THE INFLUENCE OF ANÆSTHESIA ON THE RESTORATION OF THE VOLUME OF THE BLOOD AFTER HÆMORRHAGE AND AFTER TRANSFUSION.*

By A. E. BOYCOTT and C. PRICE JONES.

From University College Hospital Medical School, London.

However greatly you may vary your experiments, you will not succeed in causing the quantity or composition of an animal's blood to continue abnormal for any length of time.—J. F. Cohnheim, Lectures on General Pathology, *New Syd. Soc.*, London, 1889, p. 415.

It is well known that in normal animals the volume of the blood is quickly restored after external hæmorrhage by the uptake of liquid from the tissue fluid. In the ordinary laboratory animals (rabbits, cats, dogs) this takes place quite quickly, liquid coming in during the first few minutes of the hæmorrhage and the process being generally complete in a few hours; in man it may take several days as far as the rather indifferent data go. The precision with which the normal volume is regained is such that Vierordt (1854[1]) proposed to use measured hæmorrhages as a means of estimating the quantity of blood in the intact body, pointing out that if a = red cells per unit volume before bleeding, and b = the lowest count of red cells obtained after bleeding (*i.e.*, the point of maximum dilution), and v = the quantity removed by bleeding, then the volume of blood would be $v \left(1 + \dfrac{b}{a-b}\right)$.

Good accounts of these earlier methods for estimating the blood volume will be found in Tarchanoff (1880-81[2]), Melassez (1874-75[3]), and Limbeck (1901[4]) : in the last the formula (p. 2) is misprinted $\dfrac{ac}{b-c}$ for $\dfrac{ab}{b-c}$, and there is another misprint of 6·0 for 6·6 in the illustrative experiment (p. 67).

Methods.

Rabbits are anæsthetised with ether, and 1·5 grm. per kilo of urethane in 50 per cent. solution is given at once subcutaneously and the necessary operation for taking blood from the carotid completed. No further ether is given and, kept on a water-bath operating table with a regulator, the animal remains in good condition for many hours. After the effect of the ether has passed off, the conjunctival reflex remains fairly or quite brisk, though it requires pretty severe stimulation of the skin to evoke any response : the depth of anæsthesia is

therefore only moderate. The amount of urethane is by itself enough to keep an intact animal very sleepy for about twenty hours, but never sufficiently anæsthetised for a cutting operation on the skin. After examining preliminary carotid samples, the animal is bled a known amount, and thereafter the progress of restoration is followed by sampling a few drops of blood from time to time. In each sample the red cells are counted and the hæmoglobin determined by a Haldane-Gowers instrument, the same instruments being used throughout the work.

The rabbits used have varied in weight from 2000 to 2850 grms. We have assumed that they had, before bleeding, 45 c.c. of blood per kilo crude body-weight. The data previously given (1912 [5]) show that this is a probable average figure and the variability of the blood volume being about 10, it follows that about 70 per cent. of normal rabbits of between 2 and 3 kilos would have a volume between 40 and 50 c.c. per kilo, and that not more than 5 per cent. of animals would have more than 54 or less than 36 c.c. The probability that this assumption is correct is increased if animals are used which have a fairly normal concentration of hæmoglobin. The average (1914 [6]) is 76 per cent. on the human scale, and we have rejected animals which by a preliminary examination had less than 65 per cent. As a matter of fact it makes no difference to our main results what assumption within reasonable limits is made as to the blood volume as any one may find who will try the calculations. The interpretation of these simple observations is best illustrated by an example. Weight 2250 grms. : blood volume 101 c.c. 10.13 A.M. ether and urethane. 10.45 A.M. : Hb 71 per cent. 11.5 A.M. : Hb 70 per cent. : mean value before bleeding 70·5 per cent. 11.12-11.16½ A.M. : bled from carotid into 10 c.c. oxalate solution to a total volume of 49 c.c. which gave Hb 50 per cent. = 39 c.c. of blood with $50 \times \frac{49}{39} = 63$ per cent. Hb = 35 c.c. of the blood before bleeding which had 70·5 per cent. Hb, *i.e.*, 35 per cent. of original blood was removed. If the blood volume were completely restored by the addition of tissue fluid, the hæmoglobin percentage would be reduced by 35 per cent. of the original value, *i.e.*, the hæmoglobin percentage in the blood would be $70·5 - \frac{35 \times 70·5}{100} = 46$ on the scale. During the four and a half minutes occupied by the bleeding, the hæmoglobin fell from 70·5 to 63, and of the liquid removed 35 c.c. was blood and 4 c.c. tissue fluid taken up while the hæmorrhage was going on. 11.16½ A.M. : Hb 62 per cent., at which point the volume of the blood, relative to the normal taken as 100, was $\frac{46}{62} = 74$ instead of 100 − 35 = 65. 11.32 A.M. : Hb 59 per cent. = relative volume 78. 12 A.M. : Hb 57 per cent. = relative volume 81. 12.30 P.M. : Hb 57. The red cell counts are dealt with in the same way. Thus in this case the normal figure was 5·173 millions per cubic millimetre : full restoration of volume would reduce this to $5·173 - \frac{35 \times 5·173}{100}$ = 3·362 : the lowest figure reached was 4·160, *i.e.*, relative volume $= \frac{3·362}{4·160} = 81$.

