Transcapillary Pulmonary Exchange of Water in the Dog[1]

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THE CAPILLARIES in the lungs, in contrast to capillaries elsewhere in the body, are in effect bathed in air rather than water. There is little histological evidence for a substantial pulmonary interstitial space; instead, the bulk of the lung tissue appears to consist of cells and blood vessels. Perhaps because of these anatomical peculiarities little or no consideration appears to have been given to the possibility that large amounts of water might exchange across the walls of these capillaries. The data presented below, which are part of a more general study of capillary permeability, suggest that, in the normal dog, water does cross these capillaries in amounts per unit time which approach in magnitude the pulmonary blood flow.

METHODS

Experimental Procedure. The general approach outlined briefly elsewhere (1, 2) has been used. Mongrel dogs were anesthetized by the intravenous administration of sodium pentobarbital in the dosage of approximately 24 mg/kg of body weight and kept in a supine position for the duration of the experiment. Additional sodium pentobarbital was administered as required. The jugular vein and the carotid or brachial artery were exposed. A solution of the sodium salt of heparin was given intravenously in the dosage of 100 USP u/kg of body weight. A polyethylene catheter, filled with 0.85% NaCl solution, was then inserted through a small slit into the exposed artery and advanced so that the tip was well within the lumen of the aorta. In most experiments it was possible to avoid placing a ligature around the artery and catheter as bleeding generally did not occur from the point of insertion of the catheter. The diameter of the catheter used was determined from the diameter of the blood vessel and was approximately one-third the latter. In most experiments, therefore, blood flow distal to the point of insertion of the catheter was not stopped. Blood was collected from the tip of the catheter in flat-bottomed

shell vials measuring approximately 10 x 40 mm and containing 0.03–0.06 ml of sodium heparin solution (1000 USP u/ml). The shell vials were held in a rack which was advanced at a pre-set rate so that blood was collected in each vial for a given period of time (generally 0.5–2.0 seconds). (Details of the collection device will be published later.) The experimental procedure was then to insert the needle of the syringe containing the mixture to be injected into the jugular vein, the collecting device was started, and injection was made within 1 second after sufficient blood had flowed through the catheter to clear the latter of NaCl solution. (The volume of solution injected was no greater than 1.5 ml; in later experiments approximately 0.1 ml/kg of body weight have been injected.) Between 0.4 and 2.0 ml of blood was collected in each vial. The animals were killed on completion of the experiments.

Analytical Methods. *T-1824.* Of whole blood, 0.2–0.5 ml was diluted with 1.5 ml of 0.85 % NaCl in colorimeter tubes. The blood and NaCl were well mixed, the tubes then centrifuged, and the optical densities of the supernatant solutions determined at 620 mμ in a Beckman Model B spectrophotometer. No detectable hemolysis was present when this determination was carried out on the day of the experiment.

Polyvinylpyrrolidone (PVP). Whole blood samples were analyzed as described elsewhere (3).

Deuterium and tritium. Whole blood samples of approximately 0.01 ml were placed in the special reduction tubes and the procedure described in (4) was followed. Deuterium determinations were carried out in a mass spectrometer designed and built by one of us (T. E.); tritium determinations were carried out by means of an ionization chamber. The ratio D/H was determined for deuterium; activity per unit pressure in the ionization chamber after opening the sample tube was determined for tritium.

