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THE CIRCULATION TIME IN THE CAT, STUDIED BY A CONDUCTIVITY METHOD

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The time course of the action of certain drugs in the cat, particularly histamine liberators (MacIntosh & Paton, 1947), suggested that its relation to the circulation time should be investigated. We could find in the literature, however, no measurements of circulation time in the cat, and these experiments were designed to remedy this deficiency. The method used was to record the changes in the electrical conductivity of the blood after injection of substances of varying conductivity into the circulation. Stewart (1894), using rabbits and dogs, also used this method to measure the time between an injection and the first change in conductivity in the blood at various points. Others (see Ruskin & Decherd, 1947, for references) have recorded circulation times by the injection of substances known to act at various sites (e.g. decholin, saccharin, histamine, sodium cyanide, nikethamide). But all these methods have the disadvantage that they measure only one particular time; in general, that of the shortest path from the site of injection to some specific organ. It is clear that there may be a wide range of paths by which blood may travel between two points in the body, and we have tried by continuous recording to demonstrate the resulting range of circulation times, both in the greater and lesser circulation. In this paper we describe the method of recording time-concentration curves of a substance injected into the circulation and of deducing from these curves the average circulation time and the cause of its variation. It is hoped to discuss in a later paper the relation of such curves to the action of certain drugs (cf. Gray & Paton, 1948).

METHODS

Preparations. Cats under chloralose anaesthesia have been used in all experiments; they were eviscerated when necessary. After all dissection had been completed, time was allowed for capillary oozing to cease, and for firm clots to be formed. Heparin (2 ml. of 1% sodium salt) was then injected intravenously, and not less than 15 min. later, cannulae were inserted. Electrode cannulae, which were tied in continuity with a vessel, were inserted as quickly as possible to minimize disturbance of the circulation. The cannulae for injection were either glass cannulae tied

into a vein or were blunt needle cannulae which were inserted through small side vessels so that their tips lay in the vessel into which it was desired to inject. In the latter case adjacent branches of the vessel concerned were tied and divided.

Injecting system. The injecting system consisted of a graduated pipette fitted to a two-way tap. One arm of the tap was connected with a metal adaptor which could be plugged into the needle cannulae, and the other with a reservoir. In order to minimize distensibility, the junctions were kept as short as possible and made with plastic tubing. The other end of the pipette was connected with a pressure reservoir which enabled any chosen rate of flow to be maintained uniformly. In some experiments injections were made from a pipette into a glass cannula tied into the main vein.

Electrode cannulae. It was necessary to measure the resistance of the blood without interference from the resistance of the vessel wall or of the external fluid, or from changes in the length and diameter of the vessel. For this reason it was decided to mount the electrodes in a cannula which could be tied in continuity with the vessel. Three electrodes were used, the outer two being con-

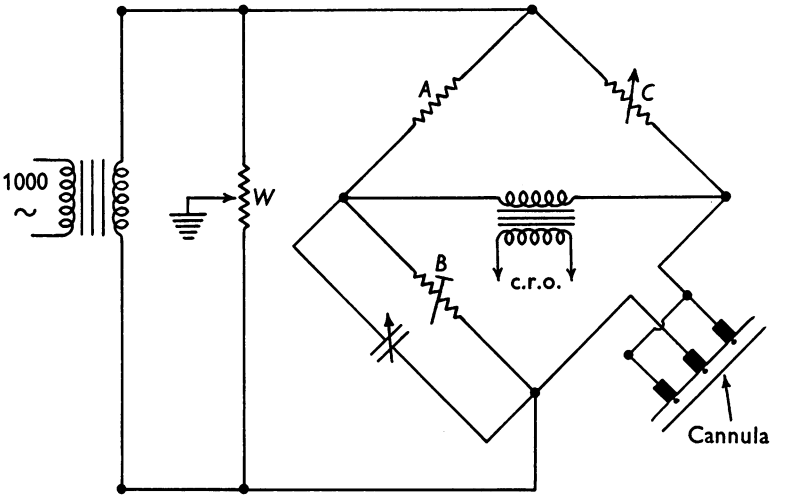

Fig. 1. Diagram of a.c. impedance bridge and cannula. Normal working conditions; $A = B = 2000\ \Omega$. Final balance of resistance obtained with C and the bridge balanced against earth with the potentiometer W.

nected, thus short circuiting the parallel resistance provided by the blood and tissues in contact with the ends of the cannula. As shown in Fig. 1, a glass tube of 3 mm. internal diameter was provided with three side arms 10 mm. apart into which 0·5 mm. diameter silver wires were fixed with de Khotinsky's cement. When filled with blood the cannula had a resistance of about 2000 Ω.; the true capacity of blood at low frequencies has been shown by Fricke & Morse (1926) to be of the order of 100 $\mu\mu$F. for a cubic centimetre of blood, the same volume having a resistance of the order of 200 Ω. This capacity would imply a reactance at 1000 cyc./sec. of about 10 MΩ. in parallel with our resistance and can clearly be ignored. Various minor modifications in the cannula were made at different times.

Bridge and recording system. The recording cannula formed one arm of a resistance bridge (Fig. 1). Resistances A and B were fixed equal to each other, usually at 2000 Ω. The bridge was balanced with resistance C, and the variable condenser was used to balance the capacity in the cannula. 3 V. r.m.s. 1000 cyc./sec. was supplied to the bridge through a transformer, and the balance was recorded through a transformer coupling with amplifier and cathode-ray oscillograph. A double-beam oscillograph was used without time-base deflexion and the spots were photographed on

moving paper. One beam was connected to the output of the bridge and the other to an oscillator to give a time scale. A second tube was fitted with time-base deflexion and was used for balancing the bridge. The only deliberate connexion from the bridge and animal to earth was through the Wagner earth (potentiometer *W*). This was adjusted so that, at balance, the output of the bridge was also balanced against earth.

Calculations

The amplifier and bridge were calibrated at frequent intervals by replacing the cannula with a decade resistance box. The calibrations were plotted so that a change of width from the null point represented the percentage change of resistance in the cannula. The calibrations were sufficiently linear near the null point for the change of width to be used over the range of the initial widths employed. Changes of capacity in the cannula were not detectable with the type of deflexions discussed in this paper.