The correspondence between the hæmoglobin determinations and the red cell counts is not always as good as in this example. But we have met with no case where the two sets of data were grossly discrepant. We have generally used the hæmoglobin figures for the calculations, because it is simpler and because the determinations appear to have a rather smaller error than the counts. Thus a series of 100 consecutive observations on himself by Price Jones gives coefficients of variation of 3·7 for red cells and 2·1 for hæmoglobin. The colour index is liable to a considerable error of observation, and we should not regard an occasional or sporadic variation of necessary moment unless it was at least 0·1 : periodic differences of less than this would mean more. The several observers

(*e.g.* Scott (1917 [23]), Lamson (1915-16 [24])) who have commented on discrepancies between hæmoglobin figures and red cell counts appear to have expected too much from the methods. No one would be much surprised if, on making twenty sets of determinations on the same blood, the hæmoglobin figures varied from 98 to 102 and the counts from 4·8 to 5·2 millions, a variation which entails occasional colour indices as divergent as 0·94 and 1·06.

Our method of experiment is valid only if the samples of blood examined are representative of the general circulation and if the total quantity of red corpuscles in the circulation remains, except for abstractions or additions of measured amount, the same throughout the experiment. It has been suggested from time to time (*e.g.*, Hawk (1904 [25]), Schneider and Havens (1915 [12])) that these conditions are not always fulfilled, and that there are in the body accumulations of red cells which may under special conditions (*e.g.*, hæmorrhage) be brought into the circulation. The existence of some such depots seems necessary to explain the increase in the concentration of corpuscles which has been observed in the later stages of large interrupted bleedings in horses by Martin([7]) and Penfold (1920 [8]). But we know of no clear evidence that anything of the kind occurs in the smaller experimental animals, whose circulation may be presumed to work on a much more active plan than that of man and other large mammals. Scott (1917 [10]) and Lamson (1921 [26]) examined the point carefully and concluded that in cats and dogs the blood is homogeneous and that there is no evidence of any depots of red cells hidden in the organs. Boycott and Douglas (1909 [9]) found the blood in rabbits well mixed up and uniform. In the present experiments we have considered the colour index closely, because it is possible that reserve cells might differ in this respect from those already in the circulation, but we have found no indication of their entrance into the blood. We think, therefore, that our observations are satisfactory in these respects. Nor can we easily believe that Cohnheim (1889 [11]) is right when he suggests that blood obtained from the carotid is not representative of that in the vessels, because the specifically heavier red cells escape in larger numbers than would correspond to their proportions in the circulating blood.

In experiments without anæsthetics blood is taken from the ear or the heart. Even under favourable conditions and with a helpful subject a hæmorrhage from the ear of about two-fifths of the total blood occupies about quarter of an hour, and the precise rate is largely beyond control. For comparison with quick carotid bleedings we have therefore taken blood from the heart with a needle. The subsequent changes have been followed as in anæsthetised animals, the samples being taken either from the heart or the ear. If the latter method is used, it is most essential that really free-flowing arterial-coloured blood should be obtained, if necessary with the help of heat friction, xylol or some other device. We have been dismayed to find, after a good many years of testing blood from rabbits' ears, that what we had thought was a rather strict criterion in this respect was in fact less stringent than it should have been by comparison with simultaneous heart samples. If the circulation through the ear is only moderately good, ear samples will often give readings on the hæmoglobinometer four or six points higher than heart blood.

To facilitate comparison of one experiment with another the results throughout are expressed in terms of the assumed normal blood volume before bleeding taken as 100.

