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FACTORS CONTROLLING THE WASHOUT OF THE INTERSTITIAL SPACE OF THE ISOLATED, PERFUSED RAT HEART

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SUMMARY

1. The time course of the washout of the extracellular markers, inulin, raffinose, sucrose and sorbitol, has been determined for the isolated rat heart perfused for 20 min with Krebs bicarbonate medium containing the marker, and washed out with marker-free perfusate.

2. The filtration problem for the perfused rat heart was found to be due to omission of the gassing of the concentrated sodium bicarbonate solution with CO_2 in the preparation of the perfusate. This probably caused calcium phosphate to be precipitated. The reduced contractility of the heart was reflected in the washout of the extracellular markers.

3. The logarithmic plot of the heart content of the marker against time showed two components. The slower was an exponential process, the rate of which was linearly related to the diffusion coefficient for the various markers.

4. The faster component was absent in the quiescent heart, and in the beating heart its contribution to the washout of the interstitial space was related to the strength of contraction.

5. Similar washout curves were obtained for Evans Blue-albumin conjugate, which readily penetrates the interstitial space of the isolated rat heart.

6. It is suggested that the two components of efflux are a diffusion process limited by perfusate flow, and bulk movements of fluid.

INTRODUCTION

The capillaries of the isolated, perfused rat heart, in contrast to those of the organ *in situ*, are freely permeable to bovine plasma albumin (Sutherland & Young, 1966). This change in capillary permeability occurs on

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excising the heart, and is probably brought about by a permeability factor released from nerve endings. It was of interest, therefore, to examine the washout of the extracellular space of the heart in the light of this finding, and so obtain some information on the exchange of solutes between the interstitial space and the vasculature of the perfused preparation.

METHODS

Animals. Male rats of the Hooded Lister strain, weighing between 200 and 300 g, were used.

Perfusion fluid. The perfusion fluid was Krebs bicarbonate medium (Krebs & Henseleit, 1932) equilibrated with 95 % O_2/5 % CO_2. The preparation of the medium is discussed in detail later in this section.

Perfusion technique. This was essentially that described by Bleehen & Fisher (1954), except that the preparation of the medium and the problem of filtration were re-investigated.

Extracellular markers. The markers used were: [U-^{14}C]D-sorbitol (8·5 mc/m-mole; Radiochemical Centre, Amersham), non-radioactive sorbitol, sucrose, raffinose and inulin (all from British Drug Houses Ltd., Poole, Dorset). Details of the preparation of the conjugate of Evans Blue and albumin have been given elsewhere (Sutherland & Young, 1966).

Procedure

Hearts removed from rats under ether anaesthesia were perfused for 20 min with medium containing one of the extracellular markers, and washed out with marker-free medium, the effluent samples being collected at timed intervals commencing 30 sec after the start of the washout. At the end of the perfusion the hearts, together with the effluent samples, were analysed for the marker, and the pattern of elution expressed as the decrease in the heart content of the marker with time of washout.

The concentrations of the markers used were: [U-^{14}C]D-sorbitol, 50 μc/100 ml.; carrier sorbitol, 50 mg/100 ml.; sucrose, 1 g/100 ml.; raffinose, 2 g/100 ml.; and inulin, 2 g/100 ml. Hearts were homogenized, and the homogenate deproteinized with cadmium hydroxide (Fujita & Iwatake, 1931). Although inulin from some sources is removed by this procedure, that used in these experiments was not; differences of this type in the properties of inulins from various sources have been described before (Bassir, 1956). [U-^{14}C]D-sorbitol was determined by liquid scintillation counting using the medium of Bray (1960). Sucrose, raffinose and inulin were determined by the method of Bacon & Bell (1948). Hearts perfused without marker gave a blank reading corresponding to 100 μg of raffinose (or sucrose) or 27 μg inulin/g tissue. Details of the use and determination of the conjugate of Evans Blue and albumin have been given in detail elsewhere (Sutherland & Young, 1966).

