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Bone blood flow in the rat determined by the uptake of radioactive particles

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Summary

1. Two different suspensions of radioactive particles, $^{99}Tc^m$-labelled microspheres and ^{59}Fe-labelled resin particles, were injected into the rat central circulation in order to study the distribution of blood flow to the skeleton.

2. Measurements of particle uptake showed that the mean proportion of cardiac output flowing to the skeleton was 3·1%, a figure much lower than the value of 27% found by Brookes (1970) in a similar experiment.

3. The blood flow per unit mass to individual bones covered a wide range.

Key words: bone blood flow, microparticles.

Introduction

Bone blood flow is a factor of importance in the study of mineral metabolism and the homeostatic role of bone. In addition, it is important in determining the distribution of those radiopharmaceutical products being increasingly used for bone scintigraphy.

Two principal methods have been used to study total or regional skeletal blood flow in experimental animals: the indicator fractionation technique and the plasma clearance and skeletal uptake of bone-seeking radionuclides. Only the latter is likely to be applicable to human studies. The indicator fractionation technique was introduced for this purpose by Kane & Grim (1966), with ^{42}K or ^{86}Rb as the tracer. The same workers used radioactive microspheres to validate their method for canine hind-limb bone (Kane & Grim, 1969). Brookes (1970) extended the use of radioactive particles to examine the blood flow to the whole skeleton in rats.

His value of 27% of the cardiac output was much higher than that deduced by those workers who used bone-seeking radionuclides, so we considered that his work should be repeated, with another form of particle in addition to the labelled resin he employed. Studies were made of uptakes of individual bones or groups of bones, as well as the whole skeleton. It was hoped that, in addition to shedding light on disparities between estimates of bone blood flow in experimental animals, the results would help towards the development of a method of measurement of bone blood flow in humans. A preliminary account of some of this work was presented at a meeting organized jointly by the British Institute of Radiology and the Bone and Tooth Society in February 1974 and an abstract of this communication was published (Tothill & McCormick, 1975).

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Methods

Ten male Sprague–Dawley rats were used, ranging in age from 3 to 4 months. With the exception of rat no. 1 (225 g), weights were in the range 400–500 g. The rat was anaesthetized with ether, the left common carotid artery dissected out under microscope observation and a thin (2 FG) cannula inserted. This cannula was used to inject the radioactive agents into the aorta and to collect blood samples after flushing with sodium chloride solution (154 mmol/l; saline). In all the animals, a first injection of one or more bone-seeking radionuclides was made, followed by the withdrawal of up to eight blood samples (approximately 0·5 ml each) at

intervals of 1–2 min. Each time, a similar volume of saline was injected to maintain the blood volume.

The final blood sample from five of the rats was analysed for pH, Pco_2, Po_2 and bicarbonate. There were no changes to suggest a severe reduction in circulating blood volume.

It had been planned to use measurements of blood clearance and bone uptake of ^{18}F and ^{85}Sr to derive a measure of bone blood flow for comparison with the particle-uptake results and with those of other workers. Unfortunately it was subsequently discovered that although the cooking procedure used to facilitate dissection did not lead to the elution of these radionuclides from bone, it did result in appreciable migration of radioactivity into bone from soft tissue. The bone-uptake results with ^{18}F and ^{85}Sr were therefore invalid, but the manipulations are described here in view of possible influences on the subsequent distribution of radioactive particles, and as a warning to others contemplating this technique.