For the comparison between anæsthetised and non-anæsthetised animals we have a series of experiments in which the duration of the bleeding was between four and five minutes and the quantity of blood removed about 40 per cent. of the total. This is about as much as can be got quickly from the carotid of an anæsthetised animal, and is quite

as much as can be taken with a good chance of survival.   Examples of
the results obtained without anæsthetics are shown in Fig. I., where

FIG. I. NO ANAESTHETIC.

FIG. II. ETHER AND URETHANE
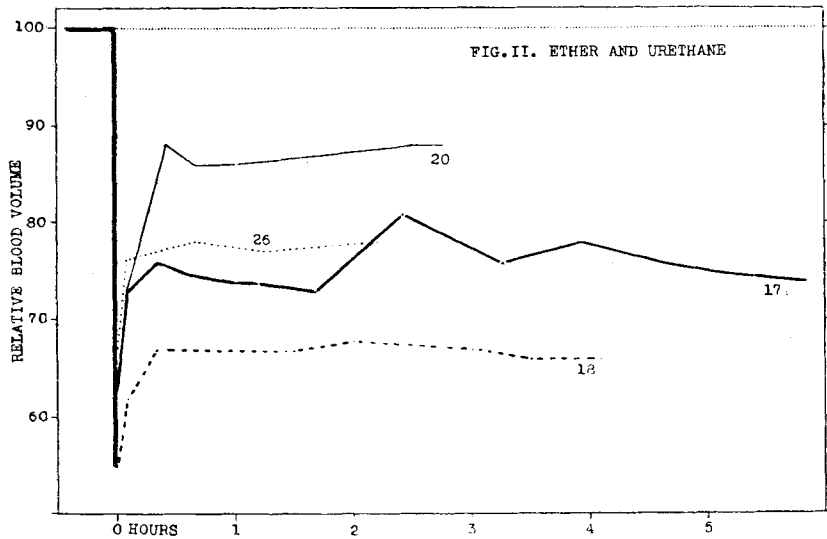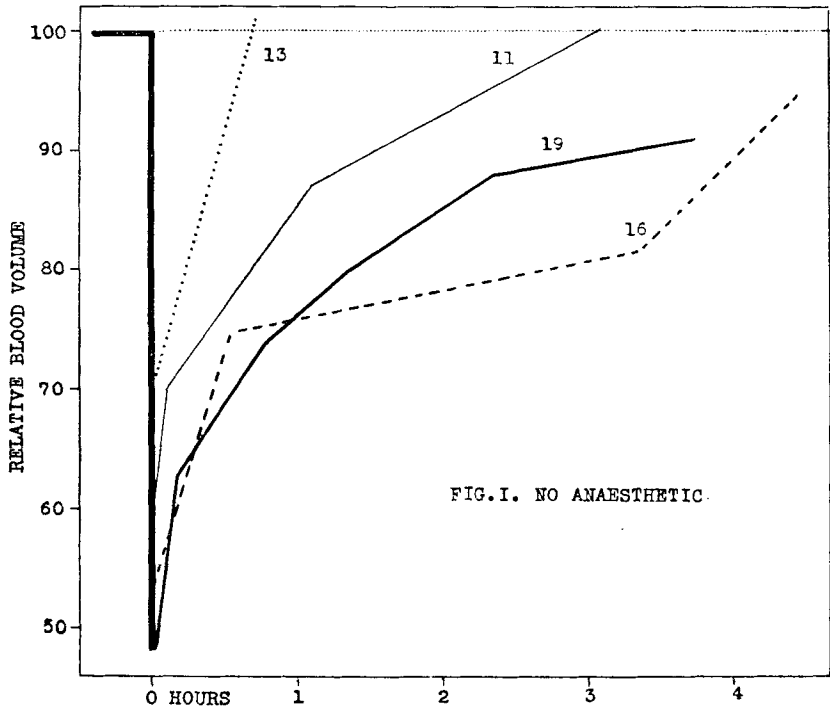
11 and 19 represent what may be taken as usual rates of restoration,
13 a case where restoration of volume was exceptionally quick, and
16 one where it was unusually slow.   Fig. II. gives comparable results

for anæsthetised animals, other examples being shown in 21 and 33 of Fig. IV. Most rabbits under the conditions of the experiments suck in enough fluid from the tissues to bring the volume back to about 75 per cent. of the normal: more rarely it remains at or below 70 per cent. and sometimes reaches nearly 90 per cent. (20 in Fig. II.).

