Distribution of Circulation Rates within a Single Tissue Type

Abstract. *An analysis is undertaken to determine the continuous distribution of circulation rates which would be necessary for a single tissue type to display a simple exponential response for the exchange of a solute with blood. It is shown that there can be no more than one real solution. This indicates that heterogeneous circulation cannot be invoked to explain anomalies to the popular assumption that blood perfusion is the process limiting the rate of blood-tissue exchange.*

It has been generally accepted that circulation is the process limiting the rate of exchange of inert substances between blood and tissue (*1*, *2*). If this assumption is made, then the following simple expression for tissue tension (p) may be used to determine the local blood perfusion rate (Q) from data for the "washout" of an inert tracer (see *3*):

$$p = P_A \cdot \exp(-\lambda Q t) \qquad (1)$$

for a step in arterial tension (p_A) defined by $p_A = P_A$ for $t \leq 0$ to $p_A = 0$ for $t > 0$, where t represents time; λ is the blood : tissue partition coefficient for the tracer, and P_A is constant.

The "washout" of tracers from the whole body (*1*), or from whole organs (*4*), can be analyzed into several exponential terms which have been attributed to component tissue types in parallel to the same blood supply. However, this simple explanation does not provide an adequate correlation of much quantitative data, an example being that recorded (*5*) for the simultaneous elimination of tracers of similar molecular weight yet greatly different lipid-aqueous partition coefficients.

Explanations for such discrepancies include the following:

1) parallel arteriovenous pathways within each tissue type (*6*);

2) inter-tissue diffusion (*7*);

3) heterogeneous permeability of each tissue type (*8*), bulk diffusion within cells making a far more significant contribution to limiting blood : tissue exchange than that estimated assuming radial diffusion in a homogeneous medium (*9*).

The first two explanations should be tested more critically if applied to a single tissue type. One of the closest practical approaches to such an ideal case is afforded by skeletal muscle for which the curve for the washout of tracers, such as ^{133}Xe (*10*) and ^{85}Kr (*8*), can be analyzed into two or three exponential components. Advocates of parallel, yet dissimilar, arteriovenous pathways within skeletal muscle have attributed this format to:

1) heterogeneous perfusion, such as postulated in liver (*6*);

2) a dual circulation (*11*); and

3) direct arteriovenous shunts (*12*), although this would appear most unlikely from anatomical and other evidence collected by Barlow *et al.* (*13*).

The "dual circulation" explanation would appear equally unlikely in view of the experimental fact that a single exponential can be obtained from the uptake of radiosodium from the skeletal muscle of a cat (*14*).

The query which arises is how such a simple linear response to arterial change can be interpreted by the more popular tissue model based upon heterogeneous perfusion. Van Liew (*15*) has indicated the need to consider whether any linear response obtained from a biological system is contributed by a continuum of exponential processes. The vital question is therefore whether it is possible for any such spectrum of circulation rates to give an overall format comprising one exponential term and, if so, what the relative preponderance of each perfusion rate would need to be.

Quantitatively, the question may be resolved by determining the distribution of circulation rates as the cumulative fraction (R) of tissue capacity for solute versus the corresponding blood perfusion rate (Q).

Consider a finite-stage model of m tissue regions in parallel for which the general region, the nth, has a total solute capacity R_n, perfusion rate Q_n, and partition coefficient λ_n. For such a tissue to give an overall single exponential (time constant K), as reported by Walder (*14*):

$$\sum_{n=1}^{m} R_n \cdot \exp(-\lambda_n \cdot Q_n \cdot t) \equiv \exp(-Kt) \qquad (2)$$

since this relationship must hold for all values of t, and, by definition,

$$\sum_{n=1}^{m} R_n = 1$$

Reverting to a continuous distribution of perfusion rates, Eq. 2 becomes

$$\int_0^1 \exp(-\lambda Q t)\,dR \equiv \exp(-Kt) \qquad (3)$$

Taking successive derivatives with respect to t, and putting $t = 0$:

