Table XV, of an average weight of 3460 grammes, and containing seven females and one male, we find an average blood constant k of 1·20, as compared with 1·58 in our own experiments, which is to say that the CO method in this case gives a blood volume more than 31 per cent. greater than it should be for animals of this weight if calculated according to our formula.

In the light of what has been said, and independently of whether the experiments are calculated according to our formula $B = W^{\frac{2}{3}}/k$, or as percentage of the body weight, it is evident that while the CO method, in smaller animals of about 2000 grammes in weight, gives approximately the same results as we have found, there is a definite variation as the animals increase in size, a variation which can only be accounted for by the fact that there must be substances in the organism other than the circulating hæmoglobin which have the power of combining with CO, and which are present in a quantity that increases not only absolutely but relatively as the animals increase in weight, which will in normal animals mean increase in age also.

This being the case, it is clear that, as we have already stated in our preliminary communication, great caution must be exercised both as to the results obtained by the CO method, and the value of the deductions based upon them.

On the other hand, the method might be useful to show the difference between the amount of circulating hæmoglobin, and of the extravascular hæmoglobin or substances behaving like hæmoglobin in its avidity for CO. It is impossible to express any certain opinion upon this matter until definite experiments have been carried out for its elucidation.

If we now ask how the differences which we have just described are to be accounted for, we turn naturally first of all to the muscle. It is now known that, as animals of the same species increase in weight, *i.e.*, become older, the ratio of muscle to body weight increases more than proportionately (Ranke), and it has long been known that the muscles of old animals contain relatively more muscle hæmoglobin than the muscles of younger ones. Lehmann in his experiments upon rabbits, for instance, has found that the dark muscles of the hind leg have, in an old rabbit, slightly less than 2½ times as much hæmoglobin as in young rabbits of about 1000–1500 grammes. This, of course, fits in very well with what we know from the difference between veal and beef, where the same observer finds that the muscles of grown-up cattle contain 2½–3 times more hæmoglobin than the muscles of young cattle, having examined the muscles of the skin, the chewing muscles, the biceps, those in the lumbar region, the heart, and the diaphragm.

This may to a certain extent account for the results obtained by Boycott and Douglas by the CO method, according to which as the animals increase in size an increasingly greater relative amount of blood is found.

At the present moment we are not prepared to express an opinion how far the muscle hæmoglobin will explain the discrepancies noted above, but as far as can be seen it appears likely that other substances with the power of fixing CO must be present besides the muscle hæmoglobin, in a relatively larger quantity in the older and heavier animals than in the younger and lighter animals of the same species.

Having now dealt with the blood volume of rabbits, we pass on to our *experiments upon guinea-pigs and mice.*

TABLE XVI.

No.	Sex.	Body weight. (" Rohgewicht.")	Hæmoglobin per cent. (Man = 100.)	Blood volume observed.	Blood constant, k (= $W^{\frac{2}{3}}/B.$)	Blood volume expressed as percentage of body weight.
		grammes.		c.c.		
1	♂	215	85·1	11·12	3·22	5·17
2	—	275	73·0	11·75	3·60	4·27
3	—	365	84·1	16·20	3·15	4·44
4	♂	365	87·0	15·61	3·27	4·28
5	♀	385	86·3	15·46	3·42	4·02
6	♂	415	86·1	15·60	3·56	3·76
7	—	565	82·3	22·00	3·10	3·89
8	♂	565	87·1	21·82	3·13	3·79
9	♂	825	92·6	27·35	3·22	3·32
		Average . . .	84·8	—	3·30	4·10

In Table XVI are given the experimental results of the determination of the blood volume of nine guinea-pigs of weights ranging from 215 to 825 grammes "Rohgewicht," as well as the values for the blood constant, and the blood as percentage of body weight.

It is seen that, while the blood constant k exhibits only relatively small deviations, without any periodicity, the percentage amount of blood regularly decreases from about 5·2 to 3·3 per cent. as the body weight increases. The average value of k is 3·30, and the average percentage 4·1.

From Table XVII it is seen that while the maximum individual deviation by calculating from our formula is 8·2 per cent., the maximum deviation if the blood volume be calculated as percentage of body weight is 26 per cent., and while the average deviation in the first case is 4·57 per cent., in the latter it is 9·26 per cent.

In Tables XVIII and XIX the animals are grouped together in four groups according to their weights, and the blood volumes are calculated from the average value of k for the groups, 3·29, and the average percentage of the groups, 4·02 (see Table XVIII). Although the greatest individual difference between observed and calculated values, calculated according to our view, is under 4 per cent., the greatest individual variation, when calculated as percentage of body weight, is more than

Table XVII.

