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Blood flow rates in compact and cancellous bone, and bone marrow

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Several attempts are now on record at measuring rates of blood flow in skeletal tissue. All methods employed to elucidate this problem, particularly relevant to an understanding of the local control of osteogenesis, have to reckon with the complex mode of vascularization of tubular bones. Numerous arteries, epiphyseo-metaphyseal as well as the nutrient artery, form the afferent limb of the osseous circulation: the efferent pathway is even more diffuse and includes the entire surface of the shaft (Brookes, 1964, 1965; Oliveros & Palacios, 1964). To date, the methods that have been devised in individual laboratories do not evaluate rates of flow in the various anatomically distinct tissues comprising a whole bone organ, nor have they readily been taken up by other workers, possibly because of the complexity of instrumentation involved. Only one method (Edholm, Howarth & McMichael, 1945) claims applicability to man and this has not been repeated since its publication twenty years ago. The following method of measuring flow rates in various parts of the osseous circulation is therefore offered as a practical approach to the acquisition of a wider range of skeletal haemodynamic data than attempted heretofore, and in the hope that its relative simplicity (nothing more sophisticated than a scintillation counter being required) will lead to its testing by other investigators.

MATERIALS AND METHOD

The investigation was carried out on 140 young white female rats weighing 120–140 g, and in two stages utilizing a radioisotope dilution technique in which red cells were labelled with ^{51}Cr. In the first stage the circulatory red cell volume, V, in a selected tissue was determined. The second stage of the investigation obtained data relating the concentration of injected labelled red cells to time, so that thereby a rate constant, k, for flow might be determined. The product of these two factors gives an estimate of the rate of red cell flow in the tissue investigated (Veall & Vetter, 1958). This could be converted into a value for whole blood by applying an arbitrary haematocrit.

Stage 1. Red cells from heparinized donor rats were packed, washed in saline and labelled with ^{51}Cr in the form of sodium chromate (Gray & Sterling, 1950). It is essential that red cells for injection into host rats should be drawn, labelled and injected in the fresh state, so that only circulatory red cell volume be measured, avoiding both fortuitous labelling of plasma proteins by the reduced trivalent ion, and uptake of effete corpuscles by reticulo-endothelial elements in the marrow vasculature.

The precise amount of label injected into the circulation of host rats need not be

known. In practice 100 μc ^{51}Cr was used for labelling 0·5 ml of packed red cells for each rat. The labelled cells were washed repeatedly until the radioactivity of the washings was the same as background. The host rat was then anaesthetized and heparinized, and the labelled red cells injected into the superficial epigastric vein. This vessel, for quick, efficient, intravenous injection under direct vision, is better than the commonly utilized ventral caudal vein. After allowing 15 min mixing time, the tail of the rat was amputated, a drop of blood caught on a glass slide, and the anaesthetized rat dropped into a Dewar vessel containing an acetone-Drikold mixture at -50 °C. The rat was thereby rendered solid and the circulation stopped.

Three 0·005 ml blood samples were gathered in micropipettes from the drop of tail blood. Another sample was gathered for haematocrit determination by centrifuging the blood in its micropipette and measuring directly the proportion of packed cells in the column. (A pilot experiment showed no difference between the haematocrits of cardiac blood measured simultaneously in this way and in Wintrobe tubes.)

The frozen femora were removed, manually cleaned of extraosseous tissue and fixed in 2 % formalin for 24 h. Several tissue specimens were than taken, namely the middle third of the shaft cortex, the marrow core associated with it, the superior and inferior metaphyses, and the inferior epiphysis. The superior epiphysis in the head of the femur was not included because it was not invariably ossified, and at most, a centre of ossification had only just put in an appearance. Bone sampling was facilitated by using a low speed dental drill with fine burrs and circular sawblade attachments. Care was taken to remove all traces of articular and growth cartilage adherent to samples of epiphysis or metaphysis. All samples were weighed immediately after isolation. The micro-balance used gave a reading to 0·1 mg. The blood, bone and marrow samples were then metered in a well-type scintillation counter. From these data the circulatory cell volume, V, was calculated by simple proportion

$$V \text{ ml}/100 \text{ g tissue} = \frac{\text{radioactive concentration counts/g} \times 43{\cdot}4}{\text{counts}/0{\cdot}005 \text{ ml. tailblood} \times 200}.$$

were $43{\cdot}4 \pm 0{\cdot}095$ (2·2 %) was the mean haematocrit of tail blood (Table 2).