These individual comparisons must not of course be pressed too far, because the results in detail depend on the average fact that the normal blood volume is 4·5 per cent. of the body-weight; only the average results, therefore, are valid. Thus in 20 of Fig. II. the final figure of a relative volume of 88 would become 100, if we started with the assumption that the volume was 6·4 per cent. of the body-weight, and 75 if we took 37 c.c. of blood per kilo as the datum line. Either assumption is just possible and quite improbable.

FIG. III.
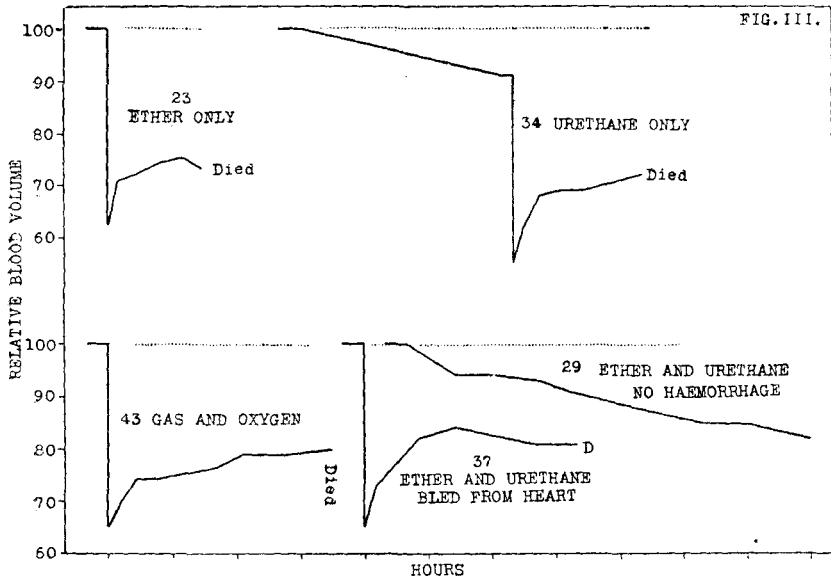
In the non-anæsthetised animals restoration of volume begins at once and proceeds fairly steadily until it is complete in about three hours. In the rabbits under ether and urethane the intake of fluid from the tissues during the bleeding and for some half hour afterwards is about as quick but afterwards slows off or stops altogether, and the volume remains without any substantial alteration for several hours forming on the curves what may be called the "plateau stage." A proportion of the animals die, generally about one and a half to two hours after the bleeding, presumably from circulatory failure from defective filling of the heart due to the diminished blood volume: the blood becomes progressively more venous and respiration fades away and finally stops. This is particularly liable to happen if restoration fails to restore the volume beyond about 70 per cent. of the normal.

The nature of the anæsthetic seems to make no great difference. Our customary procedure with ether and urethane has been described,

and gives a distinctly light anæsthesia after the first half hour of the experiment. Ether without urethane (Fig. III., 23), urethane without ether (Fig. III., 34) and, rather unexpectedly, nitrous oxide and oxygen (Fig. III., 43) all gave the same general results. The dose of urethane had to be increased from 1·5 grms. to 2 grms. per kilo to carry the animal through the initial operation: this larger quantity is not fatal to rabbits. The possibility that the operation necessary to get carotid samples might have some shock effect and so influence the results, was examined in a series of experiments in which under anæsthesia the animal was bled from the heart as in the non-anæsthetised rabbits (Fig. III., 37). On the whole restoration of volume was perhaps a
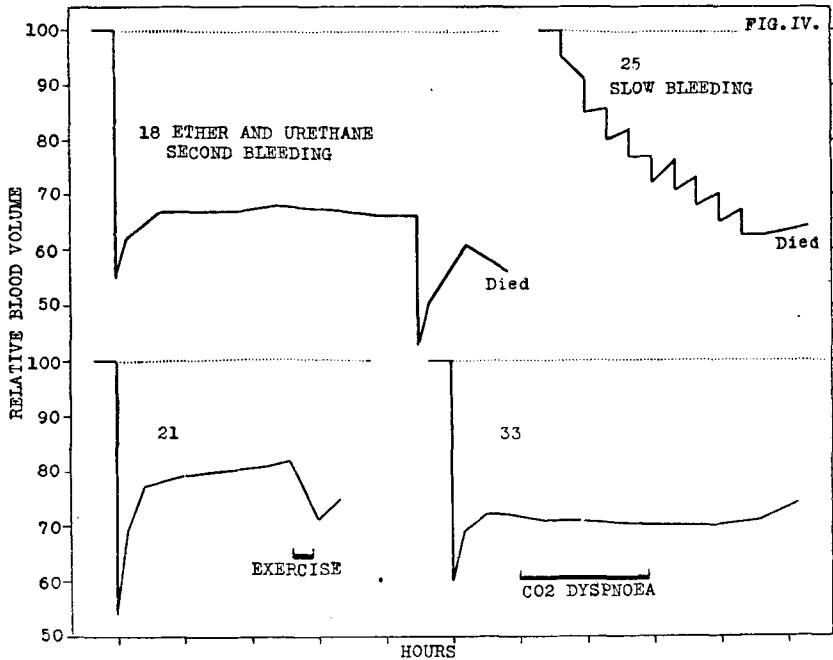
little more complete than in the similar operated animals; but the difference was too small to be sure about, and the response did not approximate to the non-anæsthetised reaction.