$$\int_0^1 dR = 1 \qquad (4)$$

$$\int_0^1 (\lambda Q)\,dR = K \qquad (5)$$

$$\int_0^1 (\lambda Q)^2 dR = K^2 \qquad (6)$$

such that for the general (nth) derivative:

$$\int_0^1 (\lambda Q)^n\,dR = K^n \qquad (7)$$

Since (λQ) must be real, K is also real, and

$$\int_0^1 (\lambda Q - K)^2\,dR = K^2 - 2K^2 + K^2 = 0.$$

But, since $(\lambda Q - K)^2 \geqq 0$,

$$\lambda Q = K \qquad (8)$$

is the only real solution.

The others must be complex; Eqs. 4 to 7 can be satisfied by the more general solutions:

$$\lambda Q = K + a_1 \cdot \exp(2\pi i R) + a_2 \cdot \exp(4\pi i R) + a_3 \cdot \exp(6\pi i R) + \ldots \qquad (9)$$

or

$$\lambda Q = K + b_1 \cdot \exp(-2\pi i R) + b_2 \cdot \exp(-4\pi i R) + b_3 \cdot \exp(-6\pi i R) + \cdots \qquad (10)$$

where a_1, a_2, a_3 . . . and b_1, b_2, b_3 . . . are arbitrary constants, and $i = \sqrt{-1}$.

To avoid any imaginary component to the general solutions,

$$a_1 = a_2 = a_3 = \cdots a_n = 0, \text{ and}$$
$$b_1 = b_2 = b_3 = \cdots b_n = 0,$$

when Eqs. 9 and 10 revert to Eq. 8. This reaffirms that there is only one real solution to Eqs. 4 to 7, which is $\lambda Q = K$.

This implies that, for an aqueous tissue type where λ is constant, Q can have only one value. Thus, for skeletal muscle to give the pure linear response to changes in arterial concentrations of isotopic sodium recorded by Walder and others, there must be uniform circulation if blood perfusion is the rate-limiting process. However, there would seem to be no reason why the circulation distribution should be any different for the exchange of inert isotopes as for Na^{24}.

Hence, the analysis above would indicate no justification for invoking heterogeneous perfusion to explain anomalies from Eq. 1 in the interpretation of data for the elimination of

inert tracers from single tissue types. This would suggest that diffusion is making a more significant contribution to limiting the rate of blood-tissue exchange than is generally accepted.

B. A. HILLS
Department of Chemical Engineering, University of Adelaide, Adelaide, Australia

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15 May 1967

Vitamins D_2 and D_3 in New World Primates: Influence on Calcium Absorption

Abstract. *In* Cebus albifrons *monkeys it was demonstrated that vitamin D_3 promotes the intestinal absorption of calcium-47 and that vitamin D_2 does not increase absorption above that seen in monkeys deficient in vitamin D. These data support previous observations that vitamin D_2 is not effective in preventing metabolic bone disease in this species.*

The relative ineffectiveness of vitamin D_2 in the chick has been well established. It was demonstrated in 1930 that in this species vitamin D_2 has 10 percent (or less) of the activity of vitamin D_3 in the prevention and cure of rickets, promotion of body growth, egg production, and other parameters (*1, 2*). Approximately 30 years ago several reports presented clinical evidence indicating a similar but quantitatively smaller difference in the activity of vitamin D_2 and D_3 in human infants (*1*). These reports have been questioned, and whether or not a significant difference in activity does exist in man has been unresolved.

We have reported evidence (*3, 4*) which indicates that vitamin D_2 is not utilized or is significantly less effective than vitamin D_3 in the prevention of rickets, osteomalacia, and osteodystrophia fibrosa in several species of South American primates. These data were based on the development of bone disease in *Cebus albifrons* and several species of tamarins (*Saguinus oedipus, S. nigricollis, S. mystax*) maintained on a purified diet containing vitamin D_2 and the reversal of the disease process by substituting vitamin D_3 for D_2. We now report the influence of vitamin D_2 and vitamin D_3 on calcium absorption, which provides additional evidence that vitamin D_3 is more active than D_2 in the Cebus monkey.

