Radioisotopic Assessment of Extravascular Albumin as an Index of Lymph Transport

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During the recent investigations into dosimetric problems of endolymphatic therapy we became acutely aware of the need for a quantitative and physiologic means for assessing lymphatic transport (1). Cannulation of efferent lymphatic ducts to observe flow is the most quantitative method available, however, this method is neither physiologic nor practical as a routine clinical tool.

The rate of isotope appearance in a point distal to an interstitial isotope injection has been used by many as an index of lymphatic flow (2). Radiocolloid injection into the foot followed by assessment of isotopic activity over the groin as a radiocolloid transit time, although advocated by some, is neither physiologic nor quantitative. The toe to groin transit time is easy to perform, but limited by the problems of inconstant variables i. e. isotope diffusion from the interstitial injection, peripheral lymphatic pick-up of the radiocolloid which may be influenced by the interstitial injection, and lymph node trapping of the radiocolloid (e. g. popliteal nodes) which may not be constant and thereby, affect the ultimate shape of the isotopic accretion curve at a site cephalad to the node. In view of these considerations, if one wishes to study only lymphatic transport, it would seem more reasonable to select a labeled substance which is not accreted to variable extents by the lymphatic nodes in the interspersing area from the sites of injection to the site of assessment.

Intradermal injections of radioalbumin as proposed by *Taylor* et al. (3) overcome in part the objection to the colloid injections. The problem of isotope diffusion at the site of injection limits the use of this technique when measuring the isotope disappearance rate from an injection site. Although lymphatic transport is one parameter of this measurement, the addition of the variable diffusion precludes quantitative physiologic studies. In addition, the probability that interstitial injection itself alters the kinetics of regional lymph transport must be considered.

With these points in mind, we sought a more physiologic means to assess regional lymphatic transport, primarily directed at eliminating the distortion of regional lymphokinetics by the measuring procedure. *Rossing* (4) has shown a difference in the percentage of total albumin located in the extravascular space between ambulatory and bed ridden patients by analyzing the disappearance of intravenously injected

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radioiodinated albumin. His ambulatory patients had 47% of total albumin located intravascular whereas hospitalized and bed ridden patients had 44% of total albumin located intravascularly. The difference was significant ($p = <.01$) and was felt to represent a difference in the extravascular turnover rate of albumin which may be used as an index of total lymphatic flow. As *Casley-Smith* (5) has pointed out some organs (e. g. intestines, kidney) may have fenestrations which may allow a capillary return of large molecules (e. g. albumin), although in most organs the major portion of extravascular albumin is returned to the blood stream via the lymphatic system (7, 8). It is in these structures (e. g. muscle) that tracing the extravascular albumin would give an index of regional lymphatic transport (fig. 1). In addition,

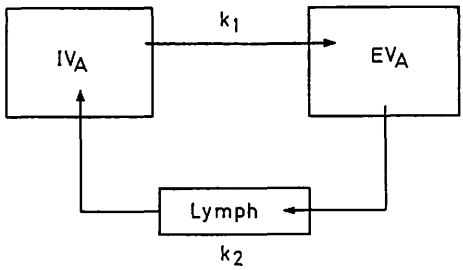

Fig. 1 Experimental model. IV_A = Intravascular albumin, EV_A = Extravascular albumin, which returns to the intravascular space via lymph transport. This model is applicable only in areas where this prerequisite is fulfilled. If one can measure IV_A, EV_A, and k_1 then k_2 can be calculated from the equation

$$k_2 = \frac{k_1 \times IV_A}{EV_A}$$

total lymph turnover as discerned by compartment analysis of intravenous albumin injection, may provide an index of total lymph flow which could be useful in certain experimental situations. In a total kinetic study, however, one must acknowledge that the measure is an integration of extravascular albumin return rates and may encompass organs where the albumin may not be returned via lymphatics. The theoretical considerations of a new method to assess regional lymph transport in areas where extravascular albumin return is dependent upon lymph transport will be presented herein.

