J. Physiol. (1966), **186**, *pp.* 535–546
With 3 text-figures
Printed in Great Britain

THE VOLUME OF VASCULAR COMPARTMENT IN RAT HIND LIMB MUSCLES

BY R. O. LAW* AND C. F. PHELPS

From the Department of Biochemistry, The Medical School, University of Bristol

(*Received* 11 *March* 1966)

SUMMARY

1. A non-recirculatory perfusion system has been developed suitable for the perfusion of the hind limbs of small experimental animals.

2. By means of it a solution of T. 1824-labelled serum albumin has been introduced into the vascular compartment of the hind limbs of female rats under isogravimetric conditions. Excision and analysis of certain muscles has been used to provide information concerning the percentage distribution of the labelled albumin within these muscles.

3. Experiments have been carried out *in vivo* employing [^{131}I]labelled serum albumin and [^{51}Cr]labelled erythrocytes in order to compare the vascular volumes determined under *in vivo* conditions and in perfusions, and to estimate the capillary haematocrit *in vivo*.

4. The physiological validity of the methods used and the results obtained has been discussed.

INTRODUCTION

This paper describes a new approach to the determination of the fluid volumes of distribution of marker molecules within tissues, using a simple non-recirculatory perfusion system on isolated rat hind limbs whose weight changes can be monitored throughout the course of the experiment. In particular an account is given of the determination of the vascular volume of certain individual skeletal muscles within the hind limbs.

Previous methods of measuring vascular compartment volumes fall into two categories. Krogh (1929) injected Prussian Blue gelatin and Indian ink into the blood vessels of the horse, the dog and the frog, sectioned muscles and counted the capillary density within the sections. Similar work by Stoel (1925) and by Duyff & Bouman (1927) suggested vascular volumes in rabbit muscle of between 0·34 and 6·33 % of the muscle volume, with a wide scatter of values. The second method has re-

* Present address: School of Agriculture, University of Cambridge.

course to the analysis in comminuted muscle of a marker molecule presumed to be vascularly confined, previously introduced into the muscle either by infusion or by suspension of the isolated muscle in a medium containing the marker molecule. Boyle, Conway, Kane & O'Rielly (1941) and Danielli (1941), using estimates of the haemoglobin content of blood and muscle, suggested vascular volumes of 2·3 and 5% respectively in frog muscle. The use of specific plasma labels (e.g. Evans Blue, T. 1824) added to whole blood requires a knowledge of the haematocrit of muscle, as shown by Rieke & Everett (1957), who used [^{59}Fe]labelled red cells and [^{131}I]–labelled plasma to estimate these separately in various tissues in the rat.

Several authors have shown that perfusion techniques may produce oedema, and the importance of a precisely defined equilibrium between arterial and venous pressures and the colloid osmotic pressure in the maintenance of an isogravimetric state was shown in the sophisticated work of Pappenheimer & Soto-Rivera, (1948). The work described here is the measurement of the vascular space of muscle by a perfusion technique which introduces T. 1824-labelled serum albumin while maintaining the limb in such an isogravimetric state.

The results so obtained have been compared with those obtained from the *in vivo* injection of [^{131}I]labelled serum albumin and [^{51}Cr]labelled erythrocytes.

METHODS

The perfusion apparatus. The perfusion apparatus is shown in Fig. 1. *G* was a gas inlet (95% oxygen: 5% carbon dioxide), which provided the driving force for the perfusate; *X*, a distilled water humidifier; WB, a water bath maintained at 40° C; PR, a mercury reservoir permitting rapid and accurate control of perfusion pressure; *R*, the reservoir containing the perfusion fluid. After leaving the reservoir, the fluid passed pH flow electrodes, *P* (E.I.L. microflo type SMF 23). The pH of the influent fluid, as measured on an E.I.L. Vibron Electrometer pH meter was 7·3–7·4. The pH of rapidly analysed samples of venous effluent was never less than 7·2 and was usually identical with that of the influent fluid. *H* was a secondary heating chamber, containing two 24 V post office bulbs. By varying the current through these bulbs, the temperature of the fluid in this chamber could be controlled with considerable accuracy. This temperature was recorded by means of a thermistor, *T* (Standard Telephone Co., Ltd., Type F 23) connected to a calibrated millivoltmeter by means of a Wheatstone bridge network. *M* was a mercury manometer. *C* was the cannula. This consisted of 7 cm of flexible, widebore polythene tubing, and 2 cm narrow-bore tubing (Portex No. 48, Portland Plastics, Ltd., Hythe, Kent; New Cat. ref, PP. 30; internal diameter 0·5 mm). The flexibility of this cannula permitted weight changes of as little as 75 mg to be recordable on the single pan balance, *B*, upon which the hind-limb preparation was placed.