When a second hæmorrhage is inflicted on an anæsthetised rabbit which has ceased to increase its volume after a hæmorrhage and is keeping at a steady level of, say, 70, there is an immediate quick intake of fluid (Fig. IV., 18) just as there is after a primary bleeding. This shows that in the plateau stage the capacity of response is not altogether lost but can be brought into action by a sufficient stimulus. Taking the stimulus as being the difference at any moment between the volume of the blood and the capacity to which the vascular system is adapted, slow bleeding should give worse restoration than a single quick hæmorrhage. This we found to be the case. A characteristic result is

shown in 25 of Fig. IV. where the rabbit under ether and methane was bled from its carotid 5 per cent. of its original blood volume every twenty minutes: it was not till after the fifth bleeding that any substantial restoration occurred.

The plateau condition of the anæsthetised animals suggests that at this stage the blood volume has settled down to a readjusted level. The rate of circulation is of course slow, and it seemed possible that if it were increased restoration of volume might proceed more as it does in a normal animal. We roused up the circulation in two ways. (*a*) General faradisation of the limbs, and to a less degree the trunk, for ten minutes or so had a clear effect but in the wrong direction (Fig. IV., 21), for the blood became more concentrated and the volume diminished. This is the same effect as has been described by Scott, Herrmann, and Snell (1917 [23]). (*b*) Another series of animals were given pretty violent CO_2 dyspnœa, either throughout the experiment or when the plateau stage was reached, by being made to rebreath their own expired air. This had no perceptible effect (Fig. IV., 33).

Analysis of the distribution of internal pressures in the vascular system might perhaps throw light on the anæsthetised state were it not impossible to do it on the non-anæsthetised controls. The presumption is, however, that arterial pressure would be lower in anæsthesia, and it is well known that the blood volume tends to vary inversely with the arterial pressure over short periods of time (Zuntz and Cohnstein (1888 [13]), Sherrington and Copeman (1893 [14]), Mummery and Symes (1907 [15]), Scott (1917 [10])). On this ground, therefore, we should expect the anæsthetised animals to bring fluid into the blood more readily than the controls.

Taking the facts as a whole it seems legitimate to conclude that anæsthesia, even of a light description, alters the permeability of the capillaries in such a way that liquid tends to accumulate in the tissues. The phenomenon is independent of hæmorrhage, for if a rabbit is anæsthetised with ether and urethane and not bled the blood concentrates and the volume decreases (Fig. III., 29).* The normal distribution of liquid between tissue fluid and blood is the resultant of the quantity passing from the blood to the tissues (perhaps from the arterial end of the capillaries), and that coming from the tissues to the blood (by the lymph and perhaps through the venous parts of the capillaries). The volume of the blood might be diminished by a relative increase of permeability from within outwards (*i.e.*, from blood to tissue fluid) or by a relative decrease of permeability from without inwards. Anæsthesia seems to have this latter effect. The facts obviously link on to those of traumatic toxæmia and secondary shock worked out by Bayliss (1919 [17]), Cannon (1919 [17]), Dale, Laidlaw, and

* Starling (1909 [16]), probably using dogs, says that chloroform narcosis lowers blood pressure and dilutes the blood : the degree of anæsthesia likely makes a good deal of difference.