Methods and Results

Total extravascular albumin return rate: The total extravascular albumin return rate can be calculated from the intravascular albumin disappearance time measurement by plotting the $\ln \frac{(IVA_0 - IVAeq)}{(IVA_t - IVAeq)}$ against time (9). Where IVA_0 = initial intravascular albumin activity, IVAeq = intravascular albumin activity at equilibrium and IVA_t = intravascular albumin activity at time t. Figure 2 shows an experiment where the intravascular albumin activity plotted against time is corrected for catabolic rate of 8.5% per day[4] and Fig. 3 shows a straight line ln plot. From this we can calculate k_1 and k_2 and in this instance $k_1 = 0.057\ hr^{-1}$ and $k_2 = 0.041\ hr^{-1}$ which will give a $t^1/_2$ of total body (integrated) extravascular albumin return rate of approximately 17 hours.

Regional extravascular albumin return rate: The experimental data has given excellent whole body calculations, but this integrated total body extravascular albumin half time is not applicable for regional studies. We have, therefore, sought a method to assess regional lymphatic transport. This would be possible if we could measure

the regional extravascular albumin activity. Our regional method consists of double label to define the blood volume (^{51}Cr tagged RBC) and the total albumin content (^{131}I albumin). The albumin should be lightly iodinated after the technique of *Mac Farlane* (6) with no or very little free iodine. We now use 100 μc of ^{51}Cr and 10 μc of ^{131}I. A change in regional activity is measured over the area of interest (e. g. gastro-

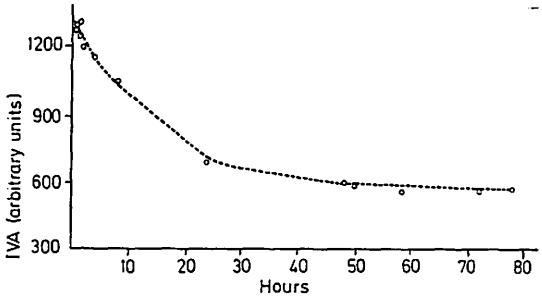

Fig. 2 Linear plot of the intravascular albumin activity disappearance with time in a control patient who received 10mc ^{131}I labeled albumin intravenously for blood volume determination. This plot is corrected for catabolism as described in the text.

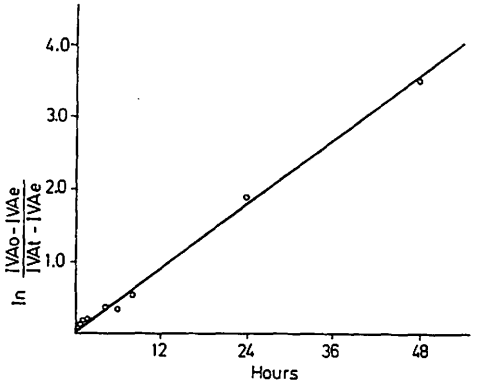

Fig. 3 Standard kinetic plot which in this instance gives a linear relationship supporting the experimental model.[9] The slope of this line equals $k_1 + k_2$ and k_2 can, therefore, be calculated as described in Fig. 6.

cnemius muscle) at 5 minutes and every 2 hours thereafter. We use simultaneous anticubital vena punctures to obtain blood samples and, thereby, correct for changes in regional blood volume, thus, discerning extravascular albumin. The initial regional blood volume is calculated from the Chromium and Iodine activity under the probe at 5 minutes, when little if any albumin is extravascular. From this 5 minute ratio the regional hematocrit is also determined. The ratio of regional to initial hematocrit (obtained by venapuncture) can be used to correct for possible subsequent changes in ^{51}Cr to ^{131}I ratio due to changes in hematocrit. The Chromium to Iodine activity is separated spectrophotometrically by assessing the upper limits of the Chromium activity and taking the iodine counts from above this region. For this purpose it is necessary to have a highly discriminating crystal (preferably 2" x 3") in order to discern the subtle variations in energy differences between Chromium and ^{131}I. Although the albumin could be labeled with other isotopes (e. g. ^{99m}Technetium) it would seem preferable to select an albumin label which is of a very close energy to the ^{51}Cr so that the anatomic sites discerned would be similar and not dependent upon variations in self absorption. ^{51}Cr and ^{131}I energies are close enough that most external detecting