The perfusion fluid. The perfusion fluid used was the saline–bicarbonate–phosphate Ringer suggested by Krebs (1950), containing tricarboxylic acid cycle intermediates (prepared fresh weekly by neutralizing solutions of the acids with sodium bicarbonate and stored at 0° C). Bovine serum albumin (b.s.a.) (4·5%, w/v) was added to this medium as colloid osomotic support. All reagents were Analar Grade where possible. All analytical volumetric glassware conformed to British Standard Specification B.S. 1797 (1952). All water used was glass-distilled, and pH measurements were standardized against M/20 potassium hydrogen phthallate buffer, pH 4·00, at 15° C.

Preparation of animal and cannulation. Female albino rats, which had been maintained on a diet of P.R.M. (Christopher Hill, Ltd., Poole, Dorset) *ad libitum*, were anaesthetized by the intraperitoneal administration of Nembutal (8 mg/100 g body weight). For a variety of reasons it was not always possible to obtain rats of similar weight for different series of experiments. Heparin (1000 i.u.) was injected into the right basilic vein as a precaution against possible intravascular clotting.

The abdominal wall was incised longitudinally to the level of the xiphisternal cartilage. The uterine horns and the intestinal tract were reflected laterally, and the former were ligatured. The inferior mesenteric artery and the ileolumbar vessels were also ligatured. A common, loose ligature was placed round the descending aorta and the inferior vena cava approximately 2 cm above the level of the bifurcation of these vessels. This ligature was left untied until just before cannulation itself. Midway between this ligature and the bifurcation, the aorta and the vena cava were separated, and a loose ligature placed round the artery. The function of this ligature was to hold the cannula in position after insertion.

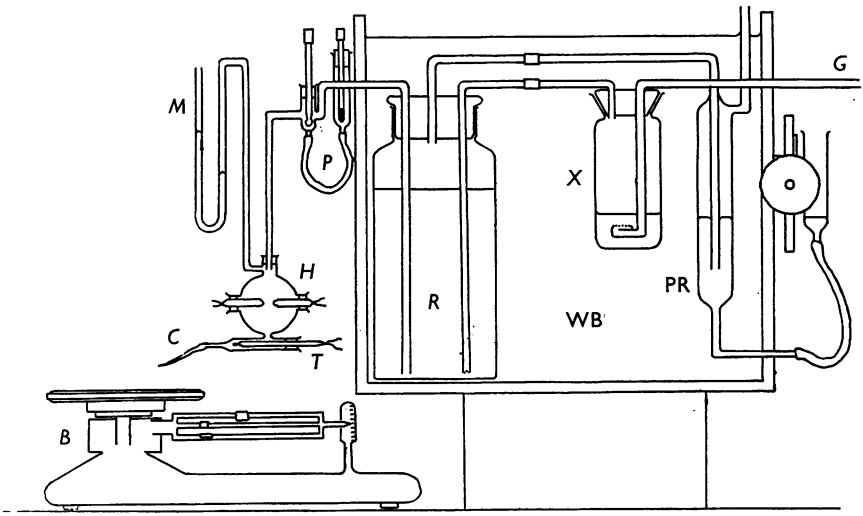

Fig. 1. Apparatus for maintaining an isogravimetric perfusion while introducing a labelled marker *G*, gas supply; PR, pressure regulator; *X*, humidifier; *R*, perfusion fluid reservoir; *P*, pH electrodes; *M*, Hg manometer; *C*, cannula; *B*, balance; WB, water bath; *H*, supplementary heater; *T*, thermistor. For further details see text.

The animal was then placed on a laterally sloping waxed cork board, in a manner somewhat similar to that employed for frog hind limb perfusions by Hyman & Chambers (1943), and the board placed upon a single pan balance.

The common ligature round the aorta and vena cava was tightened, thus isolating the caudal part of the animal. The arterial cannula was immediately inserted, a process requiring 30 sec or less. During insertion, a slow flow of warm perfusate through the cannula was maintained, thus eliminating the possibility of introducing air embolism into the preparation. The inferior vena cava was severed at the level of arterial cannulation, and the effluent fluid collected as it dripped from the sloping board. It was not observed that this method of collection, if carried out with due care, involved any accumulation of effluent fluid either on the preparation itself or in the board.