Radioactive sodium, phosphorus and C[14]-labeled urea. Of whole blood, 0.09 ml was mixed with 0.01 ml of 1% sodium dodecyl sulfate (for hemolysis) and the resultant mixture quantitatively transferred to $\frac{7}{8}$-in. diameter Whatman No. 2 filter paper discs cemented with rubber cement to 1-in. diameter copper planchets. The planchets were then allowed to dry. P[32] activity was determined by means of a conventional end-window Geiger-Müller tube and scalar assembly. Na[22] activity was determined either as was the P[32] (positrons), or by means of a scintillation detector (γ particles), or in a windowless flow counter (positrons). The C[14] activity was determined in a windowless flowcounter. The number of counts recorded for each sample was such that the error, $100 \times \sqrt{N}/N$ where
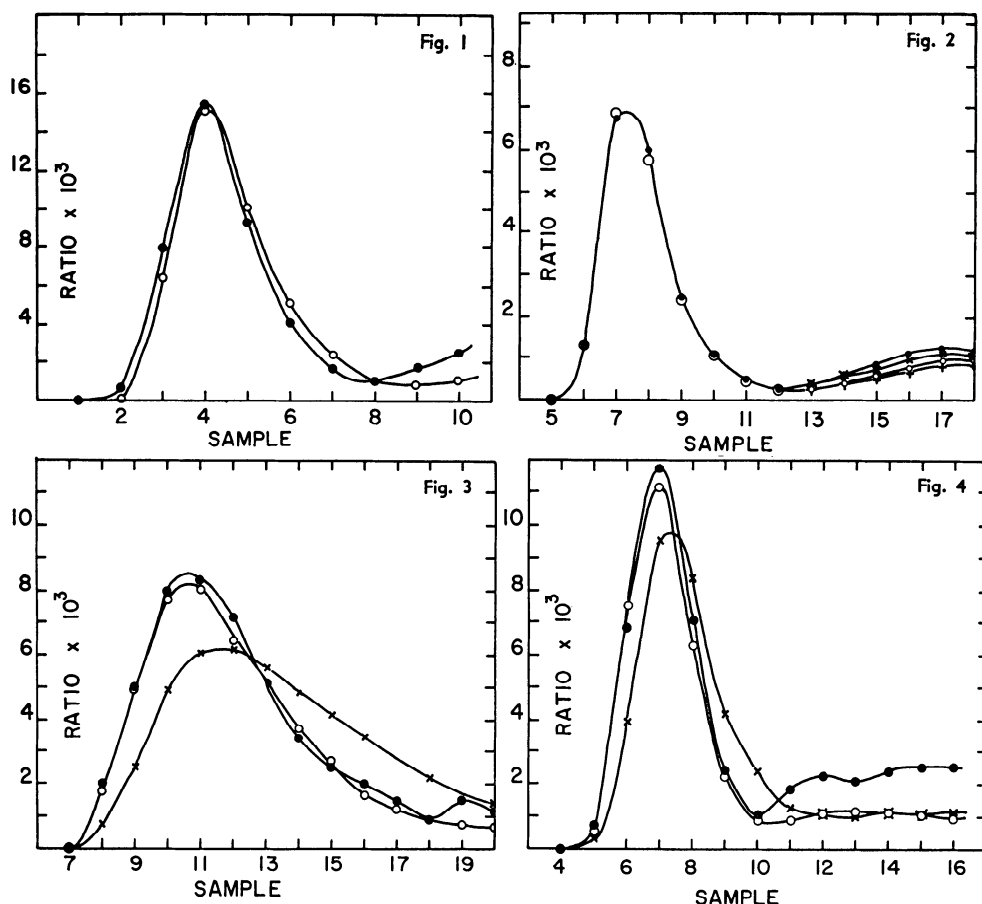
FIG. 1. Time-concentration ratio relationship following the 'instantaneous' injection, immediately prior to the collection of the first sample, of a mixture containing P^{32}-labeled red cells (*dots*) and PVP (*circles*). Collection time for each sample was 1.12 seconds.

FIG. 2. Time-concentration ratio relationship following the 'instantaneous' injection, during collection of the third sample, of a solution containing T-1824 (*dots*), inulin (*crosses*), thiocyanate (*circles*), and *p*-aminohippurate (*plusses*). The ratios for inulin, thiocyanate and *p*-aminohippurate are nearly identical during the first part of the curve and fall within the areas of the large circles. Collection time for each sample was 1.14 seconds.

FIG. 3. Time-concentration relationship following the 'instantaneous' injection, during collection of the third sample, of a solution containing T-1824 (*dots*), Na^{22} (*circles*) and deuterium oxide (*crosses*). Collection time for each sample was 0.96 seconds (*exper. 3* in table 1).

FIG. 4. Time-concentration ratio following the instantaneous injection, during collection of the third sample, of a solution containing T-1824 (*dots*), C^{14}-labeled urea (*circles*) and deuterium oxide (*crosses*). Collection time for each sample was 2.0 seconds (*exper. 6* in table 1).

N is counts/min., was less than ± 2%. The error was less than ± 1.5% for most of the experimental points.