TABLE 1. Resistance of mixtures of (A) blood and 0·9% NaCl, and of (B) blood and 5% NaCl

Mixture	Observed resistance (Ω.)	Expected resistance (Ω.)	Error (%)
	A		
Whole blood	2440	—	—
0·9% NaCl	1390	—	—
98% blood	2405	2405	Nil
95% blood	2350	2355	−0·2
90% blood	2270	2265	+0·2
80% blood	2145	2120	+1·2
50% blood	1775	1770	+0·2
	B		
Whole blood	1015	—	—
5% NaCl	113	—	—
99% blood	963	940	+2·4
98% blood	890	876	+1·6
90% blood	597	565	+5·7

If it be assumed that the final resistance in the cannula is the resultant of the parallel resistances due to the blood and the injected substance, then the final resistance (R) can be calculated as

$$\frac{100-F}{r_1}+\frac{F}{r_2}=\frac{100}{R}, \tag{1}$$

where r_1 = resistance of the cannula when filled with blood at the temperature of the mixture; r_2 = resistance of the cannula when filled with injected substance at the temperature of the mixture; and F = the percentage concentration of the injected substance in the cannula blood at the specified time.

Stewart (1894) and Fricke (1933) have shown that this relation is not accurate if r_1 and r_2 are taken to represent the resistivities of cells and plasma respectively. However, when whole blood is diluted, as much as 50% with 0·9% NaCl or 10% with 5% NaCl, the simple assumption holds within the accuracy of our method (Table 1).

In practice r_1 is measured from the balance of the bridge immediately before the injection; r_2 is measured in the same cannula immediately after the experiment, the necessary temperature correction being applied. The percentage change of resistance (x) is obtained from the record and equals $\frac{100\,(r_1-R)}{r_1}$. Substituting for R in equation (1) and transposing, we obtain

$$F=\frac{100xr_2}{(100-x)\,(r_1-r_2)}. \tag{2}$$

If the volume flowing through the cannula in unit time is h, then the total volume, (V), of the injected solution which has passed through the cannula is given by the equation

$$v = h\int F dt = \frac{hr_2}{r_1 - r_2}\int \frac{100x}{100 - x}\, dt. \tag{3}$$

The full numerical evaluation of this integral is tedious, but approximation is possible. In a typical conductivity trace, x may not exceed 5–7%, and the mean value is considerably less. It is then possible to replace $\frac{100x}{100-x}$ by x in equation (3), with an error less than 5%. $\int x dt$ represents the area of the tracing and can be conveniently measured with a planimeter. If the rate of blood flow is known, the volume of the injected solution passing through the cannula can thus be calculated. Conversely, if the volume injected is known, the rate of blood flow can be calculated when recording is from the site of injection.

For the case where a steady state is obtained, using a constant rate of injection (I) over a time t, we have

$$v = It = hFt. \tag{4}$$

This equation is used in the calculations of blood flow, described at the end of the next section.

It is interesting to consider what happens at a junction or division of the blood stream. At a junction, the concentration F in one branch at any time t is diluted by the blood from another branch in which $F = O$, giving $\frac{kF}{k+K}$ as the concentration in the mixed stream, where k is the volume in unit time flowing in the former and K that in the latter. The area of the time—concentration relation therefore equals $\int \frac{kF}{k+K} dt$, and the volume of injected substance is $(k+K)\int \frac{kF}{k+K} dt$, which equals $k\int F\, dt$, the volume of injected substance in the branch before mixing.

At a division the concentration F at a given time t must be the same at the mouths of both branches. Therefore $\int F dt$ will be the same in both mouths. Under constant conditions of blood flow this integral must remain constant as it passes down each branch. The volume of injected substance in each branch will be proportional to the two rates of flow, being equal to $c\int F dt$ and $C\int F dt$, where c and C are the volumes in unit time flowing in the two vessels.

It should be noted that the area $\int F dt$ is not altered by being measured in a branch (although its shape may change); for although the amount of injected substance flowing down one branch is $\frac{c}{C+c}$ times that passing down the main trunk, the rate of flow past the recording cannula is also $\frac{c}{C+c}$ times the rate of flow in the main trunk.

These considerations can be conveniently illustrated by a typical example. (In what follows it is assumed that the injected substance is not distributed in the blood by the process of flowing; even if this occurs, however, the main argument is still valid.) Suppose that after an injection into a vein there is at time t a concentration F' of the injected substance in the blood of the vein. If the rate of flow in the vein is k then the volume injected (V) will equal $k\int F' dt$. During its passage to the heart this blood will be mixed with uncontaminated blood from other veins giving a concentration of $\frac{kF'}{k+K}$ as it enters the heart; $(k+K)$ being the total volume of blood entering the heart. It is this concentration which leaves the heart and passes into the arteries, so that the value of F recorded in the carotid is equal to $\frac{kF'}{k+K}$. If c equals the volume of blood flowing through the carotid

in unit time then the volume of injected substance in the carotid will equal $c\int \frac{kF'}{k+K}dt$. This volume could, if desired, be calculated from the conductivity record, if c was known. Usually, however, the total volume (V) of injected substance is of more interest. This volume (V) must all pass through the heart, and since the concentration at the aortic valves is $\frac{kF'}{K+k}$ and the cardiac output is $k+K$, then $V=(k+K)\int \frac{kF'}{k+K}dt$. V is known and $\int \frac{kF'}{k+K}dt$ can be obtained at any arterial branch: hence the cardiac output $(k+K)$ can be calculated (cf. White, 1947). This can be rewritten as

$$V=U\int F dt, \tag{5}$$

where V is the total volume injected, U is the cardiac output, F the percentage concentration measured after it has all passed through the heart and t is the time. More generally this formula gives the relation between the volume of substance (V) injected at any point, the area $\left(\int F dt\right)$ of the time—concentration relation at any other point (either in a main trunk or in a branch), and the rate of flow (U) of all that blood with which the injected material mixes between the point of injection and the point of recording.

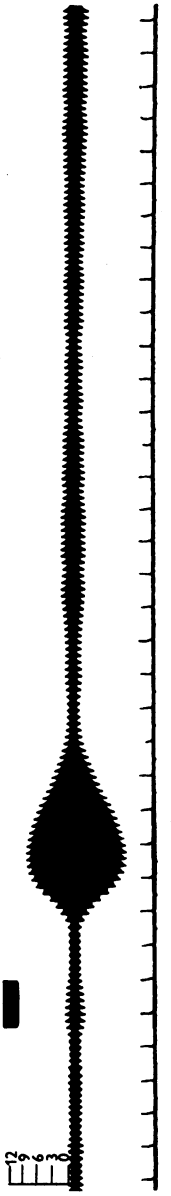

Fig. 2. Record of change in conductivity of carotid blood after the injection, during the signal, of 2 ml. of 0·9% saline into the femoral vein. Time, 1 sec. Ordinate = concentration % (F).