The drop in pressure occurring across the narrow part of the cannula was estimated by measuring the difference in fluid height in two vertical manometers connected by the

cannula; the drop in pressure was found to be linearly related to the rate of fluid flow. In this paper the term 'applied perfusion pressure' will be taken as referring to the pressure as read on the mercury manometer, *M*, whereas 'arterial pressure' will refer to the pressure at the arterial end of the cannula as derived from a knowledge of the flow rate brought about by the given value of the applied perfusion pressure.

Perfusion. A solution of bovine serum albumin labelled with T. 1824 (100 mg/100 ml. Ringer) was perfused into the hind limb preparation for a predetermined length of time, after which the perfusion was discontinued; both gastrocnemii were rapidly excised, gently blotted and weighed. Weights varied from about 350 mg to just over 500 mg. Each muscle was then finely scissor minced and allowed to stand for 24 hr in a solution of 0·88 ammonia (3 ml.) (Young, 1964). The extracts were then spun at 15,000 *g* for 15 min, after which time any supernatants which were cloudy were discarded. The clear, pale blue supernatants were read spectrophotometrically at 612 mμ (the absorption brought about by a suitable diluted 0·88 ammonia extract of the perfusion fluid providing a basis for estimating the effective dilution of the dye within the muscle, i.e. the apparent dilution due to the vascular confinement of the dye).

The sample of T. 1824 used was shown by chromatography on Sephadex G–25 (10 cm × 4 mm), to be free of red impurities such as demonstrated by Leeson & Reeve (1949) and Cooley (1954).

Radio-iodinated serum albumin. Radio-iodinated serum albumin (r.i.s.a.) was supplied from the Radiochemical Centre, Amersham, in a sterile solution containing 0·9 % benzyl alcohol and 43·6 mg albumin in 2·36 ml. isotonic saline. The specific activity of the whole sample was 2·00 mc (approx.) on the day of receipt. The sample used for this work was stated to contain up to 5 % free $^{131}I^-$ at the time of receipt, and since the iodide ion is an extravascular marker (Flear & Graber, 1963) it was necessary to remove the free ions by column chromatography before the plasma space determinations. Elimination was carried out on a Sephadex G-25 column (4 cm × 4 mm) in isotonic saline. By calibrating this column using a coloured molecule (cytochrome c) a routine was established whereby over 90 % of the original radioactive protein could be regained, diluted about 1·5–1·6 times. Fresh columns were used for each separation, and elimination of the free radio-iodide ions was carried out before each plasma space determination.

Injection and sampling of r.i.s.a. Weighed female rats were anaesthetized with Nembutal and then heparinized exactly as in the perfusion experiments. The left basilic vein was injected with 0·1 ml. r.i.s.a. solution. Since this was essentially a comparative experiment, it was not necessary to know the precise specific activity of the injected r.i.s.a. Fifteen minutes were allowed for circulation of the injected protein. At the end of this time the gastrocnemii were rapidly excised, lightly blotted and weighed. Immediately following this, two cardiac blood samples were taken. The gastrocnemii were homogenized in 3 ml. 0·9 % saline, spun at 2000 *g* for 20 min on an M.S.E. Multex centrifuge. Samples of the clear pink supernatant were weighed, diluted with 3 ml. 'Dioxane' (dioxane containing 120 g/l. naphthalene, 7 g/l. 2,5-diphenyloxazole, 50 mg/l. 1,4-bis-[2-(5-phenyloxazolyl)]-benzene) and analysed on a liquid scintillation counter (Nuclear Chicago).

The cardiac (central) blood samples were spun at 1000 *g* in an M.S.E. Minor centrifuge in Wintrobe tubes for 10 min until a clear supernatant was obtained (packed cell volume = 43 %, red cell volume = 42 %). The specific activity of weighed samples of plasma was estimated by the same method as employed for the muscle homogenates (assuming sp.gr. of plasma = 1·03).

^{51}Cr-labelled sodium chromate. $[^{51}Cr]Na_2CrO_4$ was supplied by the Radiochemical Centre, Amersham, as a sterile, saline solution containing 9·25 μg ^{51}Cr/ml., specific activity approximately 1·05 mc/ml. at the time of receipt.