Preparation of Krebs bicarbonate medium

Bleehen & Fisher (1954) showed for the rat heart perfused with Krebs bicarbonate medium that unless an adequate filter (a cellulose extraction thimble) was incorporated in the perfusion circuit, the flow rate fell gradually to zero over 30–40 min. However, since earlier workers (Locke & Rosenheim, 1907; Bodo & Marks, 1927) using perfusion media that contained no phosphate and very much less bicarbonate obtained adequately perfused rabbit heart preparations without any such means of filtration, the particulate material encountered by Bleehen & Fisher (1954) may have arisen from the use of a bicarbonate medium containing phosphate and calcium ions. In the preparation of the bicarbonate medium it was recommended (Krebs & Henseleit, 1932) that the sodium bicarbonate solution be gassed with CO_2 before the final combination of the salt solutions, to avoid the formation of insoluble calcium salts. However, in the absence of such precaution a visible

precipitate is generally not encountered, and consequently many prepare the medium without gassing with CO_2; this course would seem even more acceptable when 1·27 mM-Ca^{2+} is used instead of 2·54 mM, the former being closer to the Ca^{2+} concentration of the plasma (McLean & Hastings, 1935).

Krebs bicarbonate medium was prepared either without the prior gassing of any of the constituent salt solutions before complete mixing, or with prior gassing of the sodium bicarbonate with 100 % CO_2 for 15 min, and the rest of the medium with 5 % CO_2/95 % O_2. The effect of this difference in the preparation of the medium on the coronary flow is shown in Fig. 1, and it is evident that a precipitate of particle size sufficiently large to block small vessels in the heart must be formed when the prior gassing with CO_2 is omitted.

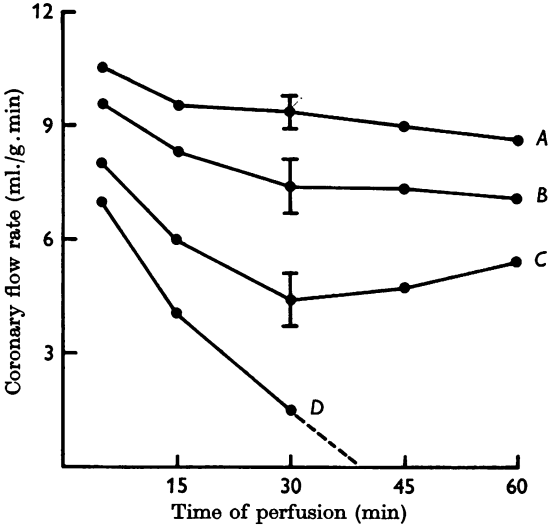

Fig. 1. Effect of preparation and filtration of perfusate on coronary flow. *A*. Medium prepared with gassing of the conc. sodium bicarbonate solution with CO_2; no filter. *B–D*. Medium prepared without gassing bicarbonate with CO_2: *B*, filtered through sintered glass filter (pore size 10 μ); *C*, filtered through Soxhlet extraction thimbles; *D*, not filtered. Ten hearts per group (except *D*, four hearts); vertical lines indicate S.E. of mean.

Furthermore, since these difficulties were not encountered if phosphate was omitted from the medium, it would appear that the precipitate formed is calcium phosphate. Since calcium ions have an important role in heart contraction, the precipitation of some Ca^{2+} as calcium phosphate might, depending upon the initial Ca^{2+} concentration, reduce the strength of contraction. Perfusing hearts with adequately prepared media of various Ca^{2+} concentrations showed that a change from 2·54 to 1·27 mM made little difference to the contractile activity, but that any further reduction produced obvious changes, and at 0·5 mM contraction ceased; these observations are similar in trend to the data for the perfused frog heart (Clark, Percival & Stewart, 1928). Thus, if the lower Ca^{2+} concentration were used, the omission of the gassing of the bicarbonate with CO_2, and the subsequent precipitation of calcium phosphate, could produce a reduction in contractility. Comparison of the contractility of hearts perfused with media prepared with and without gassing with CO_2, clearly showed that in the latter, although the medium was adequately filtered (sintered glass filter, pore size 10 μ; Zachariah, 1961), the contractility was less after 10 min perfusion, remaining fairly constant for the succeeding 25 min. Moreover, analysis of the latter