No.	Body weight. ("Rohgewicht.")	Blood volume observed.	Blood volume calculated. $B = W^{\frac{2}{3}}/k$. ($k = 3{\cdot}30$).	Difference between blood volume calculated and observed.	Blood volume calculated as per cent. (4·10) of body weight.	Difference between blood volume calculated and observed.
	grammes.	c.c.	c.c.	per cent.	c.c.	per cent.
1	215	11·12	10·88	2·21	8·82	26·08
2	275	11·75	12·80	8·20	11·28	4·17
3	365	16·20	15·50	4·52	14·97	8·22
4	365	15·61	15·50	0·71	14·97	4·28
5	385	15·46	16·04	3·62	15·79	2·09
6	415	15·60	16·87	7·53	17·02	8·34
7	565	22·00	20·70	6·28	23·17	5·28
8	565	21·82	20·70	5·41	23·17	5·83
9	825	27·35	26·65	2·63	33·83	19·15
Average . . .				4·57	—	9·27

Table XVIII.

Group.	Guinea-pigs from Table XVI included in group.	Average body weight. ("Rohgewicht.")	Average blood volume observed.	Blood constant,* k ($= W^{\frac{2}{3}}/B$).	Blood volume* expressed as percentage of body weight.
		grammes.	c.c.		c.c.
A	1—2	245·0	11·44	3·42	4·67
B	3—5	371·7	15·76	3·28	4·24
C	6—8	515·0	19·81	3·24	3·85
D	9	825·0	27·35	3·22	3·32
Average . . .				3·29	4·02

* The figures in these columns are calculated from the average body weight and the average blood volume of the group.

17 per cent., and although the average deviation in the first case is under 2 per cent., in the second it is more than 10 per cent. (see Table XIX).

Our experiments upon *guinea-pigs* therefore prove, as clearly as do the rabbits, that our formula $B = W^{\frac{2}{3}}/k$, indicating that the *blood volume is a surface function*, represents the experimental facts in an extremely satisfactory manner. At the same time, it is obvious that it would be quite erroneous to attempt to express the blood volume for a given species as percentage of the body weight, as has hitherto been usual.

TABLE XIX.

Group.	Guinea-pigs from Table XVI included in group.	Average body weight. ("Rohgewicht.")	Average blood volume observed.	Blood volume calculated, $B = W^{\frac{2}{3}}/k$. (Average k for the groups, 3·29.)	Difference between blood volume calculated and observed.	Blood volume calculated as percentage of body weight. (Average for the groups, 4·02).	Difference between blood volume calculated and observed.
		grammes.	c.c.	c.c.	per cent.	c.c.	per cent.
A	1—2	245·0	11·44	11·91	3·95	9·85	16·14
B	3—5	371·7	15·76	15·77	0·06	14·94	5·49
C	6—8	515·0	19·81	19·53	1·43	20·70	4·30
D	9	825·0	27·35	26·75	2·24	33·17	17·55
				Average . . .	1·92	—	10·87

In Tables XX, XXI, and XXII we have tabulated the results of our experiments upon the blood volume of *mice.*

TABLE XX.

No.	Body weight ("Rohgewicht.")	Hæmoglobin per cent. (Man = 100.)	Blood volume observed.	Blood constant, k (= $W^{\frac{2}{3}}/B$).	Blood volume expressed as percentage of body weight.
	grammes.		c.c.		
1	11·90	83·5	0·740	7·04	6·22
2	11·90	90·8	0·713	7·30	5·99
3	12·30	83·1	0·757	7·03	6·15
4	12·80	85·6	0·787	6·94	6·15
5	14·25	81·6	0·998	5·88	7·00
6	14·50	100·5	0·851	6·98	5·87
7	15·15	81·4	0·912	6·70	6·02
8	15·50	93·6	0·958	6·49	6·18
9	16·45	99·5	0·900	7·19	5·47
10	17·70	81·4	1·066	6·37	6·02
11	18·00	85·2	1·143	6·00	6·35
12	18·70	90·1	1·016	6·93	5·43
13	19·45	93·6	1·073	6·73	5·52
14	21·30	99·1	0·973	7·88	4·57
15	22·65	77·6	1·470	5·44	6·49
16	23·80	90·6	1·240	6·67	5·21
17	24·50	85·8	1·135	7·43	4·63
18	25·70	91·4	1·397	6·23	5·44
19	29·35	87·5	1·432	6·63	4·88
	Average . . .	88·5	—	6·70	5·77

Table XX shows the results of the determination of the blood volume of 19 mice, varying in weight from about 12 to 30 grammes "Rohgewicht." The average blood constant is 6·70, and the average percentage of blood to body weight is 5·77.