At least 20 hr before a given estimation, about 1 ml. of blood was drawn from the experimental rat, by cardiac puncture, into a heparinized syringe. A saline solution of radio-

chromate was added to the blood sample, in the proportion of approximately 200 μc/1 ml. of blood. As with the r.i.s.a. estimations, the precise activity of the radiochromate was not important. Two hours were allowed for incubation of the blood and radiochromate. The blood was then centrifuged in Wintrobe tubes for 10 min at the end of which time the plasma buffy coat and red cells immediately below the buffy coat were removed by a Pasteur pipette (Maizels, 1945). The red cells were washed and spun 3 times with isotonic saline, the supernatant fluid being discarded on each occasion. The supernatant after the third washing contained negligible levels of radioactive matter. The cells were then suspended in an equal volume of fresh isotonic saline (the final volume now being about 70–80 % of the original). This suspension was filtered through glass wool to ensure against the injection into the experimental animal of any gross cell aggregations.

Injection and sampling of radiochromate. Weighed female rats were anaesthetized and heparinized exactly as in the r.i.s.a. and perfusion experiment. [^{51}Cr]labelled blood (0·5 ml.) was injected into the left basilic vein over a period of approximately 1 min, 15 min were allowed for circulation, as with the r.i.s.a. experiments. The treatment of the cardiac (central) blood, however, was necessarily rather different. Whereas in both the r.i.s.a. and ^{51}Cr work the radioactive and non-radioactive fractions of blood were easily separable (i.e. in a Wintrobe tube) it was not possible to take a direct aliquot of red cells for analysis as it had been with the plasma. This was because (*a*) a residue of plasma unavoidably remained in the interstices of the packed red cells (Maizels & Remington, 1959), the extent of which largely depended upon the efficiency of packing, and (*b*) there was an obvious practical difficulty in transferring packed red cells with a Pasteur pipette. It was therefore necessary to estimate the specific activity of whole blood and make the necessary adjustment in respect of the 42 % central haematocrit.

RESULTS

The extent of perfusion. The preparation included the tail, the skin of the caudal part of the animal, the terminal parts of the spinal cord, and the skeletomuscular portions of the pelvis and hind limbs. Analysis of three hind-limb preparations perfused with T. 1824-labelled serum albumin, followed by dissection and weighing of the perfused tissues indicated that the tissue composition of the preparation was as shown in Table 1.

TABLE 1. Dissection and weighing of dissected masses from three rats injected with T. 1824-labelled serum albumin

	%
Muscle	71·0
Bone	10·6
Paw	8·7
Skin and fat	9·8
	100·1

The isogravimetric state. The effects of varying either (*a*) the arterial pressure or (*b*) the colloid osmotic pressure are shown in Fig. 2 (*a*) and (*b*).

As Pappenheimer & Soto-Rivera (1948) point out, an infinite number of possible combinations of arterial and venous pressure, coupled with varying colloid osmotic pressure, will all achieve an isogravimetric condition in a perfused muscle. Since the venous pressure in the present work approached zero, variations existed only between the arterial pressure and the colloid osmotic pressure. Rat plasma contains approximately 4·5 % albumin (w/v) which functions as the chief osmotic support constituent,

and this concentration was chosen throughout the further experiments. Using this concentration it can be seen that isogravimetric conditions were achieved by employing arterial pressures of 30 mm of Hg (corresponding to applied perfusion pressures of approximately 40 mm Hg); these values resulted in flow rates of approximately 3–4 ml./min, although on occasions somewhat higher values, up to 8 ml./min, were achieved.