Two different types of particle were used. One was a standard lung-scanning agent, $^{99}Tc^m$-labelled albumin microspheres. For three animals, a C.I.S. kit was used, and for five others the preparation was from an E.I.R. kit supplied by Duphar. In each case, the manufacturers claim a particle diameter range of 10–70 μm, with the majority of particles in the range 20–30 μm, and qualitative confirmation was provided by microscopic observation. The other aggregate was very similar to that used by Brookes (1970). Amberlite ion-exchange resin British Drug Houses C.G. 120 chromatographic grade II was labelled with ^{59}Fe; acid conditions were necessary. High specific radioactivity ferric chloride (approximately 10 mCi/mg of Fe) was used in the first experiment, but it was found that much of the label was removed from the resin *in vivo*. It was suggested (N. Veall, personal communication) that plasma transferrin had a greater affinity for the iron than did the resin, and that added carrier would overcome the problem. Accordingly, 2 mg of ferric chloride hexahydrate was added to 5–10 μCi of ^{59}Fe and 5–7 mg of resin. After incubation, the resin was washed and some of the finest particles were removed by sedimentation. The manufacturers state that the mesh size for the particles is 35–75 μm, but microscopic observation showed the range of diameters to be greater, and similar to that of the albumin microspheres. The resin particles were more irregularly shaped. The label was now found to be firmly bound both *in vitro* and *in vivo*. The sizes of both types of particles were such that they would be expected to lodge in the first capillaries or arterioles reached. The particles were administered after most of the blood samples had been taken and about 8 min after the first injection of bone-seeking radionuclides. When both agents were used in the same animal an interval of 1 or 2 min was left between the injections. The number of albumin microspheres injected was derived from the mass used and the particle-size distribution published by the manufacturers, and was estimated to be about 50 000. More resin particles were used and the number was estimated by suspending a known weight in potassium carbonate solution, of a density which minimized settling, and then taking an aliquot to be examined in a haemocytometer. From this it was deduced that about 300 000 particles were injected.

Ten minutes after the first injection, the continuous withdrawal of a large volume of blood led to the cessation of circulation and death. The soft organs were removed and the remainder of the carcase was boiled in a pressure cooker for about 20 min as described by Brookes (1970). The flesh could then be picked off the bones quite easily. No attempt was made to remove marrow from the bones. It was checked that this procedure did not remove appreciable amounts of $^{99}Tc^m$ or ^{59}Fe from the bones.

Radioactivity measurements were made in two ways. Individual organs, bones or small collections of bones were measured in a well scintillation counter, with appropriate corrections for the variation of efficiency with sample height. The remainder of the carcase and large groups of organs were measured with a clinical whole-body counter. The soft organs and bones were measured by both techniques to serve as a check, and good agreement was obtained. The separation of radionuclides was made from the different gamma-ray energies and decay rates, with appropriate corrections for 'cross-over'. Sufficient radioactivity counts were collected to ensure that errors due to counting statistics were negligible. The activities administered were obtained from the total of the whole-body counter measurements, and all organ activities expressed as a fraction of these totals.

Results

The percentage of the administered radioactive particles found in the whole skeleton is shown in Table 1. There was no consistent difference between

TABLE 1. *Percentage of administered particles found in total skeleton*

	Rat no.								
	1	2	4	5	6	7	8	9	10
$^{99}Tc^{m}$-labelled albumin microspheres	3·2	2·1	2·2	2·3	3·8	3·1	4·5	2·3	
^{59}Fe-labelled resin particles			2·9	2·5	3·1	3·5	3·9	3·0	3·5

the two types of particle. It is a fundamental assumption of the technique that the distribution of the particulate tracers is proportional to blood flow; with this assumption, the mean fraction of the cardiac output going to the bone was 3·1%.

When results from individual bones were examined there was again no consistent difference in the uptakes of the two types of particle, although there were large variations in the microsphere/resin ratio. The variation between animals was slightly less for the ^{59}Fe-labelled resin particles and the results for this agent are summarized in Table 2. The mean percentage and the mean uptake per unit mass are presented, together with their variations.

Incidental to the measurement of skeletal blood flow, results were obtained of the proportion of the cardiac output going to other organs. The results, which may be of value in validating the technique, are summarized in Table 3.

TABLE 3. *Percentage of administered aggregates found in various organs*

Mean values ± SEM are shown for the number (n) of animals indicated.

	$^{99}Tc^{m}$-labelled microspheres (%; $n = 8$)	^{59}Fe-labelled resin (%; $n = 7$)
Heart	0·26±0·26	0·12±0·11
Lungs	1·00±0·91	1·35±1·19
Liver	7·32±1·74	5·04±2·25
Spleen	0·57±0·48	0·61±0·45
Kidneys	9·17±4·58	7·48±3·09
Gastrointestinal tract	9·30±3·52	8·50±2·79
Skeleton	2·94±0·87	3·20±0·46

Discussion

The indicator fractionation technique, using radioactive particles, is a powerful one for studying the partition of cardiac output in experimental animals. Provided that the injection is made into the left side of the heart or the aorta, with adequate mixing, and the particles are of a suitable size, shape and number, the distribution of activity should reflect that of cardiac output. Our particles were in the general size range required. The absence of circulating activity in blood samples withdrawn after injection confirmed the lack of particles small enough to traverse capillaries and also the completeness of the labelling. There would have been advantages in restricting the sizes to a narrower range, perhaps by sieving and repeated sedimentation, as described by Wootton (1974). However, we wished to follow the published technique of Brookes (1970) as closely as possible. It is likely that the larger particles carried a disproportionate share of radioactivity, as the labelling would be expected to relate to the surface area, if not the volume of a particle. This factor is relevant to the consideration of the number of particles injected.