While the blood constant k, as in the experiments upon the rabbits and guinea-pigs, shows no periodical variation, the ratio of blood volume to the body weight decreases as the animals increase in weight.

TABLE XXI.

Group.	Mice from Table XX included in group.	Average body weight. ("Rohgewicht.")	Average blood volume observed.	Blood constant,* k (= $W^{\frac{2}{3}}/B$).	Blood volume* expressed as percentage of body weight.
		grammes.	c.c.		
A	1—6	12·94	0·808	6·82	6·24
B	7—10	16·20	0·959	6·68	5·92
C	11—15	20·02	1·135	6·50	5·67
D	16—19	25·84	1·301	6·71	5·03
			Average . . .	6·68	5·72

* The figures in these columns are calculated from the average body weight and the average blood volume of the group.

In Table XXI the experiments are arranged in four groups according to the size of the animals, averaging the weights and the blood volumes for the animals in each group. From the average weights and blood volumes are then calculated in the usual way the blood constant for each group, and the blood as percentage of body weight, giving an average blood constant of 6·68, and an average percentage of 5·72, which is seen to be practically the same as the average for the individual experiments.

In Table XXII is calculated the blood volume from the average blood constant of the groups (6·68), and from the average percentage (5·72) of the groups, and the percentage deviation between the calculated and observed values.

TABLE XXII.

Group.	Mice from Table XX included in group.	Average body weight. ("Rohgewicht.")	Average blood volume observed.	Blood volume calculated, $B = W^{\frac{2}{3}}/k$. (Average k for the groups, 6·68.)	Difference between blood volume calculated and observed.	Blood volume calculated as percentage of body weight. (Average for the groups, 5·72.)	Difference between blood volume calculated and observed.
		grammes.	c.c.	c.c.	per cent.	c.c.	per cent.
A	1—6	12·94	0·808	0·825	2·06	0·7402	9·16
B	7—10	16·20	0·959	0·959	0·00	0·9266	3·50
C	11—15	20·02	1·135	1·104	2·81	1·1451	0·88
D	16—19	25·84	1·301	1·308	0·54	1·4780	11·98
				Average . . .	1·35	—	6·38

From this table it is seen that, whilst the greatest deviation between the observed and calculated figures is under 3 per cent., calculated according to our formula, when calculated as percentage of body weight the maximum deviation is about 12 per cent., and that while the average deviation in the first case is only about 1·4 per cent., it is in the latter 6·4 per cent.