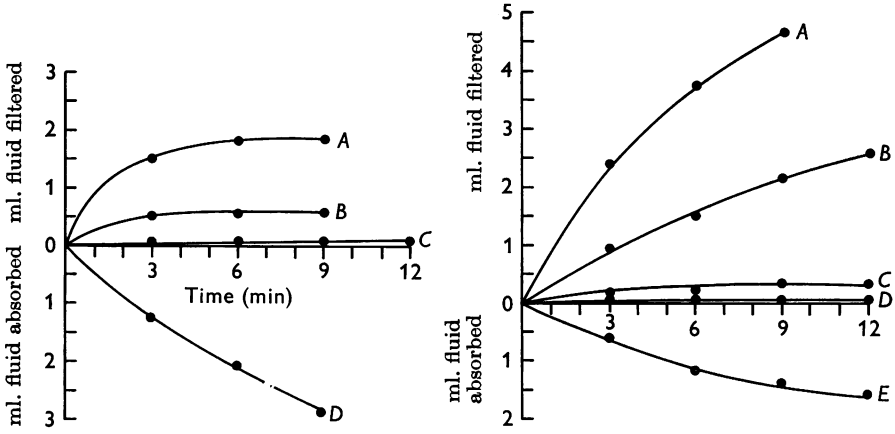

Fig. 2. (*a*) The changes in weight of the hind-limb preparation (expressed as ml. fluid filtered or absorbed) in a series of perfusions carried out at different arterial pressure with a perfusion fluid containing a constant level of bovine serum albumin (4·5 %, w/v). Arterial pressures employed were *A*, 61 mm Hg; B, 40 mm Hg; *C*, 29 mm Hg; *D*, 6 mm Hg. (*b*) The changes in weight of the hind-limb preparation (expressed as ml. fluid filtered or absorbed) in a series of perfusions carried out using perfusates containing different levels of bovine serum albumin while keeping the arterial pressure constant (29 mm Hg). Levels of bovine serum albumin employed were *A*, none; *B*, 2 % (w/v); *C*, 4 % (w/v); *D*, 4·5 % (w/v); E, 6 % (w/v).

Time course for equilibration of marker. The results of an extended series of experiments are shown in Fig. 3. These values relate to the volume of distribution of labelled protein within the gastrocnemius. A stable value of 2·74 % (S.E.M. = ± 0·12 %) was obtained. The significance of the apparently sigmoid nature of the equilibration curve is not understood.

Vascular spaces in other muscles. Values for the spaces obtained in other muscles after perfusions lasting 1 hr are shown in Table 2.

R.i.s.a. spaces. The values for the r.i.s.a. spaces obtained in the gastrocnemius are shown in Table 3. The average value was found to be 1·81 % (S.E.M. = ± 0·09 %).

Red blood cell spaces. The values for the red blood cell spaces in the gastrocnemius are shown in Table 4. The average value was found to be 0·62 % (S.E.M. = ± 0·04 %). Taken in conjunction with the r.i.s.a. space this yields a total vascular space of 2·43 %.

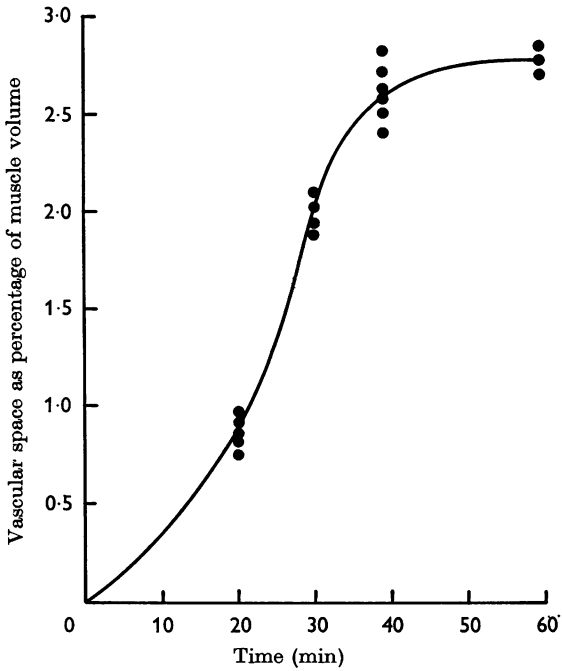

Fig. 3. Equilibration of T. 1824-labelled bovine serum albumin. The apparent volume of the vascular compartment of the rat gastrocnemius is shown as a function of time.

DISCUSSION

The chief questions to be answered in assessing the validity of the foregoing results may be summarized as follows: (1) Were all the vessels in the gastrocnemius (*a*) open, and (*b*), if open, perfused? (2) Did abnormal quantities of labelled albumin pass into the extracellular space?

Before passing to a detailed discussion of these factors, it must be stressed that, whatever the nature and variety of the factors affecting the final result, constancy of volume was attained. Since the extravascular passage of small amounts of protein is a recognized phenomenon, the term 'constancy' is not strictly accurate in this context; the plateau of the space/time curve must necessarily be a gradually ascending slope, as the labelled b.s.a. passes out of the capillaries. But under physiological conditions this slope should be sufficiently shallow to appear as a plateau during the relatively short periods of perfusion.