devices would discern the same anatomic regions if they can be adequately separated. Fig. 4 shows the pulse-height analysis of Chromium vs Iodine using a 2" x 3" crystal. The potential for ready discrimination of these two isotopes is apparent. With the experiments to date, we have been able to separate Chromium from Iodine with the following efficiencies in a well counting device (e. g. the Picker Liquimat).

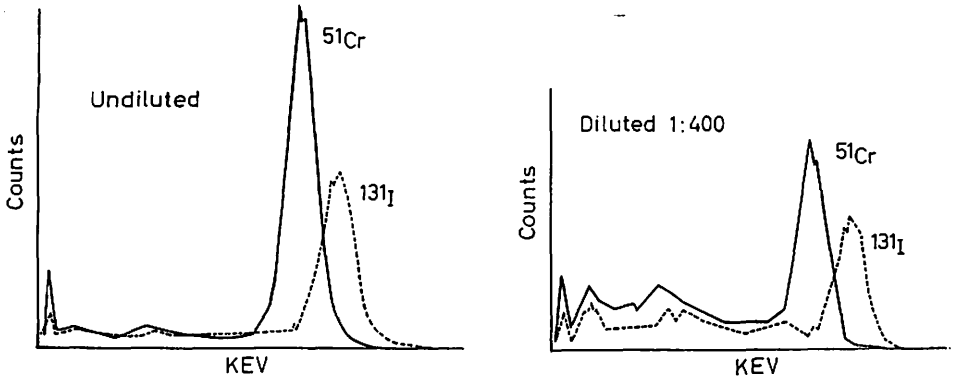

Fig. 4 Pulse-height analysis of ^{51}Cr and ^{131}I obtained with multichannel analyzer revealing the ability to separate these two isotopes (left). – The figure on the right represents specimen (diluted 1–400) demonstrating that the Iodine scatter by tissue will not significantly influence the ability to discriminate the two isotopes in this system. These observations have been confirmed in a similar experimental model using a 2" sodium thallium activated sodium crystal as for external detection.

	Cr Efficiency	Iodine Efficiency
Cr Window	45 %	7.9 %
I Window	0.65 %	25.3 %

The extravascular albumin activity is seen from the $^{131}I/^{51}Cr$ ratio obtained with the probe minus the ratio obtained in the blood sample since the probe ratio includes the combination of extravascular albumin plus intravascular albumin. Extravascular albumin activity can then be calculated from the following formula:

$$R_{Total} = \frac{EV_A^+ + IV_A^+}{C^+_{Cr}} \qquad R_B = \frac{IV_A^+}{C^+_{Cr}} \qquad R_T - R_B = \frac{EV_A^+}{C^+_{Cr}}$$

$$\therefore IV_A^+ = C^+_{Cr} R_B \qquad \therefore EV_A^+ = C^+_{Cr}(R_T - R_B)$$

where EV_A^+ = extravascular albumin activity, IV_A^+ = intravascular albumin activity, C^+_{Cr}= red blood cell Chromium activity, $R_{Total} = \frac{\text{ratio of albumin activity}}{\text{Chromium activity}}$ (external probe), and $R_B = \frac{\text{ratio of albumin activity}}{\text{Chromium activity}}$ (blood sample).

The calculated extravascular albumin activity should be corrected for biologic and physical decay both of which have been well established. The corrected extravascular activity (EVA corr) equals the extravascular activity measured (EVA meas.) plus biologic and physical decay.