Richards (1918-19 [27, 28], 1919 [17], 1920 [29]).   One of the characteristic immediate actions of the products of tissue destruction, of which histamine may be taken as representative, is to concentrate the blood and accumulate liquid in the tissue fluid.   This is greatly promoted by anæsthesia.   Indeed, doses of histamine several times larger than those which are fatal to cats under ether have relatively little effect in non-anæsthetised animals.*

The histamine effect is generally attributed to an increase of permeability from within outwards: the facts are, however, quite compatible with a decrease of permeability from without inwards, and it seems that this is probably the chief change.   We tried to get evidence on this point by measuring the restoration to normal of the blood volume after transfusion of blood.

In these experiments a volume of fresh citrated rabbit blood equal to half the blood volume of the recipient animal was injected into the jugular of anæsthetised and the ear vein of non-anæsthetised rabbits in five minutes.   Each injection contained about 0·35 grm. sodium citrate.   For example, a rabbit of 2200 grms. with 69 per cent. Hb and 6·56 mill. red cells and an assumed blood volume of 99 c.c. received intravenously 50 c.c. of rabbit blood of 67 per cent. Hb and 6·21 cells.   If nothing happened, the total volume would be 149 c.c. with 68 per cent. Hb and 6·33 cells, and if this were concentrated to 99 c.c. (by the expulsion of 50 c.c. of plasma) the hæmoglobin would rise to 103 per cent. and the red cells to 9·53 mill.   Twenty-two minutes later the hæmoglobin had risen to 80 per cent. and the red cells to 7·60, giving relative blood volumes of $\frac{103}{80} = 129$ from the hæmoglobin and $\frac{9 \cdot 53}{7 \cdot 60} = 126$ from the red cells.

The results are shown in Fig. V. for non-anæsthetised and in Fig. VI. for anæsthetised animals.   On the whole the latter made rather better restorations, and the only rabbit that brought its volume nearly to the normal level was anæsthetised (Fig. VI., 4).   The non-anæsthetised animals showed a tendency to concentrate the blood at first and then afterwards to dilute it again.   This is intelligible if we consider that concentration increases viscosity, and that the effect of viscosity on the heart is the product of its rate of production, its degree and its duration.   Judging from the figures of Denning and Watson ([19]) an increase of red corpuscles from 6 to 9 millions per cubic millimetre would increase the viscosity about 1·65 times, and this would again become considerably larger if by any incipient circulatory failure the blood became more venous and acid and the corpuscles in consequence swollen (Ferrai [20]; Price Jones [21]).   We have abundant experience that non-anæsthetised rabbits, given time, will bring their blood volume back to normal after transfusion ([22]), and will tolerate without apparent difficulty a substantial polycythæmia.   We imagine,

* Anæsthesia with nitrous oxide and oxygen prevents the histamine effect: in our hands it gave the usual result (Fig. III., 43) as regards restoration of blood volume, but our technique, in which we found it difficult to avoid some asphyxia, may have been faulty and we could not try it under increased pressure in the manner of Paul Bert (see Dale and Hill (1921 [18])).

FIG.V.TRANSFUSION.NO ANAESTHETIC

FIG.VI. TRANSFUSION.ETHER AND URETHANE

therefore, that the slackening of the rate of restoration, and its occasional reversal during the first few hours is due to the whole mechanism of response being more active and intelligent in the non-anæsthetised animals which, feeling the first difficulties of increasing viscosity, take the process slowly, while they learn that it is not necessary to send concentrated blood round the body as quickly as a more dilute suspension of hæmoglobin. The anæsthetised animal (Fig. VI., 4) which made the most complete restoration and in the process raised its hæmoglobin from 77 to 104 and its red cells from 6·80 to 9·68 millions died, apparently from circulatory failure and with an increasing venosity of its carotid blood for the last forty minutes of the experiment. These considerations make it difficult to lay any great stress on the differences, in any case not large, between the two series of animals, and we do not find in them any clear evidence that anæsthesia produces any change in the permeability of the capillaries from within outwards. The restoration of volume after transfusion differs from that after hæmorrhage in being disadvantageous in that it may cause a harmful increase of viscosity.

SUMMARY.

1. Under anæsthesia the restoration of the blood volume after hæmorrhage is slower and less complete than in non-anæsthetised rabbits.

2. This is attributed to a diminution in the permeability of the capillary wall to liquid passing from the tissue fluid to the blood.

ADDENDUM.