Vessels under vasometor control have limited stability. It has been shown by Burton (1951) that if the tension in the wall increases to a critical value, closure of the vessel will occur. It may be shown mathematically that for a vessel of given 'unstretched radius' the value of this so-called

TABLE 2. Values for the spaces obtained in other muscles after perfusion with T. 1824-labelled serum albumin for 1 hr

Length of perfusion (min)	Muscle and weight (mg)	Space (%)
60	Gluteus max. 462	2·67
60	Biceps femoris 212	2·89
60	Gluteus max. 417	3·11
60	Biceps femoris 238	2·98

TABLE 3. The plasma space in the gastrocnemius muscle

Rat no.	Weight (g)	Muscle plasma space (%)
A	270	1·86
		1·82
B	261·5	1·92
		2·08
C	274	1·70
		1·72
D	307·5	1·72
		1·74
E	250	1·80
		1·82
F	215	1·77
		1·77
G	208	1·75
		1·78
H	247·5	1·85
		1·90

Values quoted refer to left and right gastrocnemii respectively. Allowance was made for an estimated recovery of 93 %. All values are the average of two estimations. All readings were corrected for the sp.gr. of plasma.

TABLE 4. The red cell space in the gastrocnemius muscle

Rat no.	Weight (g)	Red cell space (%)
A	265	0·58
		0·62
B	241·5	0·66
		0·66
C	301	0·58
		0·57
D	252	0·58
		0·60
E	194	0·64
		0·63
F	184·5	0·53
		0·63
G	195	0·64
		0·63
H	170	0·66
		0·68

Values quoted refer to left and right gastrocnemii respectively. Allowance was made for a 42 % haematocrit in central blood and for the sp.gr. of blood. Recovery was estimated at over 98 %.

critical closing pressure is proportional to the tension developed in the capillary wall. Thus it is in the arterioles, which on account of their musculature are capable of developing relatively high tension within their walls and may possess relatively high values of critical closing pressure, that fundamental instability must be sought. The perfusions described here were carried out at arterial pressures which were considerably below normal physiological values, and it is of importance to know whether critical closing pressures were approached; were this the case, underperfusion would have resulted, and, with it, spuriously low values for the apparent vascular volume. The present arterial pressures, however, although sub-physiological, were considerably in excess of the critical closing pressures determined directly by Nichol, Girling, Jerrard, Claxton & Burton (1951) for vessels in the isolated perfused hind limbs of rabbits, and also of those obtained by extrapolation of pressure/flow curves by Whittaker & Winton (1933) and by Pappenheimer & Maes (1942).

Since the perfusion fluid contained no oxygen carrier, the only oxygen reaching the perfused tissues was that dissolved in the perfusate by physical solution. Bearing in mind the solubility of oxygen in water, as well as the flow rates normally obtained in the perfusions, it may be shown that the oxygen supply to the tissues was of the order of 3·0–3·5 μl./min/g muscle (wet weight). By comparison with the oxygen consumption values obtained by Pappenheimer (1941) for dog muscle, and by Bird, Schottelius & Schottelius (1963) for guinea-pig muscle, the present figure indicates a considerable degree of hypoxia. However, had this been such as to cause serious deterioration of the permeability characteristics of the capillary wall, then not only would a considerable extravasion of protein have been expected to occur (and with it the absence of a plateau on the space/time curve), but also the osmotic balance of the muscle would have been expected to be disrupted (thus leading to a loss of isogravimetric state). Neither of these occurrences was observed in the present work. In this connexion the work of Florey (1964) on the cyanide- and non-cyanide-sensitive oxidative processes of capillary wall cells, in relation to capillary permeability characteristics, is of interest.

The experiments which are perhaps most relevant to the present work are those which Hendley & Schiller performed in 1954. They determined the rates of oedema formation in the hind limbs of rats perfused under varying conditions of pressure and oxygenation. Employing a phosphate-buffered Ringer solution containing 20 % washed dog erythrocytes and 0·33 % ash-free gelatin they found that if the level of the oxygenation of the perfusion fluid fell below 5 % oedema occurred. Whether or not protein leakage accompanied this oedema was not determined, but in any event the oedema could be at least partially explained on grounds other than