RESULTS

Control experiments

The injection into the circulation of a small volume of hypertonic sodium chloride solution, or of a larger volume of 0·9% sodium chloride causes the conductivity of the blood to increase. Fig. 2 is a record of the change of conductivity in the arterial blood after the intravenous injection of 2 ml. of 0·9% sodium chloride. (For this record and all others shown, the balance of the bridge was arranged so that an increase in the width of the tracing indicates an increase in conductivity of the blood in the recording cannula. Conversely, a decrease in width indicates a decrease in conductivity (cf. Fig. 3).) It was necessary to show that such a change of conductivity is due solely to the appearance between the electrodes of the injected substance and that this change accounts quantitatively for its total volume.

Causes of conductivity change. In the first place, the change in conductivity depends on the conductivity of the injected material. Thus, 0·9% NaCl which has a conductivity roughly twice that of blood causes an increase; on the other hand, an injection of isotonic dextrose, whose conductivity approaches zero,

causes a transient decrease in the conductivity of the blood. The sensitivity of the method, however, is such that changes in the conductivity of the blood due to other causes became significant; for example, small alterations in haematocrit value were easily detectable. The conductivity of the blood also depends on its rate of flow through the electrode cannula (this interesting phenomenon will not be discussed further in this paper, except in so far as it concerns our results). Finally, since the conductivity of an NaCl solution changes by about 2% per degree C., serious errors could arise were temperature not controlled.

The following experiments were done to test how far these factors were sources of error. In the first place, whole blood withdrawn from the animal and then reinjected had no effect on the conductivity. Similarly, a suspension of cells in 0·9% NaCl adjusted to the same proportion of cells as in the animal's blood, was without effect. These results appeared to exclude any contribution to change in conductivity from the mechanical effects of the injection itself. This conclusion was supported by finding that injections of histamine or adrenaline mixed with blood in doses adequate to produce a large change in blood pressure were also without effect on the conductivity of the blood within our normal recording period. A small change in conductivity was, however, observed, starting not less than 40–50 sec. after the injection.

Experiments with equiconductive solutions. Injections of isotonic dextrose solution caused, as mentioned above, a decrease in the conductivity of the blood. This implied that, besides whole blood or a suspension of cells in 0·9% NaCl, there should exist a mixture of 0·9% NaCl and isotonic dextrose solution equiconductive with blood, which would give no change in conductivity when injected. It was found, however, that such mixtures, although they were equiconductive with the blood of the animal used, caused a small increase followed by a small decrease in conductivity. Further, even after the injection of mixtures of definitely lower conductivity than the blood, this small increase preceded the main change in blood conductivity. Fig. 3 shows a typical series of diphasic responses. Such experiments suggested that the earlier portion of the injected mixture after entering the vein picked up electrolytes from, and lost glucose to, the tissues, thus becoming more conductive than subsequent portions. The restitution of the tissue electrolytes would account for the subsequent depression of conductivity. We have calculated that the peak change of conductivity is approximately 5·8% after the injection of an equiconductive solution (Fig. 3*b*). When various volumes of a solution of lower conductivity than the blood were injected the size of the hump did not increase with the volume of mixture injected, although the succeeding trough became larger and longer.

Electrolyte exchange. The experiments just described suggested that the injection of any solution with a solute concentration gradient to the tissues

may lead to an exchange of solutes affecting the change in conductivity. Exchange with the blood cells, however, does not appear to be important when blood is mixed with 5% NaCl (Table 1), for the observed changes in resistance agreed closely with those calculated on the assumption that all the electrolyte remained in the plasma. After injections of hypertonic NaCl one would expect these electrolyte changes to delay and flatten both rising and falling phases of the change of conductivity against time. Thus, in one experiment the mean femoral vein to carotid artery peak times were; with 0·9% NaCl, 7 sec.; isotonic dextrose, 8 sec.; and 22·5% NaCl, 8·5 sec. But in other experiments the times did not vary significantly with different solutions. The falling phase was usually prolonged and tended to obscure the gap between the first and second circulations. The possibility of such exchanges represents an argument

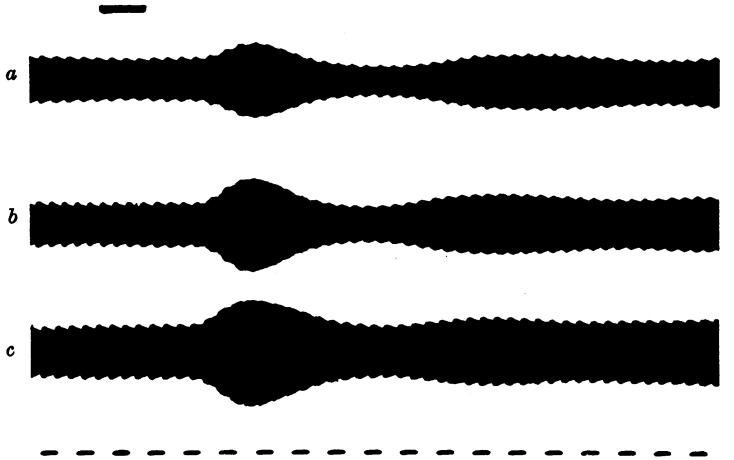

Fig. 3. Cat, 2·9 kg., chloralose. Injections into cannula tied into femoral vein of 5 ml. of various mixtures of 0·9% NaCl and isotonic dextrose; (*a*) 60, (*b*) 70, (*c*) 80% of 0·9% NaCl.

for the use of saline as an indicator, since it is isotonic and sufficiently similar in constitution to plasma not to lead to important solute exchanges, but it is clear that hypertonic NaCl can be used without serious error.

Velocity of flow. The sensitivity of the cannula to the velocity of the blood through it had two undesirable consequences; in an animal with low blood pressure there was a substantial variation in conductivity with each heart beat and sometimes even respiratory variation. The pulsation was only a technical nuisance, but the respiratory variation represented a change of a type and size sometimes to be confused with small changes due to injected saline. It was necessary, therefore, in some experiments to time the respiration and to relate it to the conductivity record, or to place the animal on artificial respiration. Unless the blood pressure was low, however, the respiratory and pulse variations were trivial and led to no confusion.

Temperature. Temperature errors can be considerable if an injection is made close to a recording cannula and the temperature is not controlled. When, however, the cannula is at some distance from the point of injection (for example, when it is in an artery and injections are made intravenously), it was found that the effect of the injected solution depended very little on its temperature. Injections of saline at body temperature or 20° C. below it produced changes in conductivity differing by less than 8%. It follows, therefore, that the injected fluid is rapidly warmed or cooled to body temperature. This heat exchange, however, cannot take place with the blood with which the fluid mixes, since this would alter its conductivity in proportion to the temperature of the injected solution. It must occur with some other heat reservoir, possibly the lungs in this instance.