Dr E. J. Salisbury points out that W. W. Lepeschkin (*Beihefte z. bot. Centr.*, 1906, vol. xix., No. 1, p. 409) found that anæsthetics much depress the permeability of plant tissues to water.

REFERENCES.

1. VIERORDT, K. . . . . . . *Arch. f. physiol. Heilk.*, 1854, vol. xiii. p. 259.
2. TARCHANOFF, J. R. . . . *Pflüger's Archiv.*, 1880, vol. xxiii. p. 548; 1881, vol. xxiv. pp. 203, 525.
3. MELASSEZ, L. . . . . . *Arch. de Physiol.* (2), 1874, vol. i. p. 797; 1875, vol. ii. p. 278.
4. VON LIMBECK, R. R. . . . "Clinical Pathology of the Blood," *New Syd. Soc.*, 1901, vol. i., p. 61.
5. BOYCOTT, A. E. . . . . *Journ. Path. and Bacteriol.*, 1912, vol. xvi. p. 485.
6.   „   . . . . *Ibid.*, 1914, vol. xviii. p. 417.
7. MARTIN, C. J. . . . . . *Unpublished Observations.*
8. PENFOLD, W. J. . . . . *Med. Journ. of Australia*, 25th Sept. 1920.
9. BOYCOTT, A. E., AND *Journ. Path. and Bacteriol.*, 1909, vol. xiii. p. DOUGLAS, C. G.   264.

10. SCOTT, F. H. . . . . . *Amer. Journ. Physiol.*, 1917, vol. xliv., p. 298.
11. COHNHEIM, J. F. . . . . "General Pathology," *New Syd. Soc.*, 1889, p. 438.
12. SCHNEIDER, E. C., AND HAVENS, L. C. *Amer. Journ. Physiol.*, 1915, vol. xxxvi. pp. 239, 380.
13. ZUNTZ, N., AND COHNSTEIN, J. *Pflüger's Archiv.*, 1888, pp. 42, 303.
14. SHERRINGTON, C. S., AND COPEMAN, S. M. *Journ. Physiol.*, 1893, vol. xiv. p. 88.
15. MUMMERY, P. L., AND SYMES, W. L. *Journ. Physiol.*, 1907, vol. xxxvi. Proc. xv. ; *Brit. Med. Journ.*, 1908, vol. ii. p. 786.
16. STARLING, E. H. . . . . "The Fluids of the Body," 1909, pp. 140, 144.
17. DALE, H. H., LAIDLAW, P. P., RICHARDS, A. N., CANNON, W. B., AND BAYLISS, W. M. *Medical Research Committee Special Report Series*, No. 26, 1919.
18. DALE, H. H., AND HILL, L. *Lancet*, 1921, vol. ii. p. 326.
19. DENNING, A. P., AND WATSON, J. H. *Proc. Roy. Soc.*, Lond., B., 1906, vol. lxxviii. p. 328.
20. FERRAI, C. . . . . . . *Archivio di Fisiologia*, 1904, vol. i. p. 385.
21. PRICE JONES, C. . . . . *Journ. Path. and Bacteriol.*, 1920, vol. xxiii. p. 371.
22. BOYCOTT, A. E., AND DOUGLAS, C. G. *Journ. Path. and Bacteriol.*, 1909, vol. xiii. p. 414 ; 1910, vol. xiv. p. 294.
23. SCOTT, F. H., HERRMANN, E. T., AND SNELL, A. M. *Amer. Journ. Physiol.*, 1917, vol. xliv. p. 317 ; see also Rakestraw, N. W., *Journ. Biol. Chem.*, 1921, vol. xlvii. p. 565.
24. LAMSON, P. D. . . . . . *Journ. Pharm. Exper. Therap.*, 1915, vol. vii. p. 169 ; 1916, vol. ix. p. 129.
25. HAWK, P. B. . . . . . *Amer. Journ. Physiol.*, 1904, vol. x. p. 384.
26. LAMSON, P. D. . . . . . *Journ. Pharm. Exper. Therap.*, 1921, vol. xvi. p. 132.
27. DALE, H. H., AND RICHARDS, A. N. *Journ. Physiol.*, 1918-19, vol. lii. p. 110.
28. DALE, H. H., AND LAIDLAW, P. P. *Ibid.*, p. 355.
29. DALE, H. H. . . . . . *Brit. Journ. Exp. Path.*, 1920, vol. i. p. 103.