Six *Cebus albifrons* monkeys were maintained on a purified diet containing 2000 international units (IU) of vitamin D_2 per kilogram of diet for 24 months (*3*). The diet was composed of (in percentages): sucrose, 62.00; casein, 25.00; corn oil, 8.00; salt mix number IV (Hegsted *et al.*, 1941), 4.00; choline chloride, 0.50; dry vitamin mix, 0.40; and inositol, 0.10. The dry vitamin mix contained (in percentages): thiamine, 0.50; riboflavin, 0.50; pyridoxine 0.50; niacin, 2.45; calcium pantothenate, 1.50; folic acid, 0.05; biotin, 0.01; and dextrose, 94.44. Vitamin A acetate and the vitamin D_2 in absolute alcohol were added to the diet in amounts of 12,500 IU/kg and 2000 IU/kg, respectively. Ascorbic acid in dilute alcohol was added to the ration daily to supply 25 mg per monkey per day.

After the development of severe osteodystrophia fibrosa, vitamin D_3 (2000 IU/kg of diet) was substituted for vitamin D_2 for a period of 5 months. After observations on the reversal of the disease process, all vitamin D was removed from the diet. After the monkeys had been on the diet lacking vitamin D for 12 months, the clinical findings, roentgenograms, and serum alkaline phosphatase signified the exacerbation of osteodystrophia fibrosa. At this point two animals (Nos. 201 and 202) were provided a daily oral supplement of 500 IU of vitamin D_3; two (Nos. 209 and 214) with 500 IU of vitamin D_2; and two (Nos. 221 and 222) were left untreated. Twelve days after the supplements were initiated radio-calcium uptake studies were conducted on each animal. After the monkeys were fasted for 24 hours, 7 μc of calcium-47 were administered via stomach tube and the animals were provided with 50 g of purified diet. Serum samples were obtained at 2, 4, 6, and 24 hours, and urine and feces were collected at 24-hour intervals for 5 and 7 days, respectively. The skull was monitored at 48 hours by holding the head beneath the probe of a scintillation counter. One milliliter of serum was counted for each time interval, in a gamma counter. The urine was heated for 1 hour with 10 ml of HCl, cooled, and made up to 200 ml with distilled water, and 2-ml aliquots were counted. The feces were mixed with a small amount of water and heated with 25 ml of HCl, cooled, and brought up to 200 ml with water, and 2-ml aliquots were counted.

To determine whether, in the Cebus monkey, vitamin D has any direct influence on the mineralization of bone, or on urinary excretion of calcium, the animals were maintained on the oral supplement of vitamin D for an additional 42 days and then injected intravenously with 3 μc of Ca^{47} (experiment 2). Serum samples were collected at 5, 10, 30, 60, 180, and 360 minutes. Urine and feces were collected and processed as in the first study and the skull was monitored at 48 hours. After the monkeys had been an additional 70 days on the vitamin D supplements, a second oral uptake study was performed (experiment 3), essentially as described above. However, a dose of 5 rather than 7 μc of Ca^{47} was given, and the animals were not fed for 6 hours.

Table 1. Thousands of counts per minute over the skull of monkeys at 48 hours.

Vitamin supplement	Thousands of counts		
	Exp. 1 (oral)	Exp. 2 (i.v.)	Exp. 3 (oral)
Vitamin D_3			
Monkey 201	17.1	6.8	12.2
Monkey 202	23.2	7.0	15.3
Vitamin D_2			
Monkey 209	2.5	6.8	9.1
Monkey 214	1.5	5.2	9.1
None			
Monkey 221	2.0	7.1	6.8
Monkey 222	0.7	5.9	6.6