those of simple hypoxia. The lowest applied pressure employed by Hendley & Schiller was 40 mm Hg which, allowing for a drop in pressure across the cannula, probably resulted in an arterial pressure not greatly differing from those employed in the present work, giving rise to closely similar rates of flow through the perfused limbs. The highest value of applied pressure employed was 205 mm Hg. Even in the presence of full colloid osmotic support such a pressure would be capable of causing oedema, and on the basis of Starling's hypothesis the presence of only 0·33 % gelatin as colloid osmotic support would increase this tendency. Even at relatively low perfusion pressures so low a colloid osmotic pressure could account for considerable oedema formation. It seems unlikely, therefore, that hypoxia was more than partially responsible for the oedema produced in Hendley & Schiller's experiments, although the fact that oxygen plays a vital role in maintaining the integrity of the capillary wall is clear from an inspection of the far greater rates of oedema formation which Hendley & Schiller found when perfusing with nitrogen-saturated Ringer under otherwise identical conditions. In the present experiments, it must be stressed that an isogravimetric state was achieved throughout perfusions lasting up to 1 hr, and this suggests that no serious deterioration of the capillary walls occurred within this time. The likelihood that hypoxia would lead to vasodilatation, and to spuriously high vascular space measurements, must be also born in mind. Thus Shadle, Zukof & Diana (1958) in blood-perfused (i.e. oxygenated) hind limbs of dogs found a vascular space of only 2·1 % using ^{32}P and T. 1824 as markers. The discrepancy between their result and the figures found in the present work might be at least partially accounted for on these grounds.

The volume of the vascular space as determined either by perfusion or by radioactive techniques is influenced by the use of anaesthetic. Rieke & Everett (1957) injected rats with [^{131}I]labelled plasma and [^{59}Fe]labelled red cells. After a 3 min mixing time (which is unaffected by anaesthesia) they froze the rats in liquid nitrogen, and proceeded to analyse a wide variety of tissues for blood content and haematocrit. They used Nembutal anaethesia in doses comparable to those employed in the present study. The rate of albumin loss from the total circulation was found to be doubled by comparison with an unanaesthetized series (Everett, Simmons & Lashef, 1956). Thus approximately 5 % of the total circulating albumin was lost during the 10 min subsequent to mixing; this figure would be very much less if referred only to voluntary muscle—thus Dewey (1959) has shown that [^{131}I]labelled serum albumin passes through muscle capillary walls at a rate which is only about 1/100th of that occurring in visceral capillaries. There is, then, no reason to suppose that the use of Nembutal significantly increased the rate of b.s.a. passage into the extra-

cellular space in these experiments. More important changes were, however, found to occur in the blood volume. For skeletal muscle, Rieke & Everett (1957) obtained a red cell volume of 0·63 %, a plasma volume of 1·64 %, thus a total blood volume of 2·27 %, and a haematocrit of 27·7 %. The present experiments rendered values, in the radioactive studies, of 0·62 %, 1·81 %, 2·43 % and 25·5 % respectively. Rieke & Everett found that their results for muscle in animals anaesthetized with Nembutal were less than controls by 12 % for the vascular space, and by 13·9 % for the haematocrit. If one accepts their findings, then the values obtained in the present study must be increased by 12 %. In the radioactive work this would lead to a vascular space value of 2·63 %. Whether such a correction should be applied to the perfusion result (2·74 %) is less certain: Nembutal acts on the muscle vessels through the spinal cord and higher centres, and these probably exert diminished influence over the hind limb vasculature during perfusion. The effect of Nembutal might thus be less significant in the perfusion experiments.

The fact that the stable value of 2·74 % for the vascular space took so long to achieve may be ascribed both in the sub-physiological (but, as has been explained super-critical) pressures applied, and also to the fact that non-particulate perfusates irrigate micro-vessels less rapidly than do particulate perfusates or whole blood (Zweifach, 1940).

One of us (R. O. L.) gratefully acknowledged the receipt of a S.R.C. studentship for the period during which this work was carried out.

REFERENCES

BIRD, J. W. C. SCHOTTELIUS, D. D. & SCHOTTELIUS, B. A. (1963). Oxygen consumption of soleus muscles from normal, vitamin-E supplemented and vitamin-E deficient guinea pigs during rest and stimulation. *Metabolism* **12**, 570–575.

BOYLE, P. J., CONWAY, E. J., KANE, P. & O'REILLY (1941). Volume of interfibre spaces in frog muscle and the calculation of concentrations in the fibre water. *J. Physiol.* **99**, 401–414.

BURTON, A. C. (1951). On the physical equilibrium of small blood vessels. *Am. J. Physiol.* **164**, 319–329.

COOLEY, G. (1954). Some observations on impurities present in Evans Blue (T. 1824) and their influence on blood-volume determinations effected by the dye method. *J. Physiol.* **123**, 16–21.