Quantitative controls. The experiments described define the conditions in which it is possible to measure the conductivity changes due to the presence in the cannula of the injected substance. There is also evidence that the injected substance can be accounted for quantitatively. In the intact animal, it has been possible to show that the areas of the curves relating conductivity change to time (blood flow being constant) are proportional to the volumes of the injected substance (Table 2). Using an *in vitro* model it has been possible to go further and, by measuring the blood flow, to calculate within 10% the volume of substance which has, in fact, been injected (Table 3).

Finally, experiments were done on the reproducibility of the changes in conductivity of arterial blood after the injection of 0·9% NaCl intravenously. Table 4 gives the results of one such experiment.

The values in Table 2 of the ratio of the area of the conductivity tracing to the volume of saline injected are of interest. This ratio for the auricle-to-carotid route is larger than for the vein-to-carotid route, and this could be accounted for by a fall in cardiac output caused by opening the chest. A similar comparison between the vein-to-carotid route and the femoral artery to femoral vein route gives an estimate of the femoral blood flow as one-tenth of the cardiac output (this is based on the assumption that the blood passing down the femoral artery is not later mixed with any significant volume of blood free of saline from another arterial source).

Properties of the circulation

Circulation time after rapid injection. Fig. 2 shows the record obtained from the carotid cannula after injecting 2 ml. of 0·9% saline into the femoral vein. In this case the conventional technique was used, a glass cannula being tied into the femoral vein and the saline injected rapidly from a syringe or a pipette. Here the injection time was 1·1 sec. as can be seen from the signal. Sharp spikes on the record indicate the heart beat and the respiration can just be seen as a shallow undulation occurring at approximately 2 sec. intervals. The

frequency and amplitude of the respiratory change are such that they cannot be confused with the slower and larger waves due to the circulation of the injected substance. The resistivity of the blood was measured immediately before the

TABLE 2. Relation of the area of the conductivity tracing to the volume of saline injected

	Injection time (sec.)	Injection vol. (ml.)	Area ($mm.^2$)	Ratio (area/vol.)
Auricle to carotid artery	2	0·267	39·8	149
	5	0·576	82·8	144
	10	1·158	152·4	132
Femoral vein to carotid artery	2	0·182	20·5	113
	5	0·391	41·9	107
	10	0·829	86·9	105
Femoral artery to femoral vein	2	0·070	74·9	1070
	5	0·191	230·3	1220
	10	0·360	352·3	980

TABLE 3. Measurement of blood flow in an *in vitro* model

Rate of injection (ml./sec.)	Rate of blood flow (ml./sec.) Observed	Calculated	Deviation (%)
0·297	1·43	1·28	−10·5
0·268	1·47	1·47	0
0·267	1·43	1·51	+5·6
0·235	1·30	1·27	−2·3
0·225	1·40	1·45	+3·6
0·202	1·47	1·47	0
0·201	1·40	1·48	+5·7
0·190	1·43	1·37	−4·2
0·148	1·43	1·36	−4·9
0·145	1·37	1·35	−1·5
0·142	1·47	1·66	+12·9
1·126	1·40	1·48	+5·7
0·0938	1·40	1·35	−3·3
0·0937	1·50	1·54	+2·7
0·0931	1·43	1·56	+9·1
0·0688	1·37	1·34	−2·2

Mean deviation = 1·03 %
Root mean square deviation = 5·81 %

TABLE 4. Reproducibility of changes of conductivity after injections of 0·9 % NaCl. Six successive injections of 2 ml. into femoral vein. Recording from carotid artery

Time to start (sec.)	Time to peak (sec.)	Peak amplitude (mm.)
3½	5½	24
3	5½	23½
3½	5½	22½
3	5	20
3	5	18½
3½	5½	21½

record was taken, and that of the 0·9 % saline at the end of the experiment. The resistivity of the saline was corrected to the rectal temperature of the animal at the time of the injection. From these figures, and the calibration of the instrument, the scale of percentage concentration of injected substance (F) was constructed (see p. 175).

The first passage of the saline past the electrode is clear; it starts $3\frac{1}{4}$ sec. after the beginning of the injection, reaches a peak $F = 8{\cdot}8$ at $5\frac{1}{4}$ sec., and returns to its original base line at 9 sec. At 15 sec. another peak with $F = 1{\cdot}1$ occurs which, because of its shape, timing and regularity of occurrence is believed to be a second circulation of the saline through the cannula. The base line does not again return to its original level during this record, but there is a slight depression at $20\frac{1}{2}$ sec. before it takes up a new steady level at $F = 1$. From this level it returns after 5–10 min. to the original base line. The value of $F = 1$ for the uniform mixture of 2 ml. of saline with the blood implies a total circulating blood volume of 200 ml., which agrees well with the expected blood volume.

TABLE 5. Circulation times in the cat

		Time from beginning of injection to peak of response (sec.)	
	No. of cats	Mean	S.E. of mean
Femoral vein to carotid or aorta	13	6·3	0·32
Femoral vein to carotid or aorta (second time round)	8	18·2	1·43
Aorta to inferior vena cava	3	12·5	1·03
Aorta to carotid or aorta	8	14·1	1·30

(Aorta; injection cannula tied into inferior mesenteric artery.)

TABLE 6. Effect of volume and speed of injection on circulation time (femoral vein to carotid artery)

Exp.	Vol.	Injection time (sec.)	Injection velocity (ml./sec.)	Start to start (sec.)	Mean to peak (sec.)
31. x. 47	1	0·4	2·5	$5\frac{1}{2}$	$7\frac{1}{4}$
	2	0·6	3·3	$4\frac{1}{2}$	$6\frac{3}{4}$
	5	1·0	5·0	4	$6\frac{1}{2}$
	5	1·1	4·5	$3\frac{1}{2}$	$5\frac{1}{2}$
	10	1·9	5·3	$3\frac{1}{2}$	$5\frac{1}{2}$
19. xi. 47	3	15·7	0·2	$5\frac{1}{2}$	7
	3	7·3	0·4	4	$6\frac{1}{2}$
	3	4·2	0·7	$3\frac{1}{2}$	$5\frac{1}{2}$
	3	2·5	1·2	$3\frac{1}{2}$	$4\frac{3}{4}$
	3	1·1	2·7	$2\frac{1}{2}$	4
	3	1·0	3·0	$2\frac{1}{2}$	$4\frac{1}{2}$
	3	0·5	6·0	$2\frac{1}{2}$	$3\frac{3}{4}$

Advantages of this method. Table 5 summarizes circulation times obtained by this method. This type of record gives a direct picture of the distribution of an injected substance in the arterial blood after a common type of injection. 0·9% NaCl is the substance injected, so that diffusion of electrolytes is unlikely to have distorted the record. The record is calibrated as percentage concentration of injected substance; hence, if a drug is mixed with the saline and it is not absorbed specifically, then the distribution of the drug in the arterial blood is known. Information about starting and peak concentration times can be obtained, and the brevity of the injections allows the second responses to be seen. Nothing can, however, be said about the physiological scatter that occurs, since much of the spread seen here could occur in the process of injection.