medium showed that the Ca^{2+} concentration (measured by the iodometric titration of the oxalate) had fallen from 1·27 mM to 1·05 ± 0·03 mM (4) after recirculation for 30 min, whereas there was no such change in the former case. Lastly, hearts perfused with an adequately prepared medium containing a Ca^{2+} concentration of 1·05 mM, had similar contractile activity to that obtained with a medium prepared without gassing with CO_2, containing 1·27 mM-Ca^{2+}.

Thus, although the formation of the precipitate must be continuous for 30 min or more (Fig. 1), the slight but significant reduction in Ca^{2+} may be complete by the first few minutes of perfusion, if not before. A possible explanation is that mixing of the ungassed salt solutions at pH 8 results in the formation of a significant concentration of PO_4^{3-} ions (Greenwald, Redish & Kibrick, 1940), and that a colloidal solution of the highly insoluble $Ca_3(PO_4)_2$ (solubility product, 1×10^{-25}) is formed, which gradually aggregates, even at pH 7·4, to give particles which block the vessels of the heart.

In practice, although an adequately prepared medium did not require filtration, a fluted hardened filter paper (Whatman, No. 54) was included in the recirculation apparatus; it was, however, a necessary precaution when Evans Blue-albumin conjugate (0·5 g/100 ml.) was present in the medium.

Treatment and presentation of the data

A semi-logarithmic plot is the usual way of testing the fit of data to a simple exponential relation. However, two important limitations must be recognized. First, the logarithmic form is an insensitive way of showing change, and a fit is only meaningful for very precise data or for data covering a wide range of values. Secondly, although the addition of a constant C to the equation $Y = Y_0 e^{-kt}$ to give $Y = Y_0 e^{-kt} + C$ theoretically changes the straight line plot ($\log Y$ against t) to a curve, in practice a value of C sufficient to decrease the slope by 48 % does not significantly alter the fit to a straight line (Hlad, Elrick & Arai, 1959). In order to overcome these limitations, the washout data were first examined by plotting $\Delta Y/\Delta t$ against Y, where ΔY is the amount of solute coming out in successive, equal intervals of time (Δt), and Y is the amount in the heart at those times. This more sensitive test gave straight lines for all the data of all experiments. In addition, any solute not washing out according to the exponential process gives rise to a positive intercept on the abscissa, corresponding to C in the above equation, and can be subtracted from the solute found in the heart at the end of the experiment. Such intercepts were small, and in most cases were within the range of the tissue blank. After correction by subtraction of the intercept, the data were expressed as the change in the logarithm of the heart content of the solute with time.

The solute space of the heart is defined as the volume of perfusate that contains the amount of solute found in 1 g wet wt. of heart tissue. The extracellular space of the perfused heart has been taken as 360 μl./g fresh wt. (Fisher & Young, 1961).

The result of a group of observations is presented as the mean ± S.E. of the mean (number of observations).

RESULTS

The time course of the washout of raffinose from the perfused heart was determined using perfusion media prepared without prior gassing of the sodium bicarbonate solution with CO_2. The data are given in Fig. 2, in the form of the change in the log. heart content with time. The curve has two components, the slower being an exponential process. Analogous washout curves were obtained for the other extracellular markers, sorbitol, sucrose and inulin. Evans Blue-albumin conjugate, which in the isolated, perfused

rat heart rapidly penetrates the interstitial space, also gave the same type of washout curve (Sutherland & Young, 1966). The rate constant of the exponential portion of the curve was different for each of these substances, and was related to molecular size, as is shown by the linear relation between the rate constant and the coefficient of free diffusion (Fig. 3).

