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Evidence of Active Transport (Filtration?) of Plasma Proteins across the Capillary Walls in Muscle and Subcutis*

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Summary

Under slight lymphatic stasis (tilting the body 15°) we measured the arrival of locally injected ^{131}I-albumin to the plasma pool. From 30 min. to 90 min. after the injection the return rate was zero i.e. local back transport in the two tissues studied viz.muscle and subcutaneous fat is very small.

Compared to a suggested steady state total ^{131}I-albumin clearance of 1,7%/hour in the horizontal body position we conclude that maximally one percent of the interstitial albumin can have a local transendothetial escape i.e. can be handled by passive forces as is diffusion and pinocytosis. As passive flux is proportional to the concentration and the interstitial albumin concentration is about half the plasma concentration then also diffusion and or pinocytosis from the plasma of albumin is negligible in the resting normal man. We suggest filtration through big leaks as the main mechanism for transendothelial protein transport.

In recent years it has become evident that interstitial fluid in most organs, except brain and retina, contains a fairly high concentration of plasma proteins. Typically the interstitial fluid (IF) albumin concentration is in the order of half that in plasma (P) (7, 12, 8). On this basis *Renkin* (10) pointed out, that if the proteins crossed the walls of the capillaries by passive transport, i.e. by diffusion or bidirectional pinocytosis, then the clearance from the interstitium must in part be local back diffusion, in part by lymphatic drainage. For a molecule with an IF/P ratio of 0.5 half of the interstitial clearance would be by back-diffusion half by lymph. *Lassen* and *Parving* (4) using data from the literature presented evidence suggesting that for the whole body the unidirectional flux of albumin from P to IF was about the same as the total lympahtic albumin drainage. The evidence was, however, not obtained by simultaneously measuring the two fluxes. And as pointed out by Levitt heterogeneity of transcapillary albumin transport would tend to invalidate the argument of *Lassen* and *Parving*.

In the present study we have measured the clearance of labelled albumin from IF in two different capillary beds in man, viz. in voluntary muscle and in subcutaneous tissue. When combined with the known non-negligible IF albumin concentration in these tissues our data demonstrate the quite predominant role of lymphatic drainage, and they rule out that passive transport by diffusion or bidirectional pinocytosis can be the main mode of the transcapillary albumin passage.

Unidirectional and hence active transport of plasma proteins across the capillary wall as by filtration through big leaks is compatible with our experimental findings.

Experimental studies

The clearance of ^{131}I-radioiodinated human serum albumin from the interstitial spaces in muscle and subcutis was studied in normal human volunteers (staff members).

The tracer used was human serum albumin ^{131}I for metabolic studies (code IK21S) from *Kjeller, Norway*. The iodination was made by electrolysis giving no discernible denaturation (*Rossing* 1967), and the content of free iodine was less than 1%. The albumin content was about 12 mg/ml and the preparation was conserved with 0.9% benzylalcohol. At the day of investigation 0.1 ml of the solution containing 10–30 μCi ^{131}I was injected with a 0.4 mm o.d. (G 27) needle and at a slow rate, the injection lasting appr. 30 seconds.

The clearance away from the local depot in the anterior tibial muscle or the subcutaneous tis-

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sue over this muscle was followed by external scintillation counting and the data expressed in percent of the initial counting rate.

The arrival of labelled albumin molecules in the plasma space was followed by taking plasma samples and counting the trichloracetica-cid precipitated proteins so as to avoid free radio-iodine. These data were also expressed in percent of injected dose using an estimated value for the size of the plasma volume of 45 ml/kg body weight.

Results

A: Horizontal body position

External clearance curve in the horizontal body position was made in six persons. The disappearance rates were almost the same in the three studies with a depot in the anterior tibial muscle as in three studies with a depot in the subcutaneous tissue over this muscle. In the first 2 hours after injection the total disappearance averaged 4.6% of the depot (range 3.5–5.5). Initially a slightly steeper slope was seen. But, from 30 min to 120 min an approximately monoexponential slope was obtained, averaging 1.7%/hours. We consider this to represent the steady state value of interstitial clearance in the resting state and at heart level. A typical external clearance curve from subcutis is presented in fig. 1.

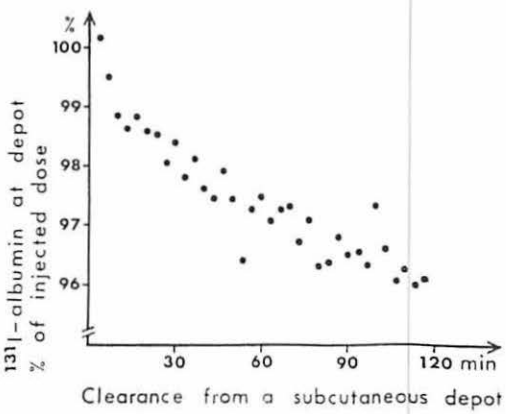

Fig. 1 Typical example of subcutaneous ^{131}I-albumin clearance in the horizontal body position at rest. The count rate is expressed every 200 sec. in percent of initial count and followed for the first 2 hours after the injection.