Stage 2. Labelled red cells were prepared as before, and rats anaesthetized in an ether chamber until regular abdominal respiration was present. Under conditions of stop-watch timing, a measured bolus of 0·3 ml packed red cells was injected intravenously into the rats. At second intervals, 3–8 s after the plunger of the syringe starts to descend, then at 10, 12, 14, 16 s thereafter, the hind quarters were amputated by guillotine in a single blow, thus stopping instantaneously the circulation in the limbs. Any blood leakage from them *post-mortem* was obviated by immediate freezing in the acetone-Drikold mixture. Bone and marrow samples were prepared as before and weighed. Samples of the labelled red cells used for injection were also taken. All samples were then metered in a scintillation counter.

Values for tissue radioactivity were corrected for decay back to the day of the experiment. No correction was made for differences in rat weight. A correction was made, however, for between-rat variation in the radioactivity of red cells used for injection, by calculating all tissue radioactivities on the basis of a standard bolus activity of 100,000 counts/100 s/0·005 ml packed cells. The actual radioactivity of the injected red cells for each rat was already known. These standardized values of

tissue radioactivities were then plotted graphically against time on Cartesian paper. Inspection of the graphs suggested that a curve was present, the first up and down-slope representing the passage of a bolus through the vascular space of 1 g of marrow, cortex, etc. (Figs. 1–5). It was assumed that radioactive fall-off during the interval of the first down-slope, was exponential and could be represented by the formula

$$\log_e C = \log_e C_0 - k.t,$$

where C is the radioactivity at time t, C_0 is a constant, and k is the *rate constant* of the curve representing the proportion of the radioactivity that is removed per second by fresh blood following the bolus. (Veall & Vetter, 1958). A regression line was calculated for this linear equation from the data readings yielded by 61 rats

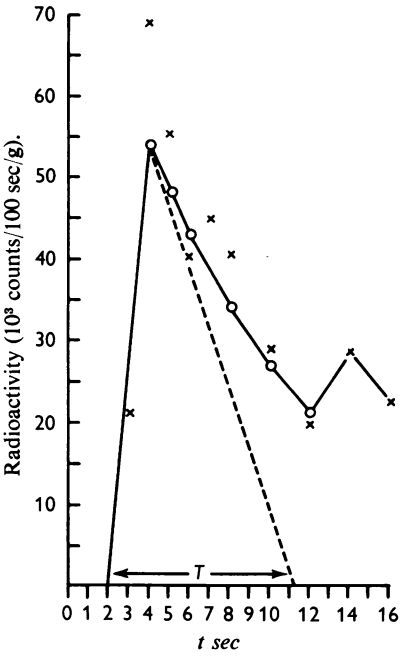

Fig. 1

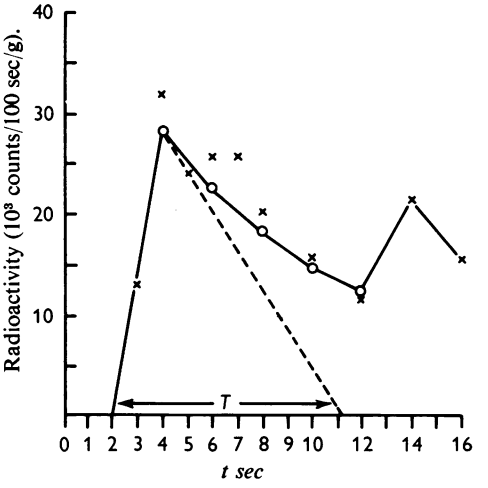

Fig. 2

Fig. 1. Cartesian plot of marrow radioactivity. ×, observed mean values; ○, values calculated by regression.

Fig. 2. Cartesian plot of cortical radioactivity.

killed in the time interval 4–10 s. The rate constant, k, was now known for each tissue. The flow rate, F, could then be calculated because $F = k.V$, both of which were now known. The calculated regression line was next plotted on to Cartesian paper (Figs. 1–5). The transit time, T, of a red cell through 1·0 g of tissue was then obtained by extrapolating the tangent to the initial down-slope of the graph to zero (Figs. 1–5). Transit times per unit red cell mass (T/V) were then calculated. The reciprocal of these times was taken as a relative measure of red cell velocity through the sinusoids and capillaries of the osseous circulation (Table 2).

