Lymphology 6 (1973) 169-174

An Indirect Method for the Estimation of Lymph Flow

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Introduction

In the course of studies on the lymphatic circulation of the hind limbs of the dog carried out in our Laboratory, a method using radioactive tracers was developed which allows the determination of some parameters related to lymph circulation: the mean transit time through a segment of the lymphatic system and the lymphatic flow.

This technique is inocuous for the subject, causes a minimal disturbance of the physiological factors involved in lymph flow, and is based on some properties of constant flows through systems of labyrinths.

Theory of the Method

Consider a system as shown in Fig. 1 where L_2 is fed by several inlet labyrinths L_1, L_1' ....., L_1^n perfused by constant flows F_1, F_1' ..... F_1^n making a total flow F through L_2.

Suppose that a step pulse injection is started at the entrance of L_1 at instant t=0. Let $h_1(t)$ and $H_1(t)$ be respectively the unit impulse response function and the step pulse response function of L_1. Similarly $h_2(t)$ and $H_2(t)$ are the response functions for L_2.

If a detection system D_1 conveniently collimated detects and records the activity at the inlet of L_2 and a second detection system D_2 records the total activity in L_2, curves such as in Fig. 2 are obtained.

Detector D_1 actually records $A_1(t)=K_1 \cdot H_1(t)$ where K_1 is a proportionality constant which takes into account the efficiency of the detector and the radioactivity injected.

The response of detector D_2 is

$$A_2(t) = K_2 \int_o^t [H_1(t) - H_1(t) * h_2(t)]\, dt \qquad (1)$$

where K_2 is a proportionality constant and the sign * means convolution.

Eq. (1) states that at instant t the mass of tracer in L_2 is the difference between the total mass of tracer which entered the system and the total mass of tracer which left it until the instant considered. On the other hand $H_1(t)*h_2(t)=H(t)$ is the response of the series system formed by L_1 and L_2 to a unit step pulse. Then eq. (1) can be written

$$K_2 \int_o^t H(t)\, dt = K_2 \int_o^t H_1(t)\, dt - A_2(t) \qquad (2)$$

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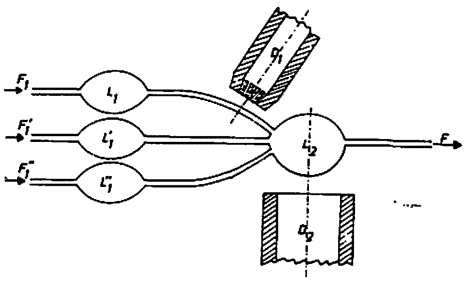

Fig. 1. Model of the lymphatic circulation of the hind limb of the dog

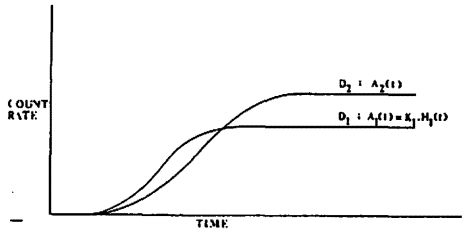

Fig. 2. Curves of count rate vs. time obtained with detectors D_1 and D_2

The first part of the right hand side of eq. (2) can be obtained graphically from Fig. 2 by integration of curve $A_1(t)$. The function $K_1 \int_0^t H_1(t)\,dt$ is thus obtained (Fig. 3). This function can be converted in $A_3(t) = K_2 \int_0^t H_1(t)dt$ using a change of scale. In principle this is easily achieved because there is a finite delay between the input and the output of L_2 i.e. the profiles of $A_3(t)=K_2 \int_0^t H_1(t)dt$ and $A_2(t)$ must coincide for a short period after t_1.

Consequently if the function $K_1 \int_0^t H_1(t)\,dt$ in Fig. 3 is multiplied by the factor b/a the two parts of the right hand side of eq. (3) are obtained on the same scale. Now subtracting these two curves the function

$$A_4(t) = K_2 \int_{t_1}^t H(t)\,dt$$

is obtained (Fig. 4).

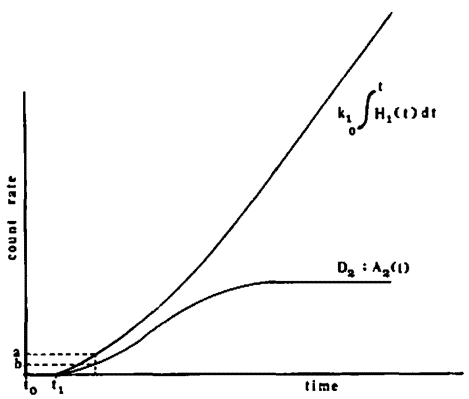

Fig. 3. Curve of the integral of $A_1(t)$ and curve $A_2(t)$

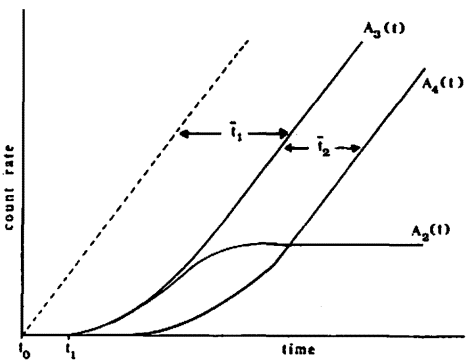

Fig. 4. Curves $A_3(t)$ and $A_4(t) = A_3(t) - A_2(t)$. Graphical determination of $\bar{t}_1$ and $\bar{t}_2$

When $A_2(t)$ is parallel to the t axis the two curves $K_2 \int_{t_1}^t H_1(t)\,dt$ and $K_2 \int_{t_1}^t H(t)\,dt$ are parallel to each other.