DANIELLI, J. F. (1941). A method for estimating the fraction of the volume of a muscle contained in the vascular system. *J. Physiol.* **100**, 239–245.

DEWEY, W. C. (1959). Vascular–extravascular exchange of ^{131}I-plasma proteins in the rat. *Am. J. Physiol.* **197**, 423–431.

DUYFF, J. W. & BOUMAN, H. D. (1927). Über die Kapillarisation einiger Kaninchenmuskeln. *Z. Zellforsch. mikrosk. Anat.* **5**, 596–614.

EVERETT, N. B., SIMMONS, B. S. & LASHEF, E. P. (1956). Distribution of blood (^{59}Fe) and plasma (^{131}I) volumes of rats determined by liquid nitrogen freezing. *Circulation Res.* **4**, 419–424.

FLEAR, C. T. G. & GRABER, I. G. (1963). Measurement of extracellular fluid volume using ^{131}I-labelled inulin. *Clin. chim. Acta*, **8**, 333–342.

FLOREY, H. (1964). The transport of materials across the capillary wall. *Q. Jl exp. Physiol.* **49**, 117–128.

HENDLEY, E. D. & SCHILLER, A. A. (1954). Changes in capillary permeability during hypoxemic perfusion of rat hind legs. *Am. J. Physiol.* **179**, 216–220.

HYMAN, C. & CHAMBERS, R. (1943). Effect of adrenal cortical compounds on oedema formation in frog's hind limbs. *Endocrinology* **32**, 310–318.

KREBS, H. (1950). Body size and tissue respiration. *Biochim. biophys. Acta* **4**, 249–269.

KROGH, A. (1929). *Anatomy and Physiology of Capillaries*, pp. 22–46. New Haven: Yale University Press.

LEESON, D. & REEVE, E. B. (1949). A method for testing the purity of commerical samples of T.1824 with observations on the amounts of coloured impurities present and the errors in plasma volume estimation caused by them. *J. Physiol.* **109**, 170–176.

MAIZELS, M. (1945). Intercellular plasma in the centrifuged erythrocytes of normal human blood. *Q. Jl. exp. Physiol.* **33**, 129–136.

MAIZELS, M. & REMINGTON, M. (1959). Percentage of intercellular medium in human erythrocytes centrifuged from albumin and other media. *J. Physiol.* **145**, 658–666.

NICHOL, J., GIRLING, W., JERRARD, W., CLAXTON, E. B. & BURTON A. C. (1951). Fundamental instability of the small blood vessels and critical closing pressures in vascular beds. *Am. J. Physiol.* **164**, 330–344.

PAPPENHEIMER, J. R. (1941). Blood flow, arterial oxygen saturation, and oxygen consumption in the isolated perfused hind limb of the dog. *J. Physiol.* **99**, 283–303.

PAPPENHEIMER, J. R. & MAES, J. P. (1942). A quantitative measure of the vasomotor tone in the hindlimb muscle of the dog. *Am. J. Physiol.* **137**, 187–199.

PAPPENHEIMER, J. R. & SOTO-RIVERA, A (1948). Effective osmotic pressure of the plasma proteins and other quantities associated with the capillary circulation in the hindlimbs of the cats and dogs. *Am. J. Physiol.* **152**, 471–491.

RIEKE, W. O. & EVERETT, N. B. (1957). Effect of pentobarbital anaesthesia on the blood values of rat organs and tissues. *Am. J. Physiol.* **188**, 403–408.

SHADLE, O. W., ZUKOF, H. & DIANA, J. (1958). Translocation of blood from the isolated dog's hind limb during levarterenol infusion and sciatic serve stimulation. *Circulation Res.* **6**, 326–333.

STOEL, F. (1925). Über die blutversorgung von weissen und roten Kaninchenmusken. *Z. Zellforsch. mikrosk. Anat.* **3**, 91–98.

WHITTAKER, S. R. F. & WINTON, F. R. (1933). The apparent viscosity of blood flowing in the isolated hindlimb of the dog, and its variation with corpuscular concentration. *J. Physiol.* **78**, 339–369.

YOUNG, D. A. B. (1964). A method for extraction of Evans Blue from plasma and tissues. *Proc. Soc. exp. Biol. Med.* **116**, 220–222.

ZWEIFACH, E. (1940). The distribution of blood perfusates in capillary circulation. *Am. J. Physiol.* **130**, 512–520.