Inulin, thiocyanate and p-aminohippurate. Conventional procedures (5–7), slightly modified and appropriately scaled down to the size of the samples available, were used.

Standards. Serial dilutions in whole blood were made of aliquots of the solution injected. These dilutions were carried through the various analytical procedures in exactly the same manner as the unknowns. The ratio of the concentration of a given substance in the experimental blood samples to the concentration of that substance in the solution injected is plotted against time.

RESULTS

Control Studies. It is well known that cells traverse the lungs in less time than does the plasma (e.g., 8, 9). In the dog, and with the technique described above, the same effect is seen in the experiment shown in figure 1. A mixture of polyvinylpyrrolidone (PVP) as a
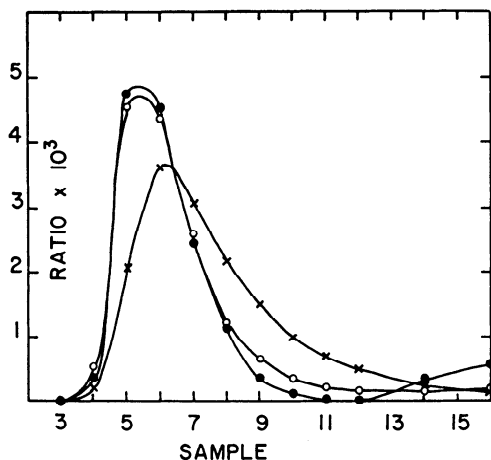
Fɪɢ. 5. Time-concentration ratio relationship following the 'instantaneous' injection, during collection of the first sample, of a solution containing T-1824 (*dots*), Na^{22} (*circles*) and tritium oxide (*crosses*). Collection time for each sample was 1.0 seconds (*exper. 2* in table 1).

3.5 % solution and P^{32}-labeled red cells was injected into the jugular vein and blood was sampled from the carotid artery. The separation of red cells and PVP (*i.e.*, plasma) though small is definite. That no loss of PVP from the circulation occurred is indicated by the fact that the areas under the two curves up to the point of recirculation are nearly identical. It is possible that substances which penetrate red cells rapidly could have a shorter circulation time through the heart-lung circuit than substances which remain in plasma. However, there is no reason to believe that marked separation could occur. Other substances have been used. Figure 2 shows the results of an experiment in which T-1824, inulin, thiocyanate, and *p*-aminohippurate were injected as a mixture. Within the limits of error of the analyses no significant separation of these substances occurred. Similar results were obtained in experiments with T-1824 and Na^{22} and with T-1824 and C^{14}-labeled urea. Some loss of these last two tracer substances occurred relative to T-1824 as can be seen in figures 3 and 4; the losses are, however, less than 10 per cent.

Experiments With Deuterium and Tritium Oxides. Typical results are shown in figures 3–5. The curves for deuterium (D) and for tritium (T) are displaced with respect to the curves for T-1824 and are lower initially;

this is taken to mean that there has been passage of D and T from blood across the capillary walls. Subsequently, until recirculation occurs, the D and T curves are higher than the T-1824 curves; this is taken to mean that there is return of D and T to the blood. The areas under the D and T curves are roughly equal to the areas under the corresponding T-1824 curves; this is taken to mean that there is no over-all loss of D or T from the circulation. In order to differentiate between passage from blood and return to blood, the procedure described below has been followed.

Calculations. Correction for recirculation of T-1824 and of D or T is made by linear extension of the experimental curve on a semilogarithmic plot. This correction is at best an approximation. However, as shown by the data in figures 3–5 most of the first passage and subsequent recirculation are quite distinct; it is unlikely that a very large error is introduced by the approximation in this correction.

It is now assumed that if a substance were lost from the circulation and if there were no return then the concentration ratio time curve of that substance would be less than but directly proportional to the curve of the reference substance at all points. To determine the curve for D or T which has not left the circulation semi-logarithmic plots are made of the T-1824 and D or T data (corrected for recirculation) on separate translucent sheets; the sheets are adjusted vertically so that the rapidly rising portions of the two curves are in as good register as possible. (This is equivalent to applying the assumption stated above and to assuming in addition that there is no significant return during the rapidly rising portions of the D or T curves. It it were possible to obtain enough data, it would be preferable to determine the ratio of the D or T concentration ratios to the corresponding T-1824 ratios for this portion of the curves; this factor would then be applied to the subsequent T-1824 data to give the value for the D or T concentration ratios which would have been obtained had there been no return. To obtain sufficient data for this mathematical procedure considerably greater amounts of blood would have been required than the size of the dogs permitted.) The D or T curve is then extended along the underlying T-1824 curve. The resultant curve for D or T is taken to represent