It is possible to show that the time delay between these two parallel lines is the mean transit time $\bar{t}_2$ through the system L_2 (see Appendix).

The mean transit time through the system L_1 can also be determined. If a straight line with the same slope as the parallel portions of the two curves shown in Fig. 4 is traced from the origin, the time delay between this straight line and the straight part of $K_2 \int_0^t H_1(t)dt$ is the mean transit time $\bar{t}_1$ from the injection point to the input of system L_2. The total mean transit time from the injection point to the output of L_2 is $\bar{t} = \bar{t}_1 + \bar{t}_2$.

Having the mean transit time through L_2 the total flow F can be calculated if the volume V of this labyrinth is known by the equation $F = \frac{V}{\bar{t}_2}$.

Experimental Model

This method was tested with a model (Fig. 5) that reproduced the main features of lymph flow through a lymph node and its afferent lymphatic vessels. The mean transit time through the model of lymph node was calculated graphically from the curves obtained with detectors D_1 and D_2 (Fig. 6) as explained in the theory of the method.

The effective volume of the model of lymph node was obtained from the difference of the weights of the model when full of distilled water and dry.

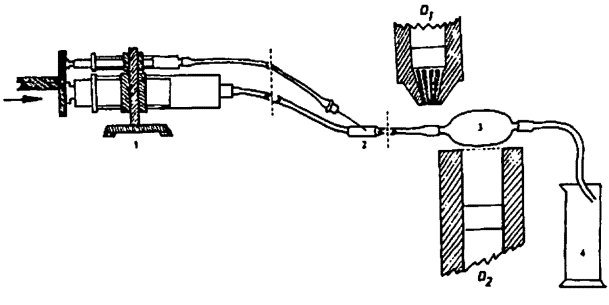

Fig. 5.
1. Device for constant velocity injection with large syringe for lymphatic circulation simulation and small syringe for tracer injection simulation.
2. Injection point.
3. Lymph node model consisting of a piece of plastic sponge inside a plastic bag.
4. Measuring cylinder.

D_1 - Scintillation detector with focused collimator.
D - Scintillation detector with parallel hole collimator.

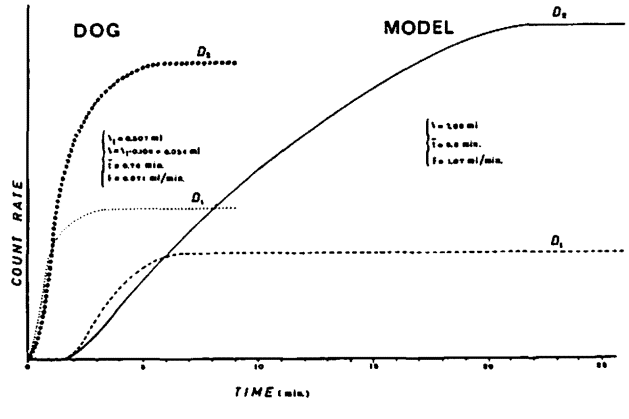

Fig. 6. Curves actually obtained with detectors D_1 and D_2 from the model and from a "in vivo" experiment. Allowing for the differences in volume and flow rate, the main features of the two pairs of curves can be seen to be very similar.

The direct measurement of the flow of liquid through the model was checked with the values obtained by the proposed method.

The results were very satisfactory as can be seen below.

Direct measurement:

- Volume of liquid collected during the test (Fig. 5-4) ---- 39.0 ml
- Time ---------- 32.47 min.
  - Flow rate ---------- 1.20 ml/min.

Indirect measurement:

- Effective volume of the model of lymph node (Fig. 5-3) ---------- 7.09 ml
- Mean transit time calculated according to the proposed method ---------- 6.6 min.
  - Flow rate ---------- 1.07 ml/min.

"In vivo"-Experiments

Normal healthy dogs of various ages and sizes were used in these tests.

General anaesthesia with a barbiturate (Pentothal) administered by intravenous injection was induced to ensure the immobility of the animals during the test.

The animals were placed in lateral or ventral recumbency and two collimated scintillation detectors coupled to ratemeters and pen recorders were positioned over one of the hind limbs, the first aimed at the popliteal lymph node and the other (a focused collimator) at its afferent lymphatic vessels as close to the first as possible.