The biologic decay for purposes of uniformity is expressed as percentage of the five minute sample lost to catabolism. The normal fractional catabolic rate (FCR) equals 8.5% per day with a standard deviation ± 1.0 (*Rossing*, 1967). This normal constant can be used unless patients have gross alterations of albumin metabolism in which case the catabolic rate can be calculated from the terminal slope obtained by following the intravascular albumin activity beyond 72 hours. Then the catabolic loss of albumin during the duration of measurement is considered the same as the loss rate following a total albumin equilibration between the intravascular and extravascular space. Thus, in the patients with abrupt changes in total albumin kinetics, this method is not feasible. Physical decay is corrected for by counting against a standard and expressing EV_A^+ as % of injected dose.

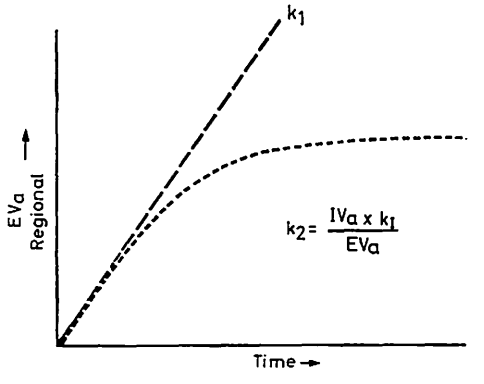

Fig. 5

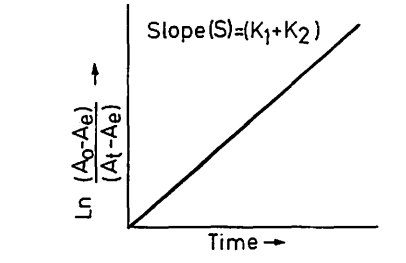

Fig. 6

Fig. 5 The plot of the regional extravascular albumin activity against time, the extravascular albumin activity being in arbitrary units. k_1 in this instance is the rate of exchange in hours^{-1} which can be converted to half time of albumin residence ($t_{1/2} = \frac{.693}{k}$).

Fig. 6 The kinetic plot of the same data as seen in Fig. 5 using the equilibration point to quantify k_1 and k_2. This approach will allow a more quantitative calculation of k_2. The plot fit of the total data suggest that the regional calculations would be kinetically valid for this experimental model. The use of the notation A_0, A_e, and A_t are for the general solution as these figures can represent either the intravascular or extravascular albumin activity depending upon the type of experiment. For regional studies extravascular albumin activity is used. This figure defines the general solution to the problem of regional extravascular transport when A = extravascular activity. Note that this plot is identical to that seen in Fig. 3.

Using the model described in Fig. 1 above where the extravascular compartment returns to the intravascular compartment via the lymphatics one can calculate k_2 by plotting the extravascular regional activity vs time (Fig. 5). At zero time the entire albumin activity is located in the intravascular space and only k_1 will be discerned. Thus, the slope of the curve at zero defines k_1. From this k_2 can readily be calculated if IV_A^+ is known (i. e. $IV_A^+ \times k_1 = EV_A^+ \times k_2$). This method of extrapolation, although

easy to understand, is rather crude and difficult to quantify. For this reason, we advocate the more standard kinetic approach as defined in Fig. 6. From the slope of the curve $\ln \frac{A_0 - Aeq}{A_t - Aeq}$ vs time, we can readily calculate k_1 and k_2 defined in units of hour^{-1}, which can be converted to half times of extravascular albumin residence (i. e. $t_{1/2} = \frac{.693}{k}$).