TABLE 1. SUMMARY OF DATA AND CALCULATIONS

Exper. No.	Area* T-1824 *a*	Area* D or T *b*	Ratio of Areas *a/b* *c*	Net D or T Remaining *d*	Net Loss of D or T† *a − d*	Net Fraction of D or T Lost *(a − d)/a*	Cardiac Output, *l/min.*	Water Crossing *l/min.*	Percent of Cardiac Output, *%*
1	52.4	54.4	0.96	19.1	33.3	0.64	1.53	0.78	51
2	13.7	15.5	0.88	4.4	9.2	0.68	1.75	0.95	54
3	48.1	52.2	0.92	22.4	25.7	0.54	2.20	0.94	43
4	64.0	65.0	0.99	33.9	30.1	0.47	1.97	0.74	38
5	27.5	28.0	0.98	17.1	10.5	0.38	2.44	0.74	31
6	29.8	31.2	0.96	12.3	17.6	0.59	1.71	0.81	47
7	28.9	26.6	1.09	13.6	15.3	0.53	1.53	0.64	42
8	26.2	23.3	1.01	10.3	15.9	0.61	1.47	0.71	48
Average			0.987						44.3

* By area (*columns a* and *b*) is meant the sum of the ratios obtained from the curves corrected for recirculation.   † Net loss of D or T could be calculated as $b − d$ rather than $a − d$. This has not been done for the following reasons. The T-1824 is the reference substance assumed not to leave the circulation; the data for D or T are compared to the T-1824 data. To establish a curve for the net loss of D or T, subtraction must be made point by point of the values calculated for the resultant curve from the corresponding points on the T-1824 curve.

the curve for D or T which has not left the circulation (net D or T remaining; *column d* in table 1). The difference between the resultant D or T curve and the experimental T-1824 curve is taken to represent the D or T which has left the circulation on a single passage through the lungs (net loss of D or T; $a–d$ in table 1). The D or T which has returned is obviously represented by the difference between the resultant and experimental D or T curves. The results of such calculations for the experiments illustrated in figures 3–5 and for other similar experiments are summarized in table 1. Cardiac outputs have been calculated in the conventional manner from the T-1824 curves corrected for recirculation. (As the concentrations of T-1824 are determined in whole blood, cardiac outputs have been calculated directly without recourse to hematocrit values.) The values for the amount of water crossing per minute are calculated from the cardiac output, the net fraction lost of D or T, and the assumption that whole blood contains 80% of water.

The uncertainty in the graphical estimation of the net D or T remaining does not exceed ±15%. The calculations for the net losses suffer from a similar uncertainty. It is evident that the results for the exchange of water given in table 1 are approximate. Other methods of calculating the net losses have been explored; these involve complicated mathematical formulations and certainly do not result in an increase of the accuracy of the results. The graphical method used here has,

at least, the advantage of being simple. The underlying assumptions made above for this graphical calculation may not be valid; no other seem to be available at present.

DISCUSSION

As noted above, the areas under the D or T curves, corrected for recirculation, are approximately equal to the areas under the corresponding T-1824 curves. The implication of this finding is that there is no over-all loss of water of any significance as the result of passage through the lungs. (The areas under the D or T curves are, as a matter of fact, significantly greater than the areas under the T-1824 curves in some experiments. This may be the result of systematic experimental errors. It may also be the result of irreversible loss of T-1824, perhaps because of binding to the vessel walls.) The loss as water vapor is obviously quantitatively insignificant with respect to the blood flow. The volume of distribution of water in the experiments described here is probably limited to the volume of the blood between the points of injection and of sampling and to the lung tissue; the heart, vessel walls, bronchial walls, and hilar structures are probably not perfused on the first circulation. The displacement and distortion of the D and T curves with respect to the T-1824 curves are interpreted to be the result of passage and return of water across the pulmonary capillary walls. As calculated above, the amount of water crossing from blood to lung tissue approaches 50% of the