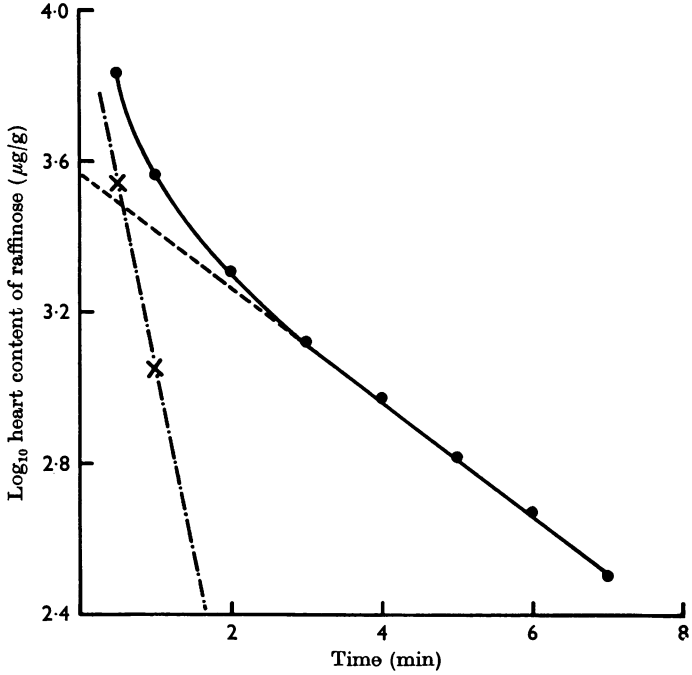

Fig. 2. The washout of raffinose from the isolated, perfused rat heart. Data are plotted as $\log_{10}$ of the heart content (μg/g) against time. The faster component (× ·–· ×) was obtained by extrapolation of slower component and subtraction. The curve comprises the values for four hearts.

If it is assumed that the two components of the washout curve for raffinose (Fig. 2) represent concurrent processes (this assumption is justified later in the Discussion), then the intercept obtained by extrapolating the later portion of the curve to zero time (Fig. 2) gives an estimate of the volume of the extracellular space accounted for by the slower component. For the washout curve for raffinose shown in Fig. 2, the intercept corresponds to a space of 190 μl./g, which is 53% of the extracellular space (360 μl./g; Fisher & Young, 1961). By subtracting from the total washout curve the values corresponding to this extrapolated portion of the slower component, the contribution of the faster component is obtained. The rate constant of the faster component is then obtained from the semi-logarithmic plot of these derived values, and in contrast to

the values for the rate constant of the slower component there appears to be little, if any, systematic change with molecular size; e.g. for raffinose the value was $0{\cdot}99 \pm 0{\cdot}08$ (8); inulin, $0{\cdot}83 \pm 0{\cdot}04$ (6); and for Evans Blue-albumin conjugate, $0{\cdot}87 \pm 0{\cdot}05$ (4).

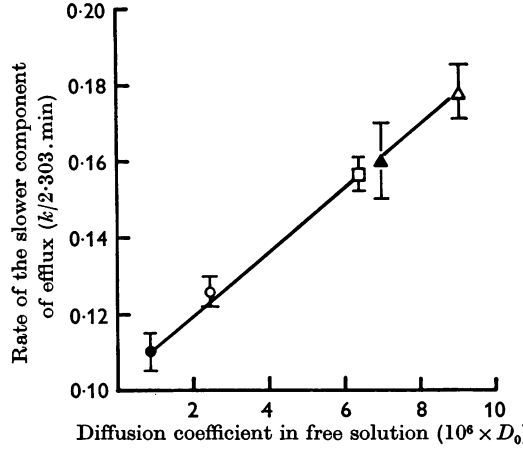

Fig. 3. The rate of the slower component of efflux for Evans Blue-albumin (●), inulin (○), raffinose (□), sucrose (▲) and sorbitol (△) plotted against their respective coefficients of diffusion in free solution at 37° C. The vertical lines indicate S.E. of mean.

Because anoxia is often cited as a cause of increased capillary permeability (Landis & Pappenheimer, 1963), the effect of a 10 min period of anoxic perfusion (medium equilibrated with 5% CO_2/95% N_2) during the loading period was investigated. There was no change in the rate of efflux of inulin nor in the volume of the extracellular space accounted for by the slower component.