RESULTS

Figures 1–5 are haemodynamic charts showing tissue radioactivity against time after intravenous injection, under direct vision, of 0·3 ml packed red cells labelled with ^{51}Cr. The data readings derive from the femora of 120 white female rats, under

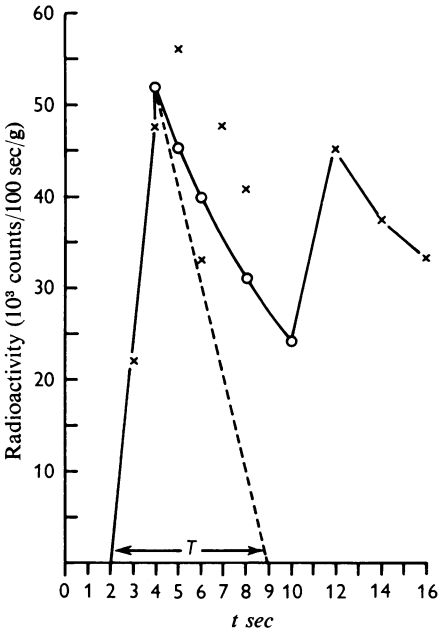

Fig. 3

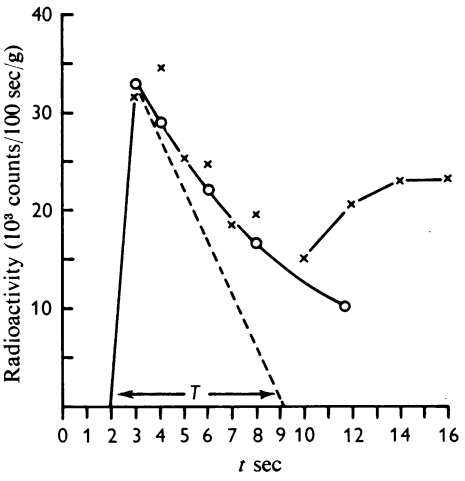

Fig. 4

Fig. 3. Cartesian plot of inferior metaphyseal radioactivity.
Fig. 4. Cartesian plot of superior metaphyseal radioactivity.

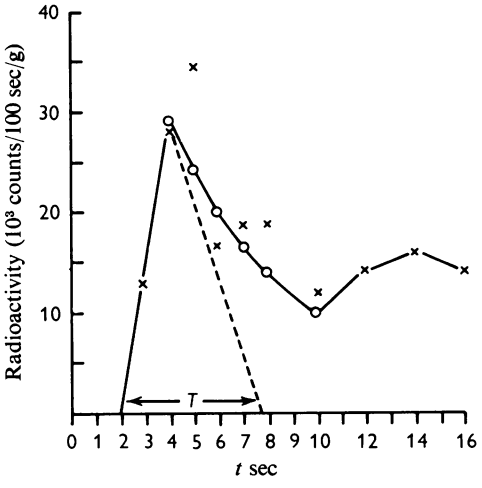

Fig. 5. Cartesian plot of inferior epiphyseal radioactivity.

light ether anaesthesia, and weighing 120–140 g each. The first downslope has been calculated by regression on data readings from 61 rats. The transit time, T, is the time taken for a red cell to cross 1 g of the tissue in question.

Table 1. *Regression constants in different parts of the femoral circulation calculated from data readings from 61 rats*

Tissue	C_0	k	S.D.
Superior metaphysis	49·7	0·14	0·03
Marrow	85·2	0·12	0·03
Cortex	43·6	0·11	0·02
Inferior metaphysis	85·1	0·12	0·04
Inferior epiphysis	61·8	0·19	0·04

mean k, 0·133; S.D., 0·031

Table 2. *Haemodynamic characteristics of the femoral circulation of the rat*

	Superior metaphysis	Marrow	Cortex	Inferior metaphysis	Inferior epiphysis
Red cell volume (Vml/100 g)	1·2	1·6	0·9	1·9	1·1
Standard deviation (%)	4·4	5·5	3·1	4·4	5·9
Red cell flow rate F = $V.k.$ 60 (ml/100 g/min)	10	13	7·5	15	9
Blood flow rate (haematocrit = 43·4 %)	22	29	17	35	21
Standard deviation (%)	27	27	28	27	28
Transit time, (T sec)	7	9·2	9·2	7	6
Red cell velocity, (V/T)	0·17	0·17	0·1	0·27	0·19
Relative velocity (%)	64	64	38	100	70
Relative flow rate (%)	64	85	50	100	60
Relative volume (%)	64	85	50	100	60

Table 1 shows for the tissues investigated, the calculated constant term C_0 and the rate constant, k, in the regression formula

$$\log_e C = \log_e C_0 - kt$$

which relates the Napierian logarithm of tissue radioactivity, C, against time, t, after injection of the bolus. No significant difference was found between the k factors when tested by Student's t test. Hence a mean value for k and its standard deviation is given in the table.

