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Regional Lymph Flow in Unanesthetized Rabbits

Anders Bill

Department of Physiology and Medical Biophysics University of Uppsala, Uppsala, Sweden

ABSTRACT

Determinations were made of the 2-hour ^{22}Na space and the 1 and 2 hour ^{51}Cr-EDTA spaces in a number of tissues. In regions where the two ^{51}Cr-EDTA spaces were similar and smaller than or similar to the ^{22}Na space the former spaces were regarded as measures of the extra-cellular volume. Using previously published data for plasma volumes, extravascular plasma-equivalent albumin and IgG spaces and the turnover rate constants for the extravascular proteins, it was possible to calculate extravascular protein concentrations and rates of net filtration from the capillaries or lymph flow in some tissues.

The albumin concentrations, probably underestimated, were about 40% of the plasma concentrations in small intestine, lung and skeletal muscle, higher in heart muscle and lower in stomach wall. The net filtration or lymph flow, probably overestimated, was 0.81, 1.5, 0.53, 0.61 and 0.12 µl/min per g in heart muscle, small intestine, lung, stomach wall and skeletal muscle, respectively.

INTRODUCTION

In most tissues there is a net filtration of fluid from the capillaries and a drainage of tissue fluid through the lymph vessels. Cannulation experiments have shown that the lymph flow varies greatly with the activity of the tissue and that elevated venous pressure increases the lymph flow markedly. Plasma protein concentrations seem to vary inversely with the lymph flow (1).

It is often difficult to delimit the region drained by a cannulated lymph vessel and to collect lymph without anesthesia. As a consequence no data seem to exist for lymph production/g tissue in unanesthetized and unrestrained animals. Also few studies have been made on the composition of lymph from well-defined regions under such conditions.

The purpose of the present communication is to report approximate values for lymph flow rates in a number of tissues and normal plasma protein concentration in lymph in unanesthetized rabbits. An indirect procedure was used to determine flow rates and these were compared with values for plasma flow obtained

under similar conditions (2) in order to obtain figures for the net fluid filtration fraction in different vascular beds.

METHODS

Theory

It has long been suspected that under normal conditions plasma proteins passing out of the blood vessels return to the general circulation mainly via the lymph vessels. In a recent study (2) strong support for this hypothesis was found both for albumin and for IgG in an number of tissues - renal cortex, stomach wall, skeletal muscle and gall bladder. In some organs, however, namely the heart, choroid plexus, lung and small intestine, some albumin also seemed to be eliminated with the blood, some of the molecules returning by diffusion or some other mechanism.

Both albumin and IgG seemed to enter the lymph vessels in a bulk flow of tissue fluid. Experiments with direct collection of tissue fluid and lymph support this notion (3).

If IgG is leaving a tissue only by bulk flow of tissue fluid into the lymph capillaries, the rate of elimination can be calculated as

$$F = L \cdot C_{tf} \qquad (1)$$

where L is the rate of lymph flow and C_{tf} is the concentration of the substance in the lymph. Elimination can also be calculated as

$$F = k_{IgG} \cdot V_{tf} \cdot C_{tf} \qquad (2)$$

where k_{IgG} is the turnover rate constant of IgG and V_{tf} the volume of the tissue fluid in which the IgG is distributed.

Thus:

$$L = k_{IgG} \cdot V_{tf} \qquad (3)$$

From previous studies (2) data were available for k_{IgG} and also for the apparent volumes of the plasma-equivalent spaces of albumin and IgG and the intravascular plasma volumes. In the present experiments estimates were obtained for the distribution volumes of ^{22}Na and ^{51}Cr EDTA. For some tissues the latter values could be used as estimates of the extracellular volume. These volumes minus the intravascular plasma volumes represent the volumes of the tissue fluids. It was assumed then that the extravascular proteins were distributed within these volumes. This is an overestimate of the true volume of distribution of the proteins but the error was assumed to be moderate. The reason why protein distribution volumes can be expected to be smaller than

those for low molecular weight substances is that connective tissue components such as collagen and glucoseaminoglycans tend to cause a steric exclusion of large molecules (4).

Experiments

On the day before the radioactive isotope experiment the animals were anesthetized with pentobarbital sodium and a marginal ear vein and a femoral artery were cannulated with polyethylene tubes for injections and blood sampling, respectively.

1. On the next day ^{51}Cr EDTA (Hoechst) was injected i.v. in different doses every 3 - 15 min to maintain a steady plasma concentration. The animal was free to move in its cage. Arterial blood samples were taken to check plasma concentrations. After 1 or 2 h of a steady plasma concentration of ^{51}Cr EDTA the animal was killed rapidly by an overdose of pentobarbital sodium followed by saturated KCl. It was then immediately dissected in order to prevent - as far as possible - redistribution of the tissue fluids. The samples were weighed and the radioactivity was determined in the tissue and plasma samples. The plasma-equivalent ^{51}Cr EDTA space in µl/g tissue was determined by dividing the radioactivity per g tissue sample with that per µl plasma. Four samples were taken from each tissue.