It was shown (Sutherland & Young, 1966) that the antihistamine promethazine hydrochloride largely prevented, at least for the first few minutes of perfusion, the increased capillary permeability found in the perfused heart. In preliminary experiments to determine whether promethazine would modify the washout of inulin and raffinose, it was found that quite independently of any action it might have on capillary permeability, the antihistamine eliminated the faster component of washout by stopping cardiac contraction, whereas the rate of the slower component was unaltered. To test this finding more rigorously, the contraction of the heart was prevented only during the washout period by raising the potassium chloride concentration of the marker-free medium used in the washout period, from 4·74 to 14·22 mM, all the media being prepared with adequate gassing of the sodium bicarbonate with CO_2. Heart contractions ceased within 15 sec of switching to the high potassium medium, and the

experiments showed that the washout curves for both raffinose and inulin had only one component, the slower one, with exactly the same slopes as had been obtained previously with contracting hearts. The data for raffinose are shown in Fig. 4.

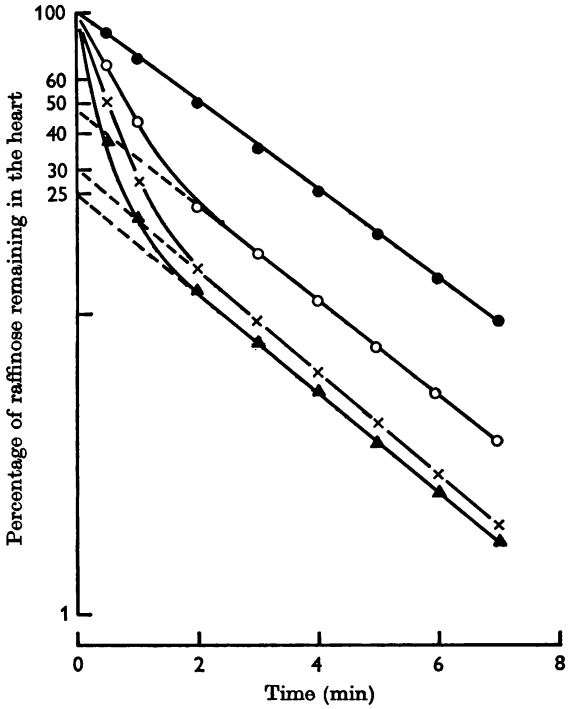

Fig. 4. Effect of contractility on the washout of raffinose from the isolated, perfused rat heart. The data are plotted as the decrease in raffinose content, expressed as percent of the initial content (log. scale) against time. ● Quiescent hearts; ○ 1·05 mM-Ca^{2+}; × 1·25 mM-Ca^{2+}; ▲ 2·5 mM-Ca^{2+}. The exponential portion of each curve has been extrapolated to zero time, the intercept indicating the total contribution of the slower component. Each curve comprises the values for four hearts.

To test further the role of heart contraction in the genesis of the faster component of washout, hearts were perfused with media (prepared with adequate gassing of the sodium bicarbonate with CO_2) containing 2·54, 1·27 or 1·05 mM-Ca^{2+} in both perfusates. The washout of raffinose was followed in these experiments, and the data are shown in Fig. 4. It is clear that the contribution of the slower component of efflux to the washout of the interstitial space was decreased by the increasing force of contraction brought about by increments in the calcium ion concentration of the perfusates; that is, the contribution of the faster component increased with

increase in contractile activity. The contribution of the slower component to the total washout was approximately the same for the inadequately prepared medium, 1·25 mM-Ca^{2+}, and the adequately prepared medium with 1·05 mM-Ca^{2+}, supporting the assertion (see Methods) that the failure to gas the sodium bicarbonate solution with CO_2 during the preparation of the medium leads to a lowering of the calcium ion concentration of the perfusate to about 1·00 mM.