Table 2 shows the results of radioisotope measurement of the circulatory red cell volume in various parts of the femoral circulation. Flow rates are also given expressed in terms of red cells or whole blood by applying the haematocrit for mixed blood (stage 1) as well as relative red cell velocities. The test showed significant differences ($P < 0{\cdot}05$) between pairs of values for red cell volume, V, in all cases except between superior metaphysis and inferior epiphysis. The k factors, however, were not significantly different. The mean value was therefore used to calculate the flow rates given by the product $F = k.V.60$ ml/100 g/min. These are significantly different at the 5% level of probability except in the case of the above pair of tissues.

VALIDATION OF THE METHOD

The method employed here assumes that each femoral tissue investigated can be treated as a simple open compartment system such that a small bolus of red cells enters at time t_0, a circulatory space V contained by innumerable capillaries. The radioactive cells are found at t_0 at a maximum concentration of C_0 in the specified tissue. Thereafter, the bolus is washed out, exponentially, by unlabelled blood following on the bolus. But the large bolus originally injected into the whole animal has had to pass through the heart and lungs before reaching the arterial system, and hence has undergone some dilution. Nevertheless, the radioactivity of arterial blood on entering the selected tissues is the same in all cases. Further dilution of the radioactive red cells occurs in the circulatory space of each tissue. At t_0, the degree of dilution of the red cells should be the same for all tissues investigated. Now C_0 can be found from the regression line for each tissue (Table 1). The graphs have been constructed for 1 g of tissue, and the red cell bolus injected into each rat had a standardized radioactivity of 100,000 counts/100 s/0·005 ml packed red cells (see stage 2). It follows that

$$\frac{C_0}{100{,}000} \times 0{\cdot}005 \times 100 = V_1$$

where V_1 is the apparent volume of red cells in 100 g of tissue at time t_0.

Table 3. *Dilution factors for a radioactive bolus on entering various femoral compartments*

	V	V_1	Dilution %
Superior metaphysis	1·2	0·24	21
Marrow	1·6	0·43	27
Cortex	0·9	0·22	23
Inferior metaphysis	1·9	0·43	23
Inferior epiphysis	1·1	0·31	27

The actual volume, V, has, however, already been measured independently (stage 1). Hence, for each tissue an initial dilution factor for the bolus on entering it can be calculated ($= V_1/V, \times 100\,\%$). Table 3 gives these calculated dilution factors for the five tissues studied. Considering the wide variation in the haemodynamic data readings to be expected under these experimental conditions, it is all the more remarkable that the five tissues, treated separately and independently, should indicate that the bolus of radioactive red cells, as it reaches them for the first time on the first circuit round the body, dilutes approximately to the same extent, namely 1 in 4, a test which suggests that the method of flow measurement adopted here is a valid one.

DISCUSSION

The following investigators have estimated blood flow rates in mixed bone and marrow. Thus, Drinker, Drinker & Lund (1922) obtained a value of 3–41 ml % min in the canine tibia as against that of Edholm *et al.* (1945) of 0·5–1·0 ml % min in

the human humerus. On the other hand White, Ter-Pogossian & Stein (1964) recently obtained a value of 16 ml % min for rabbit tibia by analysis of a radioisotope build-up curve. According to their findings, flow in compact bone alone was immeasurably small. Nevertheless, Copp & Shim (1965) utilizing ^{85}Sr clearance from the blood have obtained a value for canine compact bone of 10 ml % min. Cumming (1962) by venous effluent collection measured marrow blood flow at 52 ml % min, range 20–100.

The method utilized in the present investigation depends on the prior estimation of circulatory red cell volumes and on the construction of follow-through curves. Only the marrow value for the former can be compared with the results of other workers (Osmond & Everett, 1965). These investigators give a value of 2·69 ml % for femoral marrow in the 400 g guinea-pig against the 1·6 ml % found here in the young rat. Their method involved special haemocytometric measurements which form no part of the procedure described in this paper. The values for circulatory red cell volume found here in various parts of the rat femur indicate pronounced variability in this quantity according to the part of the osseous circulation examined. Because of the known differences in vascular pattern between different regions of a long bone, it seems likely that haematocrits too will show considerable variability. The implication of this occurrence would be that plasma shift occurs across neighbouring regions of bone. It is noted that Zamboni & Pease (1961) believe on the basis of electron miscroscopy, that the extravascular fluid of the marrow at any rate is in a highly labile state.