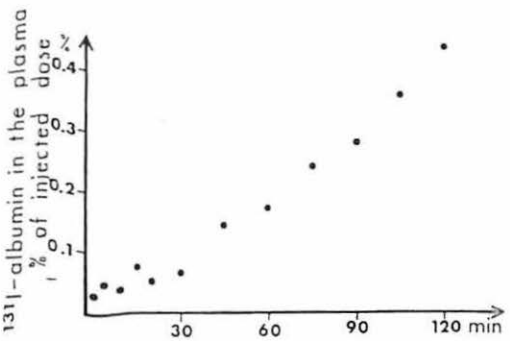

Fig. 2 Example of ^{131}I-albumin in the plasma pool as found in the horizontal body position after subcutaneous injection of the tracer. The ordinate is expressed in percent of the injected dose.

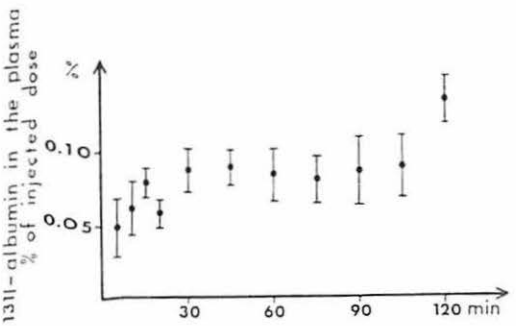

Fig. 3 ^{131}I-albumin appearance in the plasma pool expressed in percent of locally injected depot. Mean and SEM of six studies (three after injection in the tibeal anteriour muscle and three in the subcutaneous fat at the same site) in 15° tilt body position (head up). No significant increase is seen from 30 min to 90 min after the injection.

The appearance in blood of the tracer was in the horizontal body position variable often giving a steadily increasing activity. A typical curve is presented in fig. 2 giving a two hours blood recovery of 0.43% of the injected dose. The range for this value was very wide, 0.13—1.7%.

In order to study the mechanism of transport to the blood pool we made the following study:

B: 15° body tilt with leg down.

In this series we tilted the bed so that the depot was about 25 cm below heart level. This

was done with the aim of retarding lymphatic return.

Three studies were made with subcutaneous injection and three studies with intramuscular injection.

The return from interstitium to blood was on average after 2 hours 0.134% of the injected dose, and no significant difference was found between the appearance curves from the two tissues. The recovery of tracer in the blood pool is illustrated in fig. 3. It shows an initial faster increase lasting about 30 minutes followed by a 60 minutes period with a practically horizontal curve segment showing no further entrance of tracer to the blood circulation in this time interval. We consider this horizontal part to indicate that for this one hour period the lymphatic return has not reached the blood pool and that local back transport is zero. This we take also to indicate zero local back transport in the horizontal position.

Further analysis of the results is carried out in the next section.

Theoretical considerations

The body tilt data showed that local back transport is essentially zero. But, in order to analyze the data numerically a *maximal value for the local back transport of 0.01 percent per hour was estimated* taking into consideration the random statistical scatter of the body tilt data.

This estimation was performed as follows: The slope of the regression line of the average amounts of tracer in the plasma pool from 30 to 90 minutes in the body tilt experiments was $b = -0.00496$%/hour, $SD_b = 0.00489$.

At the 30 minute point an average of 0.087 percent of the dose was present in the plasma pool. If no further entrance of labelled albumin took place over the following hour then 5.6% of this amount would disappear due to the normal transcapillary escape *Parving* (5). Hence a negative slope of $-0.056 \times 0.087 = 0.00487$ %/h should have been expected.

Hence we estimate the maximal value for the local back transport by adding 0.00487 and 2 times the SD_b and obtain:

$[b]_{max} = 0.00969 \simeq 0.01$ percent/hour.

Since albumin comes from the plasma and we assume steady state, then the measured clearance away from the interstitial space must equal that going towards that space. Our data thus shows that the *unidirectional transport from plasma to interstitial fluid also averages 1.7%/hour* of the interstitial albumin mass. Hence it follows that the ratio:

$$\frac{\text{local back transport}}{\text{out transport}} < \frac{0.01}{1.7} = 0.0057 < 0.01$$

saying that less than 1% of total clearance is via local mechanism, more than 99% is via lymph, i.e. *the transcapillary albumin flux is practically unidirectional.*

This experiment result will be compared to that which could be expected theoretically in models assuming "diffusion" as an albumin transport mechanism (c.f. the comments in the introduction).

The interstitial albumin concentration is estimated to 0.5 times that of the plasma.

First assumption

Albumin transport by diffusion and/or bidirectional pinocytosis. This case, discussed by *Renkin* in 1964 (9) simply predicts:

$$\frac{\text{local back transport}}{\text{local out transport}} = \frac{C_{IF}}{C_P} = 0.5 \quad (1)$$

Our data permit to reject this hypothesis.