After recording the background, a dose of 110 μC of 125 I RISA diluted in 0.5 ml of isotonic saline was injected subcutaneously into the dorsal aspect of the foot. Care was taken in order to avoid the puncture of any blood vessel (an occasional hazard) and excessive interstitial tension caused by the injection was avoided by first inserting the needle and then injecting the tracer as the needle was slowly withdrawn. This spreading of the tracer also increases its initial absorption, in such a way that a step pulse injection is approximated. We have shown experimentally this approximation by recording the activity on the lymph vessels immediately above the injection point.

The calculation of V (the effective volume of the lymph node) was carried out by an indirect method. An image of the lymph node was obtained with a high resolution spark chamber gamma camera (1, 2) (Fig. 7). The node was assumed to be an elipsoid of revolution and its total volume was calculated using the formula

$$V_t = 0.162\ A \cdot d$$

where A is the area of the projection given by the spark chamber gamma camera and d its longest dimension.

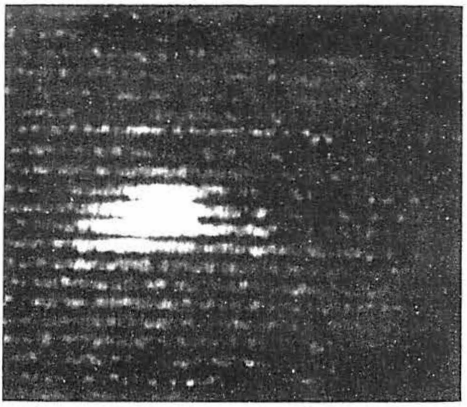

Fig. 7. Image of a popliteal lymph node of a dog as obtained with a high resolution spark chamber gamma camera.

The ratio of total volume of the lymph node to labyrinth volume or effective volume was previously obtained by aspiration of the content of several lymph nodes of normal dogs and centrifugation of the aspirated material in microhematocrit tubes.

The ratio of total volume of centrifuged material to supernatant for 6 determinations of normal nodes was 0.106 ± 0.013, (± SD).

Then the estimated value of the lymphat ic flow through the node is

$$F = \frac{V_t \cdot 0.106}{\bar{t}}$$

Fig. 6 shows one of the records thus obtained.

Discussion and Conclusions

Lymphatic flow is so strongly affected by so many factors that a method for its determination which doesn't introduce by itself disturbances in the lymph circulation is greatly desirable.

The method described here seems to show advantages with respect to the methods implying intravascular cannulation of lymphatics since the radioactive tracer is introduced interstitially. Besides these data are obtained by external detection.

This method has some potential causes of error which must be considered in order to get reliable results:

a) In the theory of the method it is assumed that the input is a step function injection. This input is obtained only approximately.

b) The detection of radioactivity at the input of the lymph node is obtained on a finite length of lymphatic vessel.

c) It is sometimes technically difficult to make the fitting of the initial portions of the two integral curves as described in the theory of the method.

d) The calculation of the lymph node capacity although reasonably accurate to a normal situation is probably an assumption in pathological situation where the ratio total volume to labyrinth volume can be altered.

The objection raised in a) does not affect the determination of the mean transit time through the lymph node and consequently the determination of the lymphatic flow but it will increase the value obtained for the mean transit time from the injection point to the lymph node. This transit time is a valuable functional index for the lymphatic circulation of the preganglionar region. Using the injection technique described before this cause of error has been considerably lowered.

To minimize the effect referred to in b) we have used a focused collimator aimed at a point as close to the input of the lymph node as possible.

In general the difficulty considered in c) is solved empirically by trials.

With the precautions mentioned above we believe that the method just described can be used with good results to determine mean transit times and lymphatic flows in situations where the conditions of the model prevail.

Appendix

Theoretically the mean transit times of dilution systems using constant acceleration injections of tracers is directly available from graphs containing the input and the output functions, as described by *Zierler* in "The Cardiovascular System" (3).

It is possible to show that the properties of constant acceleration injections are applicable to the integrals of a step function injection at the input and output of the system. If U(t) is the unit step function a constant acceleration injection can be represented by

$$M(t) = \int_0^t U(t)\, dt = t$$

we want to prove that

$$[\int_0^t U(t)\, dt\ ] * h(t) = \int_0^t [U(t) * h(t)]\, dt$$

or

$$t * h(t) = \int_0^t H(t)\, dt$$

where h(t) and H(t) are the frequency function and the cumulative frequency function of the dilution system.

Applying Lapalce transforms to the first part of last equation

$$\mathcal{L}\,[t * h(t)] = \frac{1}{s^2}\ \mathcal{L}\,[h(t)] = \frac{1}{s^2}\ \mathcal{H}\,(s)$$

but the inverse Laplace transform of this function

$$\mathcal{L}^{-1}[\ \frac{1}{s^2}\ \mathcal{H}\,(s)] = \int_0^t \int_0^\tau h(\lambda)\, d\lambda\, d\tau = \int_0^t H(t)\, dt$$

where τ and λ are auxilliary variables.

It is then valid to integrate a step function as well as the response of a system to a step function and to handle the integrated functions as if an injection at constant acceleration had been performed.

References

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