The differing flow rates within the femoral circulation presumably indicate different metabolic rates of the tissues in question. A high value for marrow is to be expected because of the considerable amount of protein synthesis occurring during haemopoiesis. The value of 29 ml % min obtained here fits into the lower limit of the range of values found by Cumming (1962) for the red marrow of rabbit. Nevertheless, bone synthesis is also a prolific energy dependent process and therefore high flow rates in compact and cancellous bone in a growing animal are likewise to be expected. In particular, the value for cortex of 17 ml % min found here agrees approximately with that of Copp & Shim (1965). This is about one half of that for marrow which is itself about one half of that found for brain tissue (Kety & Schmidt, 1945). It is known that the rate of ionic exchange between bone salt and the circulation is fast where bone is growing fast, because bone salt in young bone is in a readily exchangeable state (Neuman, 1960; Leblond, Bélanger & Greulich, 1955). The results gained in this investigation have shown that the 'growing' metaphysis has the highest rate of flow in the femur, a region known to exhibit the highest rate of new bone formation and also the site of maximal ionic exchange (Amprino & Marotti, 1964; Leblond, Wilkinson, Bélanger & Robichon, 1950). The occurrence of lower flow rates in cortex and epiphysis also agrees well with the known reduction in new bone formation found in these two femoral regions (Amprino & Marotti, 1964). Hence, it seems reasonable to utilize flow rates in bone as a relative measure of the amount of bone turnover occurring in different parts of a bone. If the two metaphyses are taken, for example, the 64 % flow rate in the non-growing end would signify that bone turnover in the superior metaphysis is two-thirds that of the inferior metaphysis. It is known that the contribution to elongation of a growing

long bone from these two parts is of this order (Payton, 1931; Bisgard & Bisgard, 1935).

The flow curves yielded the *k* factor of the blood flow within the femoral circulation. The *k* factor is a measure of the proportion of the circulatory volume that is renewed per second. The statistical equality of this quantity in the five regions studied suggests that the rate of red cell renewal in all parts of the bone is the same. Because the flow rates in the femur differ only in that the red cell volumes differ, it follows that the bigger the red cell volume, the faster must red cells travel in order that the same proportion of cells be renewed as in other compartments. It is interesting to note that the observed red cell velocities (Table 2) do in general support this conclusion, but the marrow velocity for example is lower (64%) than the 85% expected if no other factors were operative. However, different red cell velocities in various parts of the osseous circulation are of considerable significance in relation to the control of osteogenesis. The fact that red cell speeds as well as the volume of the circulatory spaces differ in compact and cancellous bone tissues, provides a firm indication of the existence of differences in pO_2, pCO_2, and pH in the femoral circulation. It is these purely vascular factors which probably play the major role in the local, as opposed to the general, control of normal bone formation, and possibly in bone repair also. As yet there are only scant observations to help elucidate the relationship of the blood–gas factors to osteogenesis. Goldhaber (1958) in hyperoxic tissue culture preparations has detected a stimulus to bone formation provided by a fall in pO_2. In experimental venous obstruction, cancellous trabeculation is increased, but in this situation it is argued that an increase in pCO_2 is a stimulus to osteoblastic differentiation (Brookes, 1966). Increased osteogenesis has been observed when the pH of a bone culture has been reduced to the lower limit of the range (pH 7–8) at which bone forms in explants (Paff, 1948). It is also known that the bulk of the blood flowing through the cortex has already passed through the marrow (Brookes & Harrison, 1957; Brookes, 1958). This suggests that compact bone is formed in an environment characterized by low values for pO_2 and pH, and a high pCO_2. On the present evidence, it seems not unlikely that variation in the haemodynamic conditions within bone, resulting in changes in the gas factors of the blood, is a primary factor affecting local osteogenesis.

CONCLUSION

The results of this investigation lead to the conclusion that differing blood flow rates exist in the parts of a long bone, together with differences in circulatory red cell volume and velocity, and bone formation rates. Although bone in general forms as the resultant of the expression of a multitude of intrinsic and extrinsic factors, local osteogenesis is presumably closely linked to local, haemodynamic conditions. The differences in haemodynamic factors observed here point strongly to the existence of differences in pO_2, pCO_2 and pH in the osseous circulation. It is probable that further investigation will show the extent to which variation in these latter blood characteristics accounts for the control of local osteogenesis in a given bone and the remarkable mutability of bone considered as a tissue.

SUMMARY

1. The rates of blood flow, circulatory red cell volumes and relative red cell velocities were estimated for several distinct parts of the femoral circulation in rats.

2. The results indicate that profound haemodynamic differences exist between the various parts of a long bone.

3. It is suggested that osseous haemodynamic conditions are intimately related to local vascular factors, such as the partial pressures of oxygen and carbon dioxide and the reaction of the blood circulating in bone. These probably exert a direct control over local osteogenesis and thus influence the anatomical character of different parts of the bone.

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