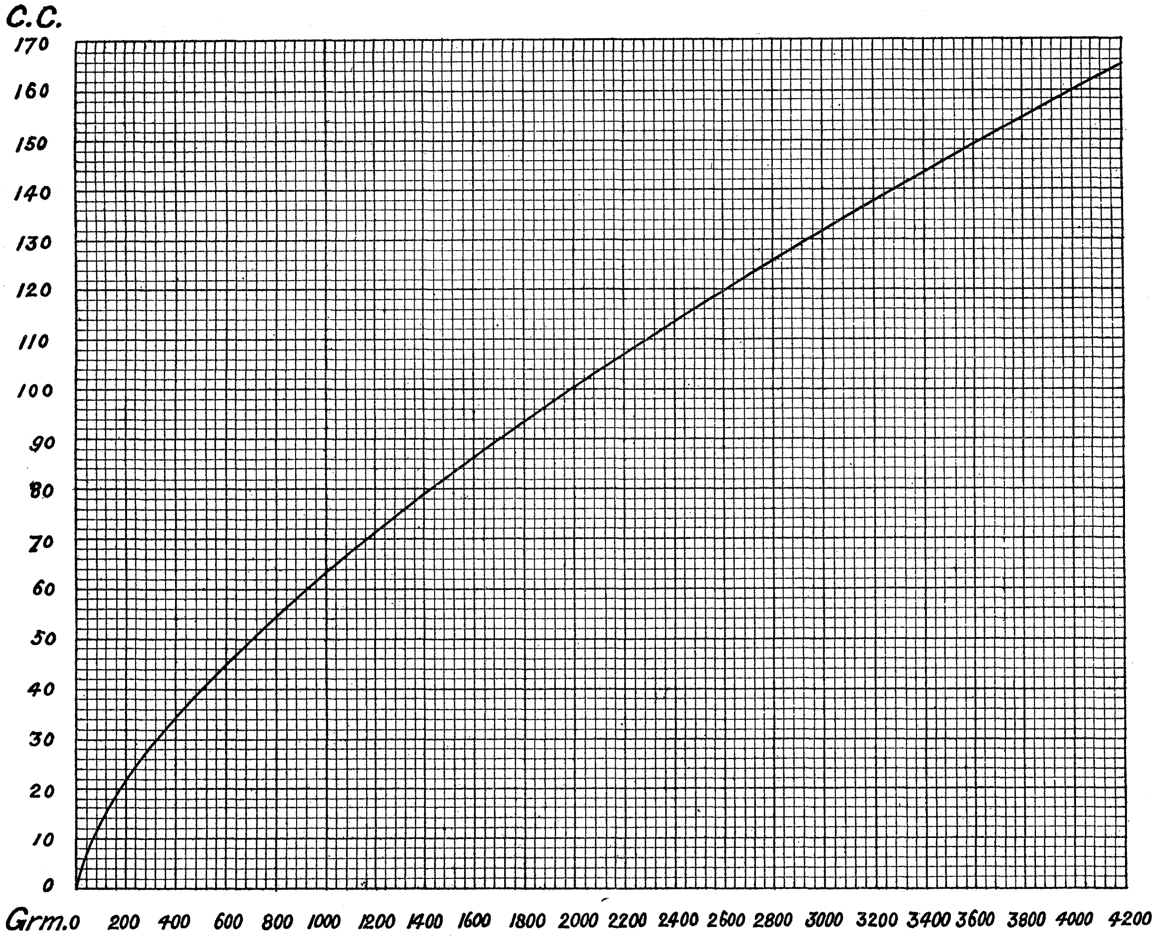

DIAGRAM A.—Blood Volume of Rabbit.

There is therefore no doubt that, in the case of the *mouse* also, the smaller individuals, having a relatively larger surface, contain relatively more blood than heavier individuals having a smaller relative surface, and that *our formula* $B = W^{\frac{2}{3}}/k$ *holds good* here as well as in the case of rabbits and guinea-pigs, and represents the experimental facts in an extremely satisfactory way, while the same facts, as we have insisted before, are absolutely distorted if the blood volume be expressed in the usual manner as percentage of body weight.

Before summarising the conclusions which we have drawn concerning the blood volumes of mammals, we wish to draw attention to a fact which is of interest from a theoretical point of view, namely, that although our formula $B = W^{\frac{2}{3}}/k$ has been proved to hold good for the calculation of the blood volume of different species of animals (rabbit, guinea-pig, and mouse) over a very wide range of weight within each species, a point appears to be reached when the individuals are very young and light, where a slight periodical deviation commences, in exactly the same way as is the case if the surface of the same individuals be calculated from the body weight according to MEEH's formula ($S = kW^{\frac{2}{3}}$).

This deviation is in both cases due to the fact that the power $\frac{2}{3}$ is slightly too small. If it be taken a little larger, the small periodic deviation disappears both from MEEH's formula and from our own.