Lastly, it was found that the washout of Evans Blue-albumin conjugate from the quiescent heart showed only the slower component of washout; and that preparation of the perfusion medium with adequate gassing of the sodium bicarbonate with CO_2, resulted in an increased contribution of the faster component compared with that obtained with perfusate inadequately prepared. This is best expressed in terms of the contribution of the slower component to the total washout, as described above for raffinose efflux; it decreased from 100 ± 5 (4) μl./g with inadequately prepared medium to 65 ± 7 (4) μl./g with the adequately prepared medium. It should be noted that the loading period for the Evans Blue-albumin conjugate was only 2 min, and that the space penetrated amounted to 150–200 μl./g (Sutherland & Young, 1966).

DISCUSSION

The washout curves for raffinose and other extracellular markers show the same biphasic pattern as that observed for Evans Blue-albumin conjugate. It was shown elsewhere (Sutherland & Young, 1966) that this vascular marker rapidly penetrates the interstitial space of the perfused heart because of a substantial increase in capillary permeability after excision of the organ. Thus it would appear that the pattern of washout of the extracellular markers is determined by the changed permeability conditions; i.e. the passage of the solutes out of the interstitial space is predominantly through the abnormally 'leaky' capillaries. The designation of capillaries as the vessels so affected requires some comment in view of the recent finding that the known chemical mediators of increased vascular permeability, including histamine and 5-hydroxytryptamine (5-HT), act specifically on the venules (Majno, Palade & Schoefl, 1961; Cotran & Majno, 1964). The involvements of capillaries and venules in the early phase of inflammation were clearly distinguished in the rat diaphragm *in vivo*, for example, by the complete suppression of venular leakage by the specific antihistamine mepyramine maleate, whereas the capillary changes were unaffected (Hurley & Spector, 1965). In the isolated, perfused rat heart the increased vascular permeability was not prevented by mepyramine maleate (Sutherland & Young, 1966), nor by 2-bromo-(+)-

lysergic acid diethylamide ('BOL 148'), a specific antagonist of 5-HT, nor did 5-HT itself cause any further increase (unpublished observations). For this reason the data are interpreted in terms of an increase in capillary permeability; and there is good evidence to support the view that this is due to an enlargement of the existing pores in the vascular wall (Landis & Pappenheimer, 1963). The transfer of lipid-insoluble substances across the capillary wall occurs through these pores, principally by diffusion, but also by bulk fluid movements. The reasons for a possible identification of the two components of washout with exaggerated forms of these processes are presented in the following discussion.

The slower component. From the linear relation between the rate constant and the diffusion coefficient, it is clear that the slow process of washout involves a diffusion process. Since this relation also applies to the washout of Evans Blue-albumin conjugate, the diffusion step cannot be transfer across normal capillaries, but must be transfer across the capillaries permeable to albumin.

Schafer & Johnson (1964) analysed the inward-passage of solutes across the capillary wall of the perfused rabbit heart. They considered three factors as possibly limiting the passage of solute: diffusion through the interstitial space surrounding each capillary; the restricted diffusion across the capillary wall; and the influence of the perfusion rate. The influence of the first is very small as can be seen from the comparison of the observed and calculated half-times of washout (Table 1). The influence of the second is more important, particularly for large molecular weight solutes such as inulin. In this case diffusion is limited to the gap between the edges of the endothelial cell, which is considered as a circular pore of average diameter 85 Å (Landis & Pappenheimer, 1963). When the diameter of the solute molecule approaches this value (e.g. albumin, 72 Å) the permeability coefficient of the wall for that solute is virtually zero. To obtain the observed capillary permeability to albumin the pores must have enlarged under the influence of the permeability factor. An increase in pore size from 85 to 180 Å could account for the observed half-time of efflux of albumin (Table 1). Such a change in pore size must have occurred, for it is implicit in any approach to this problem, that albumin passes across the wall at a rate equal to or greater than that of washout. Landis & Pappenheimer (1963) cite evidence indicating that the permeability seen in capillaries after mild injury would be most compatible with pore sizes of 150–250 Å. Such an increase in pore size will increase, although to a lesser extent than for albumin, the rate of diffusion of the smaller solutes (Table 1). However, even with the pore size found in normal capillaries, the rate of diffusion of sorbitol, sucrose and raffinose would be too rapid to account for the observed rates of efflux (Table 1). Again, if the limiting step to efflux were solely