2. On the day after the cannulation ^{22}Na (AB Atomenergi, Studsvik) was injected in different doses in such a way that the plasma concentration rose for 4 h and then remained stable for another 2 h. The plasma-equivalent ^{22}Na spaces were determined analogously to the ^{51}Cr EDTA spaces.

RESULTS

The ^{22}Na spaces in most tissues investigated were larger than the ^{51}Cr EDTA spaces and in many tissues the 1 and 2 hour spaces for ^{51}Cr EDTA were very similar (Table 1). In the liver and kidney the ^{51}Cr EDTA spaces were significantly higher than the ^{22}Na spaces, and in the brain and gall bladder wall the ^{51}Cr EDTA space was still increasing even after 1 h.

DISCUSSION

The observation that in the brain the ^{51}Cr-EDTA space was still increasing even after one hour of a steady concentration in the plasma indicates that this substance has a very low turnover rate in the interstitial space of the brain. For sodium the space was much larger. In the liver the ^{51}Cr-EDTA space was larger than the sodium space, indicating accumulation of ^{51}Cr-EDTA or some metabolite in the cells. High values for ^{22}Na spaces and increasing values

TABLE 1: Volumes of plasma-equivalent spaces.

	Plasma-equivalent space, µl/g		
	$^{22}Na_{(n=6)}$	$^{51}Cr\text{-}EDTA_{(n=6)}$ 60 min	$^{51}Cr\text{-}EDTA_{(n=6)}$ 120 min
Renal medulla	1042±76	8762±1430	9673±1690
Renal cortex	494±16	2057± 81	3081± 102
Gall bladder	890±50	245± 39	480± 59
Heart muscle	265± 6	211± 8	203± 3
Small intestine	-	210± 10	220± 19
Lung	454±29	286± 10	304± 14
Stomach wall	-	220± 17	224± 19
Skin	500±14	534± 20	550± 30
Triceps	122± 7	78± 6	76± 6
Liver	269±15	351± 42	520± 39
Brain	263± 3	11.1± 0.5	16.0± 1.4

for ^{51}Cr-EDTA spaces were found in the gall bladder wall, possibly reflecting Na transport by the cells and high ^{51}Cr-EDTA concentrations in the gall bladder bile. In the kidney the ^{22}Na and ^{51}Cr-EDTA spaces were large, as could be expected.It is well known that sodium concentrations increase towards the papilla to values three times those in the plasma. The high ^{51}Cr-EDTA spaces were most probably due to high concentrations in the urine, ^{51}Cr-EDTA not being reabsorbed in any part of the nephron.

In heart muscle, small intestine, lung, stomach, skin and skeletal muscle the ^{51}Cr-EDTA spaces at 60 and 120 minutes were very similar, indicating almost complete plasma-tissue fluid equilibration within only 1 hour. The ^{51}Cr-EDTA spaces were smaller than the ^{22}Na spaces, as could be expected if ^{22}Na entered the cells to some extent, ^{51}Cr-EDTA being restricted to the extracellular space. The skin was an exception, the spaces being similar, possibly because of a low proportion of cells with a rapid turnover of intracellular ^{22}Na.

TABLE 2: Calculated volumes of tissue fluid and albumin and IgG concentrations in tissue fluids.

	Tissue fluid µl/g	Alb.conc. tissue fl. % of plasma	IgG conc. tissue fl. % of plasma	Turnover rate constant IgG, min^{-1}
Heart muscle	163	71	54	0.005
Small intestine	194	43	40	0.008
Lung	171	42	40	0.0029
Stomach wall	202	29	18	0.0030
Skin	531			
Triceps	73	41	21	0.0017

When similar the 1 and 2 hour ^{51}Cr-EDTA spaces were pooled and regarded as estimates of the extracellular spaces. The previously obtained data for the intravascular plasma volumes in the different tissues were subtracted to obtain data for the tissue fluids. Albumin and IgG concentrations in the tissue fluids were calculated by dividing the volume of the plasma equivalent extravascular protein space by the volume of the tissue fluid (Table 2). The bulk drainage of tissue fluid with the lymph was calculated from Eqn. 3. As seen in Table 3, when lymph flow was divided by plasma flow (values from a previous study (2)) the net filtration fraction varied tenfold from the heart (0.02%) to small intestine (0.2%) - the stomach wall and skeletal muscle lying within these extremes. It is possible, of course, that in the stomach wall and small intestine there was either some net reabsorption of fluid from the lumen or net fluid loss.