Velocity and volume of injection. Even starting and peak concentration times are distorted when using this technique, since they depend on the velocity of injection. If, for example, a cannula is tied into the femoral vein the velocity of the saline cannot depend on the blood velocity until it reaches a major venous junction, such as that of the external and internal iliacs. For the early part of its course its velocity must depend entirely on the velocity of injection. The results given in Table 6 show that for the same volume injected reduction of the velocity of injection increases the measured circulation time.

These results also show that for a given velocity of injection a change in volume has little effect on the time recorded. This suggests that during injections such as these, in which the pressure is high compared with that of the venous

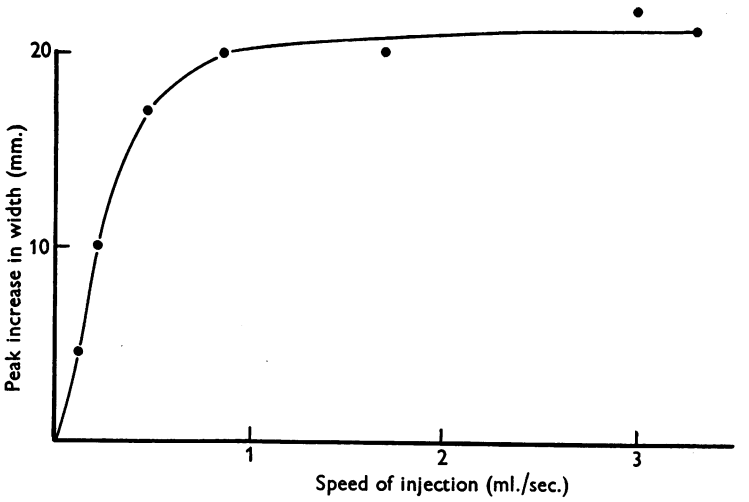

Fig. 4. Relation between peak increase in width of conductivity tracing and speed of injection of 0·9% NaCl into cannula tied into femoral vein. Cat, 3·4 kg., chloralose. Recording cannula in aorta.

blood, the injected solution spreads out into the venous bed almost equally with and against the stream; if the veins are exposed this process can indeed be seen. This implies that the errors of the method will be least if injections are made at a pressure high compared with the venous pressure and are of a volume which is small compared with that of the vascular bed between the sites of injection and of recording; the measured circulation time is then that from the first major confluence. Also, if an injection is sufficiently rapid to displace the blood entirely, a length of vessel at the main confluence will be filled with injected solution, and further increase in speed of injection will not increase the proportion of it there. Its peak concentration in the arterial blood will therefore approach a limiting value as the velocity of injection is increased, and will be independent of it over a wide range. This can be seen in Fig. 4 in which peak

concentration of saline in the aorta (expressed as increase in width of the record) is plotted against injection speed.

Measurement of the dispersion of the injected substance in the circulating blood

Preliminary observations. The experiments so far described have been concerned particularly with the size and time of the maximum change of conductivity of the blood after an injection into the circulation. These two measurements alone do not completely describe a record such as Fig. 2. For instance, in this record the conductivity begins to change 2 sec. before and does not return to its original level until $3\frac{3}{4}$ sec. after the peak, although the injection only lasted $1\frac{1}{2}$ sec. It follows that elements of injected material which entered the circulation at the same instant must have arrived at the recording cannula at widely different times. This is tantamount to saying that there are many different 'circulation times' between any two points of the circulation; and consequently a measure of this variation must be included if a complete description of the 'circulation time' is to be given.

The experiment illustrated in Fig. 5 was an early attempt to demonstrate this scatter by a direct comparison of records obtained as close as possible to the point of injection with those obtained at more distant points. Recording cannulae were tied into the inferior vena cava and aorta, and injections were made into the lumen of one or other of these vessels about 1 cm. upstream of the recording cannula. Fig. 5*a* shows the initial scatter after an injection into the inferior vena cava; this can be regarded as an approximately rectangular wave of injected material in the blood, of the same duration as the injection. Fig. 5*b* shows the wave after it has passed through the heart and lungs; Fig. 5*c* (latter part) the result of a complete circulation back to the inferior vena cava; and Fig. 5*b* (latter part) the result after $1\frac{1}{2}$ circulations back to the aorta again. Fig. 5 (*d–f*) shows a similar series starting from the aorta. These curves demonstrate clearly how the wave lengthens as it passes round the body; but since the circulation times were unusually long owing to the condition of the animal, no stress should be laid on the quantitative aspect.

These records can be regarded as frequency distribution curves of a population leaving the site of injection during the period of the injection. Ideally, it is the frequency distribution of the population leaving a single point in the circulation at a single instant under undisturbed conditions, which should be described. Experiments like the one just quoted had the great disadvantage that the operative procedures involved depressed the blood pressure and slowed the circulation rate. For describing the circulation quantitatively, therefore, a simpler procedure was adopted.

Quantitative measurements. In the first place, the necessity for recording the shape of the conductivity wave close to the site of injection was removed by limiting the speed of injection to a maximum of 0·1 ml./sec. This speed is small