diffusion, the rate constants for the various solutes would have ratios approximately equal to the ratios of the respective diffusion coefficients, and this is clearly not the case (Fig. 3). Another influence, not involving diffusion, is therefore operating so as to reduce the slope of the relation between the rate of efflux and the diffusion coefficient. For this reason the third factor, the influence of perfusion rate, must be considered. In general

TABLE 1. Comparison of the observed half-times ($t_{\frac{1}{2}}$, sec) of washout of various extracellular markers from the heart and half-times calculated (*a*) for diffusion through the interstitial space, and (*b*) for restricted diffusion across the capillary wall. For (*a*) $t_{\frac{1}{2}}$ was calculated using the Rashevsky–Schmidt approximation (Schmidt, 1952; Schafer & Johnson, 1964), where concentration in tissue element served by one capillary followed an exponential decay curve with a rate constant equal to $2D'A/RV$; where D' is effective diffusion coefficient of solute through tissue element as a whole (taken here as 50% of D_o at 37° C; Harris, 1960), A is surface area of capillary (radius 2·5 μ), R is radius of tissue element (9μ), and V is interstitial distribution volume of solute (36%). For (*b*) interstitial concentration would follow an exponential decay curve with a rate constant equal to PA/V (Schafer & Johnson, 1964); where P is capillary permeability coefficient for the solute, and A/V has the same significance as before. Values for P for normal capillaries (pore diameter, 90 Å) taken from Landis & Pappenheimer (1963); those for 'leaky' capillaries (pore diameter, 180 Å) are approximations based on increased ratio of pore diameter to molecular diameter

		Calculated for diffusion through:				
			Capillary wall			
			Pore diameter 90 Å		Pore diameter 180 Å	
	Observed	Interstitial space	$10^5 \times P$*	$t_{\frac{1}{2}}$	$10^5 \times P$*	$t_{\frac{1}{2}}$
Sorbitol	101	0·04	6	5	20	2
Sucrose	113	0·05	4	8	14	2
Raffinose	115	0·06	3	11	13	3
Inulin	143	0·15	0·3	107	3	11
Albumin	180	0·41	0·001	—	0·2	158

* Permeability coefficient (cm/sec).

it appears that the rates at which small molecules diffuse back and forth across capillary walls *in vivo* greatly exceed the rates at which they are carried to or from the tissues by the blood. Only for such large molecules as inulin can capillary permeability be considered as the primary factor limiting exchange in well-perfused tissues. Despite the high flow rates observed in the perfused rabbit heart, Schafer & Johnson (1964) identified the rate of perfusate flow as an important factor limiting the influx of sucrose. The flow rates found in the perfused rat heart (7–10 ml./min.g) were very much greater than those found *in vivo* (1 ml./min.g), and might not appear compatible with the perfusate flow limiting the rate of efflux. However, only a proportion of this increased flow would be through true capillaries; for with near maximal dilatation of arterioles and pre-capillary

sphincters that must exist in the isolated perfused heart, a substantial flow would be through the arteriole-venule shunts, that is assuming the microcirculation of the heart is similar to that seen in rat skeletal muscle (Wiedeman, 1963). It seems likely, therefore, that the slower component of efflux is the resultant of diffusion through enlarged pores (diameter, 180 Å) in the capillary walls, and subsequent removal severely restricted by perfusate flow through the capillaries.