Second assumption

Albumin transport by diffusion and sieving due to filtration and reabsorption of plasma water across an isoporous membrane. This case can be analyzed according to the equations derived by *Perl* (1975). We assume with Perl that reabsorbed fluid might carry protein back and set the reflection coefficient for filtration of albumin σ_f to be 0.95. Inserting this in his equation (12) yields:

$$0.5 = \frac{C_{IF}}{C_P} = \frac{PS + 1/2\,(1-0.95)\,L_2}{PS + 1/2\,(1 + 0.95)\,L_2} \quad (2)$$

where PS is the permeability surface area and L_2 the rate of lymph flow. Solved for PS this

equation gives PS = 0.025 L_2 i.e. PS $\simeq L_2$.

For local back flux equations (1) and (2) of Perl yield

$$j_{local\ back} = PS \cdot C_{IF} + 1/2\,(1-\sigma_f^b) j_f^b (C_{caps}+C_{IF})$$

where σ_f^b is assumed to be 0.95 and j_v^b is the reabsorption of water in the paracapillary convective fluid movement that is taken to be as large as 20 times the lymph flow (in order for the effect to be easily recoqnizable).

$$\text{thus } j_{local\ back} = PS \cdot C_{IF} + 1/2\,(0.05)\,20PS\,(3 \cdot C_{IF}) = 2.5 \cdot PS \cdot C_{IF} \qquad (4)$$

The clearance by lymph is simply $j_{lymph} = PS \cdot C_{IF}$ because PS = L_2. Hence the total back transport is $j_{local\ back} + j_{lymph} = 3.5 \cdot PS \cdot C_{IF}$

Since this must equal the unidirectional out transport it follows the ratio of the predicted fluxes across the capillary wall:

$$\frac{\text{local back transport}}{\text{out transport}} = \frac{PS \cdot C_{IF} \cdot 2.5}{PS \cdot C_{IF} \cdot 3.5} = 0.71 \qquad (5)$$

Again in distinct disagreement with the experimental results.

Third Assumption

Heteroporous membrane with unidirectional filtration of albumin from plasma to the interstitial space through big leaks. Our data can readily be fitted into the equations of Perl if we assume big leaks. Inserting into equation (3) that the local back transport is essentially zero (relative to the out transport) it follows that PS $\simeq$0 and that $\sigma_f^b \simeq 1.0$ for the albumin at the small pore sites available for water reabsorption.

As PS $\simeq$0 and no back diffusion of albumin is present at the sites of big leaks the albumin outflux is simply the water flux through the big leaks times the plasma concentration viz.

$$j_{out} = j_{water\ big\ leaks} \times C_{plasma} \qquad (6)$$

This model can account for the experimental data. The equation can further be modified with a term $(1-\sigma f)$ to allow for a small sieving effect.

Discussion

In the two tissues studied the transcapillary albumin flux was shown to be essentially unidirectional. With the fairly high albumin concentration in the the interstitial fluid (about 0.5 times that in plasma) this shows that the transport is not dominated by diffusion or by bidirectional pinocytosis. Filtration or unidirectional pinonocytosis are two mechanisms that both can explain our data.

Unidirectional pinocytosis across the endothelial cell has never been discussed in the literature. Studies with colloids that can be seen by electron microscopy have in all studies been interpreted to suggest a bidirectional proces. And in the recent study of *Johannson* (2) when ferritin was injected in paracapillary sites labeling of vesicles was fully as intense as from the plasma site.

On this basis we feel that filtration as expressed in equation (6) is the dominant transport process. This conclusion is in agreement with that of *Bill* (1) and of *Rutili* and *Hagander* (13) who have analyzed the role of saturation of the interstitium with plasma injected macromolecules in animals. Their studies are technically more involved and hence the more direct demonstration of zero back flux in our studies in man, were felt to be of interest.

So, after this "detour" of considering the dispersive transport processes of diffusion and bidirectional pinocytosis introduced by *Renkin* (9) one is back to the classical concepts: Interstitial macromolecules are drained by the lymph, and since we now know that they are normally present in non-negligible concentrations, they exit by a nondispersive mechanism presumably filtration.

In a recent study *Renkin* and coworkers (10) analyze the endogenous plasma proteins in lymph from the dog hindpaw, using quantitative lymph collection. The studies were performed by passive movement of the paw in order to have a suitably high lymph flow, and comprised measurements without and with venous stasis. Using elaborate equations for restricted diffusion and filtration they concluded that at rest diffusion was the dominant transport process while during venous stasis filtration dominated. If no trauma factors are involved then, their conclusion is in disagreement with ours. We would predict that labelled albumin injected in their preparation would solely be cleared via the lymph. If this is so, then local

backdiffusion, and by inference also forward diffusion, can both be ruled out. The discrepancy between the two analyses – the computation from the lymph data, and the clearance of interstitially injected labelled albumin – could in our opinion best be resolved by postulating that the mathematical analysis of Renkin is based on approximations that do not hold. In particular, as noted by *Rutili* and *Hagander* (13) the problem of the longitudinal gradient inside the filtering pore may explain the discrepancy if this gradient differs significantly from the linear one assumed by Renkin.

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