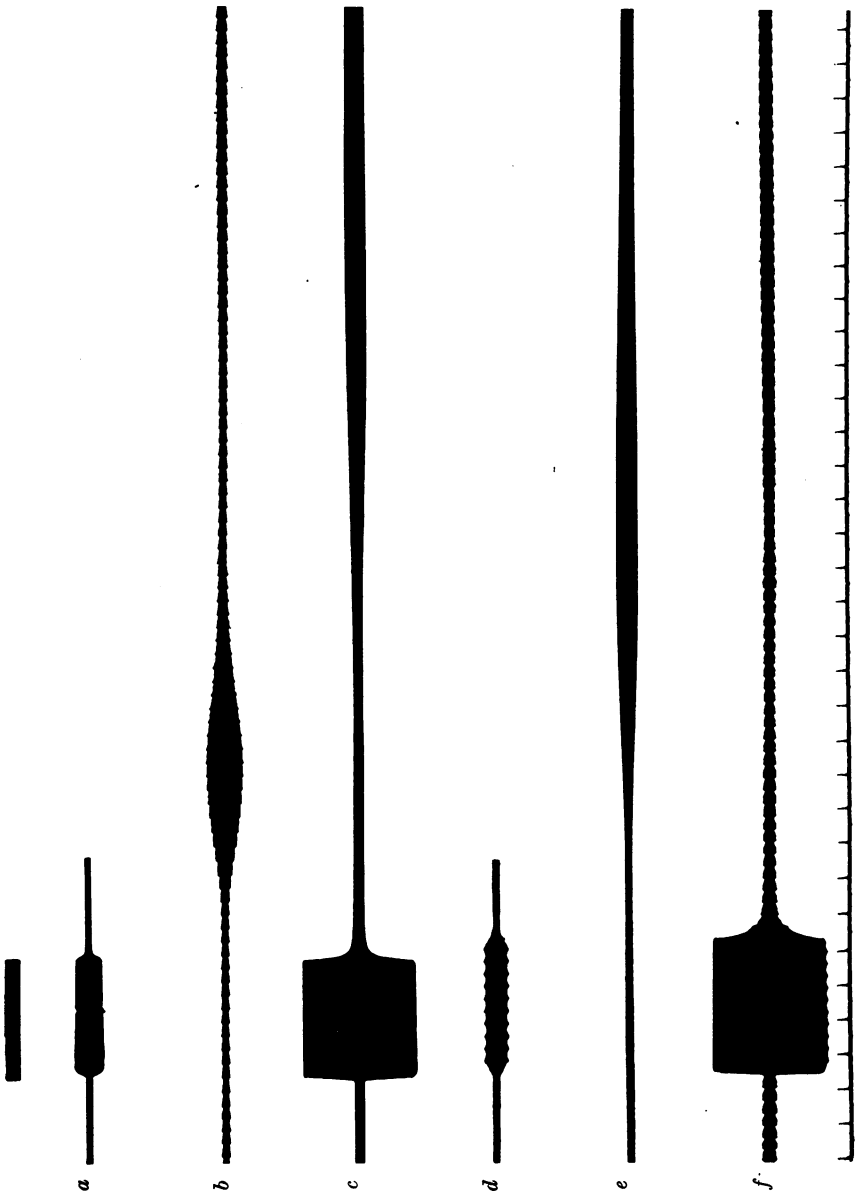

Fig. 5. Cat, 3·2 kg., chloralose. Recording cannulae in inferior vena cava and aorta. (*a*)–(*c*), injection of 3 ml. 0·9% NaCl into unobstructed inferior vena cava: (*a*) recording from vena cava (low amplification); (*b*) recording from aorta; (*c*) recording from vena cava (high amplification). (*d*)–(*f*), injection of 3 ml. of 0·9% NaCl into lumen of aorta: (*d*) recording from aorta (low amplification); (*e*) recording from vena cava; (*f*) recording from aorta (high amplication). Time, 1 sec.

compared with the velocity of blood flow in the blood vessels concerned, so that the resulting conductivity wave is rectangular, and its area proportional to the injected volume. This small injection velocity, however, meant that to obtain a measurable change in conductivity it was necessary to use injections of 3% NaCl lasting 2 sec. or longer; 2, 5 and 10 sec. injections were used, in order to allow extrapolation back to an instantaneous injection. The great advantage of this procedure was that it permitted the use of vessels accessible without major operative interference.

Three routes were investigated to show the effects of passage through the left heart alone, through both sides of the heart and pulmonary circulation, and through a limb. The details were as follows:

(*a*) A needle cannula was tied into the left auricle, and records were taken from the right carotid artery in the neck.

TABLE 7. Volumes and speeds of injection in experiments on the distribution of injected saline

	Theoretical injection time (sec.)	Injection time (sec.)	Vol. ml.	V/T
Left auricle to carotid	2	2·04	0·267	0·131
	5	5·10	0·576	0·113
	10	9·90	1·158	0·117
Femoral vein to carotid	2	2·14	0·182	0·085
	5	4·94	0·391	0·079
	10	10·01	0·829	0·083
Femoral artery to femoral vein	2	2·01	0·070	0·035
	5	5·17	0·191	0·037
	10	10·02	0·36	0·036

(*b*) A needle cannula was tied into a small branch of the right femoral vein just below the inguinal ligament, so that the tip of the cannula lay in the lumen of the femoral vein. Records were obtained from the right carotid artery.

(*c*) Injections were made into the lumen of the left femoral artery by tying a cannula into a small branch just below the inguinal ligament. The recording cannula was tied in the left femoral vein below the inguinal ligament.

The routine was carried out in five cats. In each experiment the records from the left femoral vein were taken first, followed by those from the femoral vein to carotid. The chest was not opened until these results had been obtained. In all experiments the blood pressure remained steady until the chest was opened.

Table 7 shows the mean times, volumes and rates of injection for each injection time and each route.

All records tended to have a wider, i.e. more conductive base line at the end than at the beginning. For vein to carotid and auricle to carotid records this was of the size to be expected from recirculation, but for the leg the changes were bigger than the recirculation of the small volumes used could account for, and may have been due to a slow trickle from capillary beds that were almost closed and had very long circulation times. However, before the data could be

handled it was essential that all curves should be brought to a finite end, even if a small distortion in the final result occurred. For this reason arbitrary base lines had to be adopted. With the auricle to carotid and vein to carotid records the base line was assumed to continue at its pre-injection level until recirculation began and then to rise linearly to the final base line. The point at which recirculation began was arbitrarily assumed for each cat as the time of the peak after a 2 sec. injection. This point was known to be approximately correct from other records. The limb records were corrected by assuming a linearly rising base line throughout the record.

All records were measured, the base lines subtracted, and the results plotted independently. The mean, mode and standard deviation of each individual curve was calculated. Table 8 gives the means and standard errors of these three statistics, of the original curves, together with the statistics of the

TABLE 8. Statistics of the circulation time

	Injection time (sec.)	Time from mean injection time to mean circulation time (sec.)		Time from mean of injection time to mode of circulation time (sec.)		Standard deviation of circulation times (sec.)	
		Mean of 5 exps.	S.E. of mean	Mean of 5 exps.	S.E. of mean	Mean of 5 exps.	S.E. of mean
Thoretical initial distribution	2	—	—	—	—	0·6	—
	5	—	—	—	—	1·4	—
	10	—	—	—	—	2·9	—
Left auricle to carotid artery	2	2·9	0·47	—	—	1·5	0·25
	5	3·0	0·33	—	—	2·1	0·11
	10	2·4	0·79	—	—	3·1	0·08
Femoral vein to carotid artery	2	10·1	0·59	10·0	0·64	2·9	0·29
	5	9·3	0·48	9·0	0·44	2·8	0·22
	10	8·4	0·44	9·5	0·29	3·5	0·23
Femoral artery to femoral vein	2	13·0	0·53	12·5	0·62	4·6	0·38
	5	12·2	0·44	11·1	0·83	4·7	0·43
	10	10·7	0·59	10·6	0·30	4·4	0·27

theoretical rectangular injection distributions. It will be seen that with the 2 sec. injection the standard deviation rapidly increases as the blood passes through the sections of the circulation. With the longer injection times this increase of standard deviation is obscured by the length of the injection time. The mean and modal times are shorter for long injections than for short. The fact that they do not agree within reasonable limits of error with the times given in Table 6, together with the relation of these results to the parameters of the frequency distribution of a population leaving a given point at one instant, will be discussed below.