The ultrastructure of the capillaries in the mucosa of the small intestine and stomach is very different from that in the heart and skeletal muscle, the former capillaries being thin with membrane-covered fenestrations, whereas the muscle capillaries are continuous with practically no fenestrations. It is clear from Table 3 that the values for the filtration under the conditions of study are not strongly correlated to the ultrastructure of the capillary wall.

The capillary filtration coefficient for the whole forearm in man is 0.057 $\mu l \cdot min^{-1} \cdot g^{-1} \cdot mm\ Hg^{-1}$. In the perfused dog and cat hind leg values twice as high as these have been obtained (5) and in the perfused rat hind leg the filtration coefficient is six times higher (6). These figures together with the observed lymph flow rate in skeletal muscle of about 0.12 $\mu l \cdot min^{-1} \cdot g^{-1}$,

suggest that the effective mean hydrostatic capillary pressure was close to the isogravimetric value, differing from that value by less than 2 - 3 mm Hg.

TABLE 3: Calculated lymph flow and filtration fractions.

	Calc. lymph flow $\mu l \cdot min^{-1} \cdot g^{-1}$	Plasma flow $\mu l \cdot min^{-1} \cdot g^{-1}$	$\frac{\text{Lymph flow}}{\text{Plasma flow}}$ %
Heart muscle	0.81	3 600	0.022
Small intest.	1.50	700	0.21
Lung	0.53		
Stomach	0.61	1 200	0.050
Triceps	0.12	120	0.10

It was also of interest to relate net filtration to functional capillary surface in the different tissues.

In most tissues capillary flow seems to be intermittent, due to vasomotion. The question then arises whether the whole capillary bed should be regarded as active in protein exchange and net filtration, or if only that part through which blood is flowing is involved. In vessels without continuous flow there is still a hydrostatic pressure approximately equalling that in the smallest veins . In large pores in the capillary wall there will then tend to be outflow as long as there is a hydrostatic gradient, oncotic pressure gradients playing only a minor role (7). Pinocytosis, likewise, may give a net movement of fluid out of the vessel. Thus two mechanisms resulting in outflow of slightly modified plasma are probably little affected by vasomotion. When protein exchange is discussed it then seems reasonable to consider the whole capillary bed as taking part in the exchange, even if there are differences in efficiency. It is interesting that the IgG concentration in the net filtrate is about 20% of the plasma concentration in the stomach and in the skeletal muscle and 40 - 50% of the plasma concentration in the lung, heart muscle and in the small intestine. These figures indicate that in the former tissues more than 20% and in the latter more than 50% of the net outward fluid movement occurs through relatively large pores or by pinocytosis. Other evidence also indicate that net filtration or reabsorption may occur in capillaries through which no blood is flowing, while diffusional exchange is very much

restricted to capillaries with continuous blood flow (6).

An estimate of 75 cm^2/g has been reported for the capillary surface in vasodilated skeletal muscle, and of 80 cm^2/g for heart muscle (8). These values and the present data indicate that net filtration per unit capillary surface in the heart is about 9 $\mu l/min/m^2$ and in skeletal muscle 16 $\mu l/min/m^2$. These values can be compared to the turnover rates for albumin and IgG (2). For albumin ,the values in volumes of plasma-equivalent fluid, are 16, 7.0, 3.4 and 2.6 $\mu l/min/m^2$ capillary surface in heart muscle, skeletal muscle, renal cortex and renal medulla. For IgG the corresponding figures are 5.5, 3.3, 3.3 and 1.6 $\mu l/min/m^2$, respectively. It is clear then that in the heart there is considerable return of extravascular albumin into the exchange vessels, the lymph flow accounting for only about 50% of the elimination.

It is also of interest to consider the relationship between the calculated rate of lymph flow and tissue fluid volume/g tissue: L/V_{tf} . Since $L=k_{IgG} \cdot V_{tf}$ it follows that k_{IgG} is a measure of the turnover of tissue fluid by net ultrafiltration and lymph flow. The values shown in Table 2 indicate fivefold differences between the tissues investigated, from 0.17%/min in skeletal muscle to 0.8%/min in the small intestine - the values for the stomach, lung and heart muscle ranging in between.

One important, sometimes overlooked consequence of the low turnover of macromolecules in the interstitial tissue fluid is that a change in transcapillary movements of large molecules only has its full effects on the composition of lymph many hours later - for some tissues, such as skin and skeletal muscle, it may theoretically take days to reach full steady state. For such tissues one can never expect the composition of lymph to reflect anything but a complex function of the macromolecular efflux over a period of time preceding the collection time by many hours and a plasma-tissue fluid balance with respect to water and low molecular weight substances at another time.

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Address for reprints:

Professor Anders Bill
Department of Physiology and Medical Biophysics,Biomedical Center,
Box 572
S-751 23 Uppsala,
Sweden