Discussion

The use of the extravascular return rate of albumin as an index of regional lymphatic transport is currently limited to residence $t_{1/2}$ of albumin in the extravascular space since lymphatic flow rate in terms of cc/hour cannot be defined without knowledge of the interstitial or intralymphatic specific activity. The flows of IV_A and EV_A are being measured as rate constants. This can only give values of flow in cc/minute if we know the size of the compartment. That is, that the problem of dilution in certain pathologic states may preclude the quantified assessment of lymphatic flow. For the total body we can calculate the size of the compartment easily using the standard dilution technique

$$V_2 = \frac{C_1 V_1}{C_2}$$

where C_1 and V_1 are the concentration and volume of the tracer before dilution and C_2 and V_2 are the concentration and volume after dilution. After 5–10 minute equilibration V_2 can be taken as the total intravascular albumin (IVA) while a second measurement at 48 hours will define IVA + EVA volume which must be corrected for catabolism and physical decay. From this the volume of extravascular albumin can be calculated from the equation: EVA Volume = 48 hr V_2 (corrected for biologic and physical decay) – 5 min. V_2.

For the regional values we can calculate the size of the compartment the probe is observing only if we know the size of the volume the probe is looking at. This can be determined if we could find the efficiency of the probe. This is theoreticly possible if we are looking at a limb with two probes so in fact we are observing a cylinder. If a known amount of ^{51}Cr and ^{131}I are placed in a phantom of the cylinder's dimensions the efficiency can be calculated. Using the efficiency we can calculate the % of the injected dose we are looking at with the probe as $\frac{\text{counts}}{\text{efficiency} \times \text{injected activity}}$ and hence the size of the regional compartment (from total compartment activity). If total body EVA volume is EVA_t and regional EVA volume is EVA_r, then flow from EVA_r is $EVA_r \times k_{2r}$ cc/hour, where k_{2r} is rate constant k_2 for the region. Although the extravascular albumin return rate alone in some organs and in many physiologic and pathologic states will provide a reliable index of lymphatic albumin transport, it is theoretically possible to measure regional lymph flow in cc/unit time with this technique.

We assume that the extravascular albumin half time is measured if and only if; a) there is a steady state system, i. e. the albumin lost to the extravascular space is eventually returned; b) labeled red blood cells remain in the intravascular space;

c) ^{131}I and ^{51}Cr can be discriminated externally by the probes used; d) the biologic decay of labeled albumin is constant for the duration of the measurement; e) the ratio of initial regional hematocrit to large venous hematocrit remains stable during the measurement; f) extravascular catabolism of albumin is not taking place in the area being measured.

In addition, the extravascular albumin t $^1/_2$ is an index of regional lymph flow only if lymph flow limits the turnover rate of the extravascular albumin. Whereas we acknowledge there may be a problem with diffusion, most likely capillary to lymphatic diffusion distances are not as significant a problem as one would see with interstitial injections. At least in the proposed method there is no regional alteration of lymphokinetics from the measurement procedures. Further studies will be necessary to define whether more than one first order compartment can be discerned by the regional measurement.

The method herein described provides a general solution to the problem of assessing regional extravascular isotopic content and can readily be applied to other labels which may have more or less relative affinity for lymphatic transport. With this technique, therefore, it may be possible to assess regional variations in transcapillary refill and/or lymphatic transport.

Summary

A method has been developed whereby total body extravascular albumin turnover can be measured which may well provide an integrated assessment of total body lymphatic transport. The total method, however, is limited by the fact that there may be gross differences in regional activity due to non-lymphatic return of extravascular albumin (e. g. intestines), and the fact that in many experimental situations it is far preferable to compare regional changes in lymphatic transport.

A somewhat more sophisticated double labeled technique has also been developed to discern regional extravascular albumin activity change with time as an index of regional lymphatic turnover in those organs where extravascular return is dependent upon lymphatic transport, (e. g. muscle). The general solution to the problem of double labeled extravascular and intravascular measurements is defined and advocated for further exploration into the regional assessement of interstitial kinetics. Although this method does not measure lymphatic flow in terms of cc/minute, one can reliably assess the t $^1/_2$ of label removal from tissue which in many cases is an index of lymphatic transport. Further studies into the problems of diffusion and dilution are advocated to improve upon the proposed technique.

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