The individual curves were all corrected to have equal areas and were alined on their means. The curves were then averaged over the five cats, giving nine mean curves illustrating three injection times through the three routes. These, together with the theoretical initial distribution, are shown in Fig. 6. In this figure the areas of the curves are proportional to the theoretical injection times,

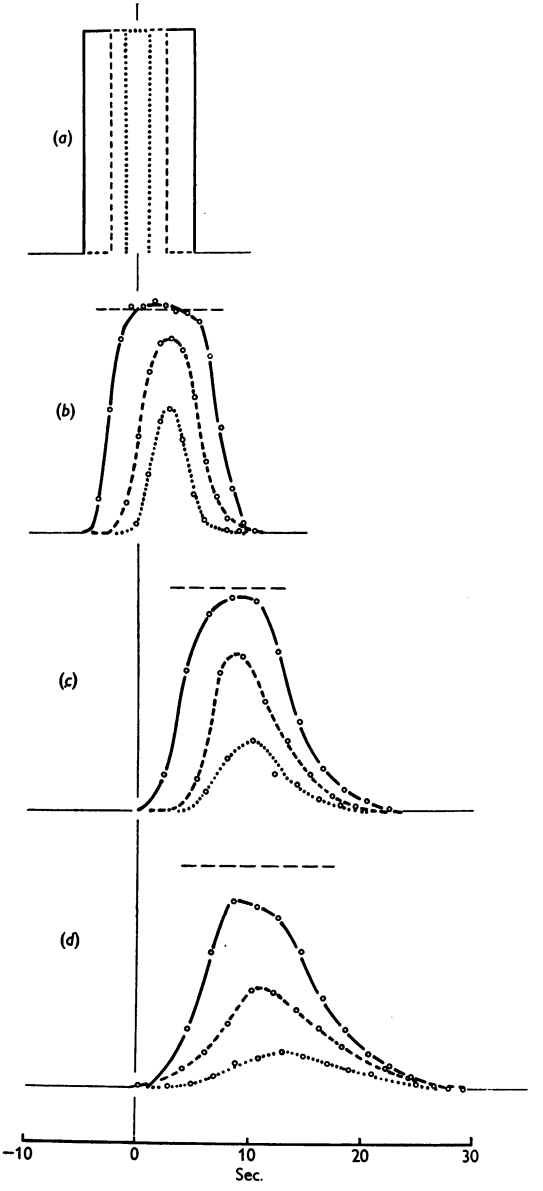

Fig. 6. Distributions of change of conductivity against time (mean of five cats); (*a*) Theoretical initial distribution; (*b*) injection into left auricle, recording from carotid artery; (*c*) injection into femoral vein, recording from carotid artery; (*d*) injection into femoral artery, recording from femoral vein. O———O, 10 sec. injection; O- - - - -O, 5 sec. injection; O . . . . . O, 2 sec. injection. For other details, see text.

and zero time is taken as the mean of the injection. Doted lines indicating the height of the rectangles have been drawn above each curve.

DISCUSSION

The main object of our experiments has been to describe how a solution is distributed throughout the circulation immediately after it has been injected into a vein. The final quantitative experiments, using a technique adequate to provide such information, are summarized in Fig. 6 and Table 8. Before discussing the results, however, it is necessary to consider how far physiological conditions have been distorted by using hypertonic saline in these experiments, and what is the cause of the discrepancy between the circulation times then observed and those observed with rapid injections of saline into a cannula occluding the femoral vein.

We have already mentioned the technical reasons for which we were obliged to use a hypertonic solution; but we believe that this has not distorted the results significantly. It is indeed probable that some exchange of electrolyte always occurs between the tissues and an injected fluid, where these differ in electrolyte content; the experiments with 'equiconductive' solutions illustrate this exchange. But it is impossible to predict from these results in which electrolyte passes from tissues to injected fluid, and in which the volume of injected fluid is large, what would happen if small volumes of hypertonic saline were introduced into the circulation. We have already shown that passage of electrolyte into red cells is negligible, but we have not studied directly the possibility of exchange elsewhere. The results in Table 2 make it unlikely that this is important, since it is improbable that the exchange would always affect the same proportion of the injected material independently of its volume. Stewart (1921), using hypertonic saline, White (1947) using 3–5% NaCl, and Hamilton & Remington (1947), using thiocyanate, give evidence that disappearance of injected electrolyte from the blood during its passage from a vein to an artery does not exceed about 10%. Finally, our experiments with isotonic dextrose and with strongly hypertonic solutions gave circulation times close to those obtained with saline, and the shape of the conductivity tracing was altered but little. Cardiovascular reactions to hypertonic solutions have been described (e.g. Weed & McKibben, 1919), but this was with larger volumes of stronger solutions than we have used, and we have observed no changes whatever in blood pressure or pulse rate as a result of our injections.

The discrepancy between the two estimates of circulation time from femoral vein to carotid artery (Tables 6 and 8) is substantial and significant. But there are at least three factors tending to accelerate the circulation after a rapid injection, of 2–3 ml. volume, into a cannula tied into the femoral vein. The first is that the injection itself speeds the passage of the saline up the vein to the iliac bifurcation, instead of leaving it to be carried by the femoral blood flow.

This might shorten circulation time by about one second. Secondly, with volumes of this size it seems probable from Table 6 that while the injected saline spreads both up- and down-stream when it reaches the iliac bifurcation (as we have discussed), it, nevertheless, spreads somewhat more towards the heart than away from it; after correcting the figures in Table 6 for differences in velocity, we estimate that the reduction in circulation time from this cause is about $\frac{3}{4}$ sec. Finally, and possibly most important, the introduction of saline into the circulation alters the viscosity of the blood considerably, since blood is at least four times as viscous as aqueous solutions of electrolytes. Direct inspection of the inferior vena cava after rapid injections of 2–3 ml. of a solution into the femoral vein shows that the blood is much diluted. There must be, therefore, a lowered resistance to flow in the vena cava and through the heart and lungs, as the column of diluted blood passes. We have estimated that this reduction in blood viscosity could well shorten the circulation time by 1–2 sec., and possibly more. These corrections taken together can account completely for the discrepancy. There is, however, one point for which we have at present no satisfactory explanation. In our final experiment the interval from the middle of the injection period to the peak or mean of the response (Table 8) diminishes as the injection period is lengthened. This does not appear to be due to electrolyte exchange, viscosity changes or recirculation; but it is of interest that the diminution of this interval appears to be associated with some degree of 'skewness' of the distribution (Fig. 6).