The faster component. The faster component of the washout is a process absent in the quiescent heart, and the rate of it is a function of the force of contraction (Fig. 4). Furthermore, for a given state of contractility the rate constant does not appear to change systematically with molecular size. Stubbs & Widdas (1959) observed in the isolated, perfused rabbit heart a loss of extravascular fluid when the force of systole was augmented by adrenaline or by an increased concentration of calcium ions; and they suggested that backward filtration from the interstitial space occurs during systole. Thus the rhythmic change in intramural pressure would give rise to net movements of fluid into and out of the interstitial space during each cardiac cycle, and the magnitude of the washout effect of this exchange would obviously be related to the strength of contraction. The faster component can thus be satisfactorily accounted for in terms of this process.

The finding that the rate constant of the slow process does not alter with change in the contribution of the fast process (Fig. 4), means that the processes of washout are in parallel and not in series, and no arrangement can account for the biphasic washout curve that does not provide a fairly complete separation of the processes. One such arrangement would be the apportionment of the length of the capillary between the two processes on the following basis.

The net fluid flux suggested as the mechanism of the fast process depends upon the rhythmic change in tissue pressure. However, as Stubbs & Widdas (1959) noted, Starling rejected backward filtration except where the sudden rise in tissue pressure would not be propagated to the neighbourhood of the large veins; and these are just the conditions found in the myocardium. Thus backward filtration will occur only at the venous end of the cardiac capillaries. If, in fact, forward filtration is confined to that part of the capillary involved in the fast process, washout by the slow process could continue at the arterial end independently (Fig. 5). That the pressure drop across the isolated, perfused heart is primarily across the capillary bed (resulting from the maximum vasodilatation in response to the low oxygen-carrying capacity of the perfusate) might account for this limitation of forward filtration to the distal end of the capillary. The rates of the two processes will be independent as long as the capillary segment involved in the slow process is much longer than the mean diffusion

distance for that process, and since the average length of a capillary is 400–700 μ, longitudinal diffusion of solute from the arterial end into the fast-emptying compartment should not have a detectable effect on the rate of the slower component of efflux, although it might be seen as a decrease in the proportion of the extracellular space emptying by the slow process.

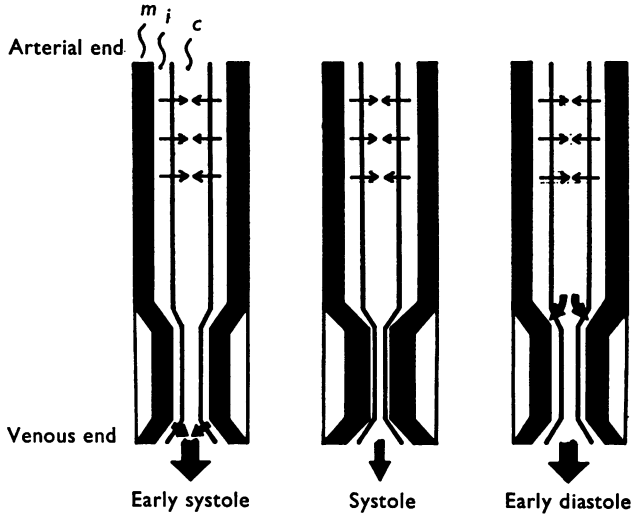

Fig. 5. Diagrammatic representation of the possible role of cardiac contraction in the efflux of solutes from the interstitial space of the isolated, perfused rat heart. The slower component of efflux (small arrows) by which solute diffuses into the capillary is independent of contraction, and would take place at the arterial end of the capillary. The faster component (larger arrows) would be due to a bulk movement of fluid out of the interstitial space, at the venous end, during early systole and a corresponding influx of fluid from the capillary during early diastole, the influx being limited to that length of the capillary surrounded by the constricted interstitial space. As the strength of contraction increases a greater length of the capillary is involved in this process. The largest arrows, at the end of the capillary, indicate the flow rate of the perfusate. *c*, capillary; *i*, interstitial space; *m*, muscle cells.

Lastly, others (Morgan, Henderson, Regen & Park, 1961) have observed this biphasic washout curve for sorbitol from the perfused rat heart, but have interpreted it as being due to efflux from two separate, anatomical compartments. The possible error of extrapolating from processes to anatomical compartments without the fulfillment of the necessary criteria is evident for this system, and may apply to others.

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