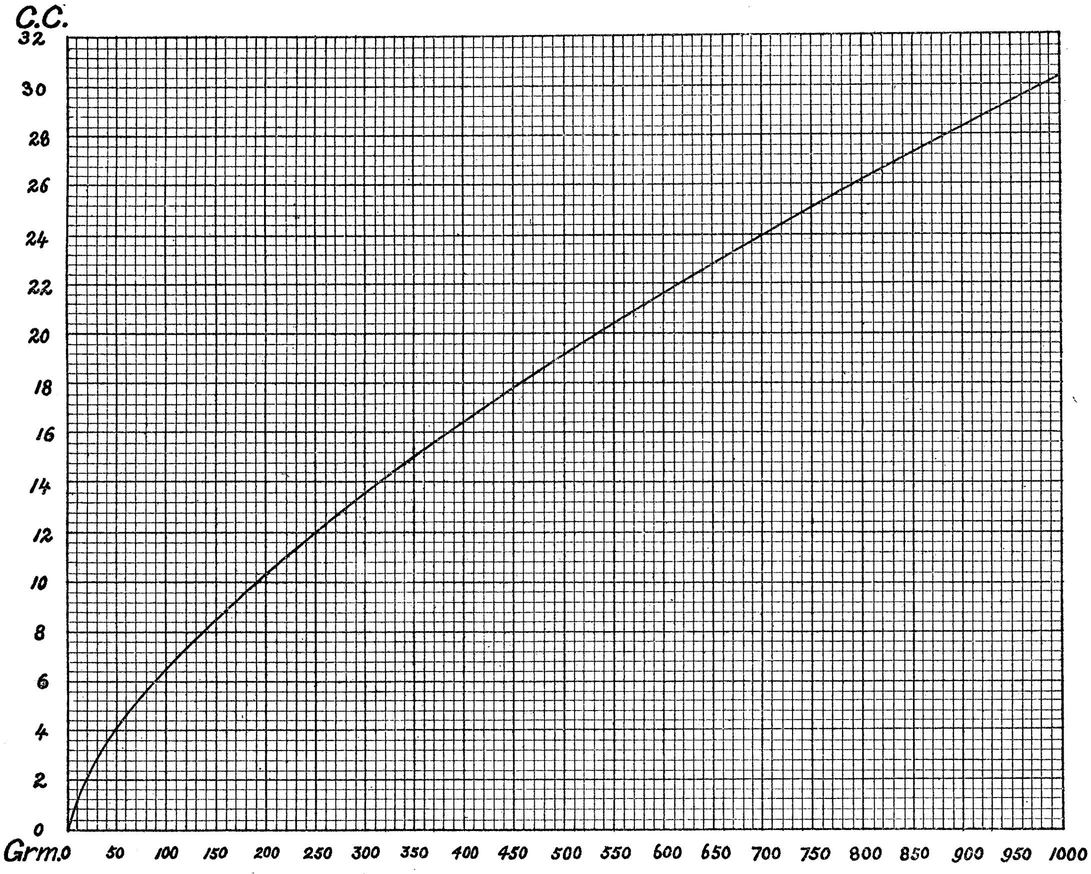

DIAGRAM B.—Blood Volume of Guinea-pig.

But in both cases the power $\frac{2}{3}$ is sufficiently accurate for all practical purposes, and, since it is more convenient to work with, we have chosen this value for n in our formula. Using this power ($\frac{2}{3}$) the small deviation referred to begins to be traceable in rabbits weighing less than about 500 grammes, in guinea-pigs below about 200 grammes, and in mice of less than about 8 grammes in weight.

In diagrams A, B, and C, the blood volume of the rabbit, guinea-pig and mouse is given graphically, as calculated according to our formula from the average constant for each species as determined from our experiments. The weights of the animals in grammes are plotted along the abscissa, and the corresponding blood volumes in cubic centimetres along the ordinate.