The discussion of these details may serve to emphasize the extent to which estimates of circulation time may vary with conditions of measurement. To those we have mentioned (salinity, volume and viscosity of injected material, and speed and method of injection) must be added such factors as anaesthesia and body temperature. The times in Table 8 represent the speed of the undisturbed circulation in a cat anaesthetized with chloralose, but are of restricted application. Those in Table 6 are of more general application, being determined with rapid injections such as are commonly used of 2–3 ml. into an occluded femoral vein. Although such comparisons are difficult, it is of interest that the values recorded are intermediate between those recorded for the anaesthetized dog (Hamilton, 1928) and for the anaesthetized rabbit (Stewart, 1894).

Besides the interval between injection and appearance of injected material at the recording cannula, two other statistics are of importance. The first is the peak concentration. This varied with the speed of injection, and from cat to cat, but as a rule the peak percentage concentration of the injected substance in the carotid blood lay between 2 and 10%, after an injection into the femoral vein; after a further passage of the circulation, the peak concentration had fallen to not more than 1%. Thus, the injected material is almost completely mixed with the blood in one circulation.

The rapidity of mixing is relevant to the discussion of the other statistic, the

dispersion in time of the concentration of injected material round its mean time. The magnitude of this dispersion for an instantaneous injection can be estimated from Fig. 6 and Table 8 by extrapolation. It will be seen that whereas the variance of the distribution increases with increase in duration of injection for the rapid L-auricle to carotid artery route, it changes less for the femoral vein to carotid artery and still less for the femoral artery to femoral vein routes. By extrapolation the approximate values of 1·1, 2·5 and 4·5 sec. respectively are obtained for the standard deviations of the distribution that would follow an instantaneous injection.

Some of the dispersion might be due to turbulent mixing of the injected fluid with blood in front of and behind it; this mixing will be at its greatest in the left heart, but after passage through the heart an injection still appears as a nearly 'rectangular wave'. Alternatively, if the flow is stream-lined, scatter might be caused by 'coning' because the velocity of the centre of a fluid stream is greater than that at the sides. The maximum velocity of the fluid passing down a tube in laminar flow is twice the average velocity, regardless of the diameter of the tube: it is to be expected, therefore, that the dispersion of injected material from this cause would depend on the distance traversed, and not on the characteristics of the vessels through which the blood flowed. But this is obviously not the case: the mean distance along vascular paths in a 2·5 kg. cat from the femoral vein cannula to the carotid cannula is of the order of 40–50 cm., while that from the point of injection into the femoral artery to the recording cannula in the femoral vein cannot have been more than 25 cm. Yet Fig. 6 and Table 8 show a much greater dispersion in the latter than in the former circumstances. The dispersion introduced by the femoral capillary bed is the less to be expected in view of the observation that breaking up a tube of large cross-section into many smaller tubes leads to flow of less dispersion (Henderson, Chillingworth & Whitney, 1915; Stewart, 1898). Therefore, although mixing and coning may well explain the dispersion observed for the left auricle to carotid and femoral vein to carotid routes, it appears quite inadequate to explain the dispersion caused by the femoral artery to femoral vein circuit.

The remaining cause of dispersion to be considered is that there may be a wide variety of vascular paths, some fast and some slow, through which the blood passes between the main vascular trunks. This is probable on general grounds, since it is unlikely that an absolute constancy of length and diameter of arteries, capillaries and veins, is maintained throughout a tissue. It is at present impossible to calculate precisely the proportion of the dispersion due to this cause. It is, however, reasonable to suppose that the variance for the femoral artery to femoral vein time-distribution curves, due to causes other than a variety of vascular paths, cannot exceed the variance of the femoral vein to carotid artery curves, and is probably somewhat less. If we then assume that the variance of the femoral artery to femoral vein dispersion is made up of two

additive components, that due to a multiplicity of paths and that due to other causes, we can subtract from the total variance observed for this route that observed for the femoral vein to carotid artery route, so obtaining a minimum value of the dispersion due to multiplicity of paths. Thus the required variance is $(4{\cdot}5)^2-(2{\cdot}5)^2=14{\cdot}0$ sec. and $\pm 2\times\sqrt{14{\cdot}0}$ gives the range of time (14·8 sec.) within which the circulation through 95% of the paths is completed. The dispersion due to the capillary bed of the lungs must in any case be relatively small compared with that due to the capillary bed of the leg: for if it contributed at all significantly to the variance of the femoral vein to carotid passage, our estimate of the variance for the leg circulation must be correspondingly increased.

This degree of dispersion in the leg is substantial, but there is sufficient evidence that it is a possible outcome of normal vascular function. The existence of arteriovenous anastomoses is well recognized, and can account for rapid transit from artery to vein. But further than this, the metarterioles and precapillary sphincteric mechanism recently analysed by Chambers & Zweifach (1944) render possible a variability of vascular path of the type and extent required by our results. We suggest, therefore, that at least that part of a dispersion which cannot be accounted for by turbulent or laminar mixing, reflects a substantial variation of length and cross-section of circulatory paths existing in the intimate vascular structure of the tissues, and that such dispersion is considerable in the circulation of the leg and slight in the pulmonary circulation.

SUMMARY

1. Certain properties of the circulation have been investigated, in cats anaesthetized with chloralose, by continuously recording the changes of electrical conductivity of arterial and venous blood caused by the injection of solutions of various conductivities into the circulation. Our technique was sensitive to a change of conductivity of 0·5%.

2. Control experiments have shown that these changes are due to the injected material, and that this can be accounted for quantitatively, provided consideration is given to the temperature of the injected substance and to constancy of blood flow through the recording cannula. Errors due to electrolyte exchange after the injection of hypertonic solutions were estimated to be less than 10% of the conductivity change.

3. With these precautions, the conductivity change observed is related by a simple formula to the concentration of the substance in the blood. A further relation is derived which enables blood flow to be calculated.

4. A brief injection of a saline solution into the circulation causes a transient change in the conductivity of the blood, which is more or less prolonged according to the vascular path between the point of injection and the point of

recording. Records of this change show the distribution of the injected material in the blood. This distribution can be conveniently described by the mean and standard deviation of its time and by its peak concentration and area.

5. Circulation times in the greater and lesser circulations have been measured by recording the time interval between the beginning of a rapid injection of salt solution into one vessel and the moment of maximum conductivity change in another.

6. The distributions in the blood of injected salt solution have been analysed. The means, modes and standard deviations have been measured for the three routes: left auricle to carotid artery, femoral vein to carotid artery, and femoral artery to femoral vein. The characteristics of the circulation which cause these distributions are discussed.

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