blood flow.[3] It is of interest that there was little net loss of C^{14}-labeled urea relative to T-1824. It may be that the capillaries or cells of the lungs are considerably less permeable to urea than capillaries or cells elsewhere in the body. The loss of Na^{22} was also quite small relative to T-1824. This result is in agreement with the findings of other workers (e.g., 11) and further validates the use of Na^{22} to obtain values for cardiac outputs. (Calculations of cardiac outputs from the D or T data presented here have also been made; the results are within a small percentage of the results based on the T-1824 data.) The small losses of Na^{22}, if not the result of relative impermeability of the pulmonary capillaries to sodium ion, may be taken to support the view that there is normally little interstitial fluid in the lungs.

Calculations of exchange rates are generally based on data obtained from serial peripheral sampling of blood following the intravascular injection of the test substance; sampling is made by means of needle and syringe at intervals of seconds and minutes (e.g., 12, 13). The time-concentration data are fitted to mathematical expressions containing two or more exponentials. Since there is no significant overall loss of water in the lungs, it is difficult to see how calculations based on such data can include the exchange of water at the level of the lungs; such calculations are therefore underestimates of the actual exchange rates and by a large factor in the case of water. (A similar situation obtains in other organs (10).)

It may be pointed out here that this passage and exchange of water must take place by a mechanism of diffusion rather than by a mechanism of filtration. (See ref. 14 for definitions of these terms.) The pulmonary capillary pressure (P'') is generally accepted to have a value of 10 to 12 mm Hg. If the pulmonary interstitial fluid pressure (P') is less than this, outward filtration could occur but it is difficult to see how water could return except by diffusion. Furthermore, since the 'colloid osmotic pressure of the plasma proteins' (π'') has a value of approximately 35 mm Hg (consider-

ably greater than the value of P'') outward filtration as conventionally defined can not take place.

It is well known, however, that water introduced by way of the trachea into the lungs is rapidly absorbed into the blood. With interstitial fluid pressure of the order of 10 mm Hg or less, this suggests that the chemical potential of water in the pulmonary capillaries must be less than the chemical potential of water in pulmonary interstitial fluid and that normally there must be little or no pulmonary interstitial fluid. In spite of this difference of chemical potentials, exchange of water can still take place by diffusion and a measure of this exchange is obtained from the rate of exchange of deuterium or tritium oxides. Injection of deuterium or tritium oxides into the blood establishes a concentration gradient (and therefore a gradient of the chemical potential) for each of these substances across the capillary walls and passage occurs. Return to the blood stream occurs with reversal of the concentration gradient (and of the chemical potential gradient) which follows the continued passage of blood now relatively free of the injected substances. If there is no secretion of water, diffusion must be the mechanism of passage. The filtration hypothesis is not compatible with the experimental results presented here; the diffusion hypothesis is.

SUMMARY

An experimental procedure for the investigation of the transcapillary pulmonary exchange of water in dogs is described. 'Instantaneous' injection is made into the jugular vein of a solution containing a reference substance (e.g. labeled red cells or T-1824), which is assumed not to leave the blood stream, and the test substances. Blood is obtained from a catheter in the carotid or brachial artery at intervals of 0.5–2.0 seconds. Analysis of these samples shows that there is negligible displacement of such materials as sodium, *p*-aminohippurate, and thiocyanate ions, inulin, and urea with respect to the reference substance. With both deuterium and tritium oxides there is significant displacement with respect to the reference substance though there is no over-all loss. The displacement without loss is interpreted as being the result of passage and return of the water across the capillaries.

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[3] The validity of using deuterium oxide and tritium oxide as tracers for ordinary water substance in such experiments will be discussed in another communication (10).

Calculations of the exchange rate of water across the pulmonary capillaries indicate that this rate approaches in magnitude the pulmonary blood flow, i.e., the cardiac output. It is pointed out that conventional sampling techniques for the calculation of exchange rates do not take this exchange at the level of the lungs into account. It is concluded that the filtration hypothesis is not compatible with the experimental data presented here but that the diffusion hypothesis is.

It is a pleasure to acknowledge the collaboration of Dr. Louis B. Flexner in the control studies and the technical assistance of Miss Barbara Rayboen in the later experiments.

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