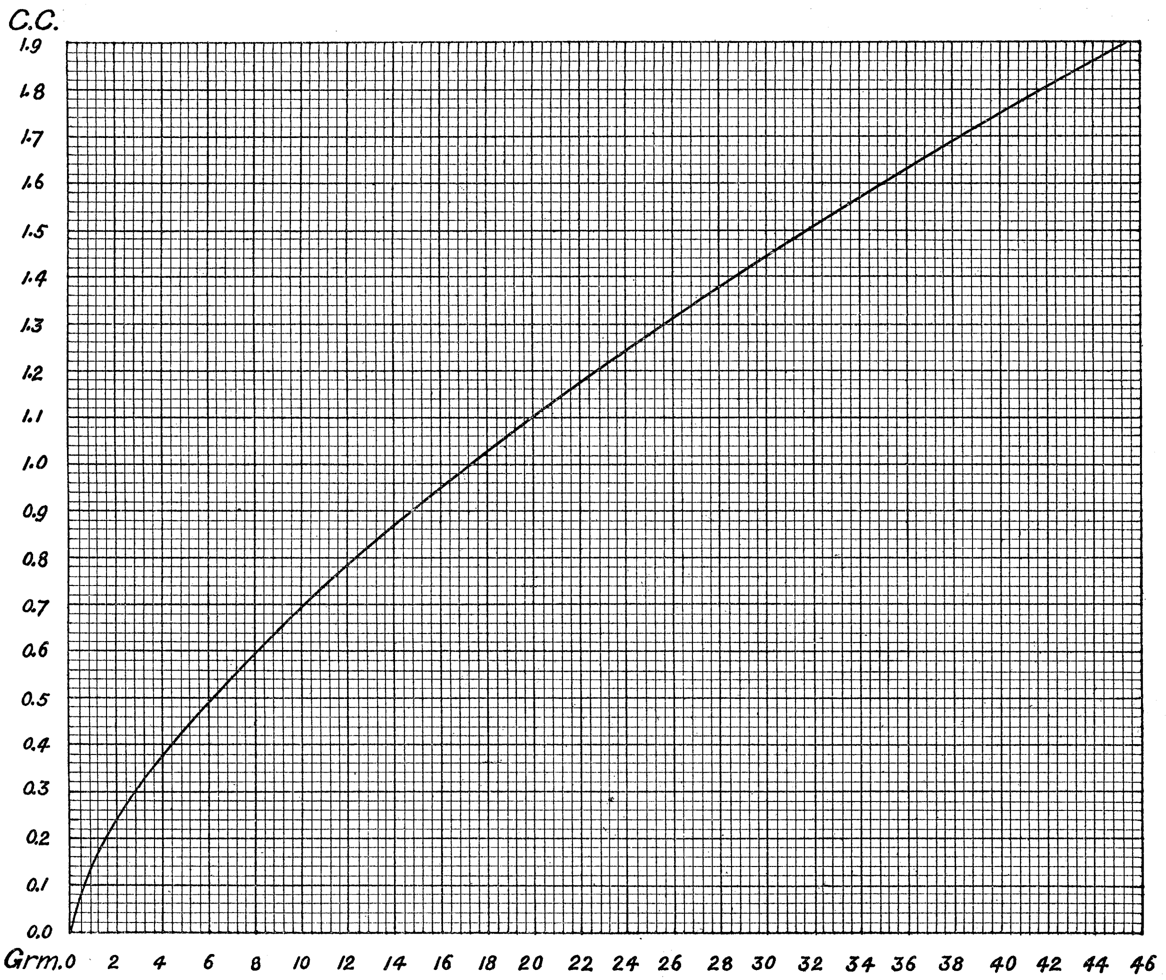

Diagram C.—Blood Volume of Mouse.

Summary.

I. The blood volume of living mammals can be determined with great accuracy by the method of bleeding and measuring the percentage fall of hæmoglobin which is thus produced.

This method when applied with the precautions which we have indicated gives results remarkably concordant with those obtained by washing out the circulation in the manner described above.

II. From our experiments carried out on *rabbits*, *guinea-pigs*, and *mice*, it is evident that the smaller and lighter animals have a relatively greater blood volume than heavier animals of the same species. This result is in full accordance with the numerous observations recorded by Ranke, Welcker, Malassez, Sherrington and Copeman and others, who made use of various different methods. It is absolutely at variance with the results obtained by Boycott and Douglas by the use of the CO method upon rabbits, according to which it would appear that the heavier animals have a relatively greater blood volume than the lighter ones.

III. In *normal* and *healthy* mammals, the blood volume is satisfactorily expressed by our formula $B = W^{\frac{2}{3}}/k$, where B is the blood volume in cubic centimetres, W the weight of the individual in grammes, and k a constant to be ascertained for each particular species of animal. This formula indicates that the smaller animals of any given species, which have a relatively greater body surface than heavier ones, have also a relatively greater blood volume. That is to say, that the blood volume can be expressed as a function of the surface area.

IV. The constant k by means of which the blood volume can at once be calculated from our formula ($B = W^{\frac{2}{3}}/k$) is for

Rabbit	1·58
Guinea-pig	3·30
Mouse	6·70

V. From II and III above, it will be apparent that the practice of expressing the blood volume as a percentage of the body weight is both erroneous and misleading. The ratio of the blood volume to the body weight in normal healthy mammals of any given species is not a constant, but we have here shown that its ratio to the body surface is approximately constant in animals of the same species.

VI. Since the results obtained in rabbits by the use of the CO method (by Douglas and Boycott) are entirely out of accord, not only with our own observations, but also with those recorded by previous workers on the blood volume, it would seem that the method in question must be liable to serious and at present unknown errors. We believe that the discrepancies noted are largely due to the extravascular hæmoglobin (muscle, bone-marrow, etc.) and probably also to other substances in the body which take up CO. These sources of error seem not to have been taken into due account in employing the CO method for the determination of the blood volume. They are, however, of very great importance, since it appears not only from the results obtained by Douglas and Boycott, using this method on rabbits, but also from observations by others on muscle hæmoglobin, that both the extravascular hæmoglobin and the other substances are present in increasing relative amounts as the animals become heavier and older.

Hence it is evident that in such individuals the figures obtained by the CO method for the blood volume must be too high, and increasingly so as the animals advance in weight and age.

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