Examination of Table 2 reveals that with the injection of about 300 000 resin particles some indi-

TABLE 2. *Fraction of injected ^{59}Fe-labelled resin found in individual bones and collections of bones*

Mean values ± SEM for seven rats are shown.

Bone	% of resin	% per g
Femur shafts	0·151±0·075	0·092±0·047
Femur epiphyses	0·087±0·033	0·071±0·018
Tibia and fibula	0·089±0·030	0·039±0·013
Fore and hind paws	0·051±0·019	0·028±0·017
Humeri	0·140±0·058	0·113±0·049
Radii and ulnae	0·153±0·083	0·167±0·083
Ribs	0·080±0·028	0·049±0·016
Scapulae	0·126±0·108	0·155±0·131
Pelvis	0·196±0·087	0·071±0·022
Skull	0·616±0·379	0·121±0·083
Jaw	0·045±0·038	0·032±0·028
Cervical spine	0·549±0·089	0·319±0·097
Thoracic spine	0·327±0·224	0·136±0·068
Lumbar spine	0·616±0·234	0·178±0·054
Sacrum	0·341±0·120	0·216±0·090
Tail	0·235±0·174	0·040±0·022

vidual bones had fewer than fifty particles in them. There would therefore be large statistical fluctuations in these numbers, and the variations between bone groups and animals observed and listed as standard errors are not surprising. The pooling of results has reduced the effect of these variations, but no doubt an ideal experiment would require larger numbers of more uniform particles. Brookes (1970) started with only 1 mg of resin so presumably injected fewer particles and experienced bigger variations. However, he was concerned only with total skeletal blood flow and it is unlikely that this difference between our techniques can contribute to the most dramatic of our findings—the very large disparity between our results for the proportion of cardiac output going to bone. His mean value was 27%, ours 3·1%. In neither series was there a big variation between animals. Our animals were larger than his (to simplify surgery) and perhaps older. Various factors may influence the partition of cardiac output. Stress, or exercise, for example, may reduce the proportion flowing to bone. One difference between our experiments and those of Brookes was that our injection of microparticles was preceded by a period of blood sampling to determine the clearance of the bone-seeking radionuclides. As an equal volume of saline was introduced it is unlikely that the small quantities of blood removed would affect the distribution of cardiac output. The blood-gas analysis carried out to examine any metabolic changes that might have occurred as a result of decreased circulation or anaesthesia gave values within the normal range.

The proportion of injected radioactivity found by Brookes in the liver and spleen was very similar to ours (Table 3), which supports the idea that there were no gross differences of material or technique.

Use of a carotid artery for injections means that the blood flow to bones (and other organs) served by that route is interrupted. If the bone blood flow in that part of the body forms a different proportion than the average for the whole body then the estimate of the latter may be affected. The same consideration applies to our results and those of Brookes. However, he used the right carotid, whereas we used the left. There is therefore the possibility that, since the innominate artery was upstream of the point of entry of our cannula into the aorta, the right carotid and subclavian arteries were starved of particles and bone-seeking radionuclides. This is unlikely, as an attempt was made to ensure that the cannula was advanced into the aorta well beyond the junction of the left carotid artery, and appreciable activities of all the materials used were found in the skull.

There do not seem to be any other published estimates of the proportion of cardiac output flowing to the whole skeleton as determined by the indicator fractionation technique. We have some preliminary results for dogs with the use of radioactive microspheres, giving a mean value of 7·6%, between our values and those of Brookes for rats. Kane & Grim (1966, 1969) and Kane (1968) made experiments to validate the technique, but limited themselves to arterial injection, so did not obtain values of perfusion distribution for the whole skeleton.

The interest in the results presented in Table 2, of the distribution of resin particles, and therefore presumably of blood flow in different parts of the skeleton, centres on the variation between bones. When expressed as fraction of the cardiac output per unit mass of bone there is a tenfold range of the mean values, with large variations between animals. Some of this variation arises from the statistical fluctuations attendant upon the injection of a relatively small number of particles, but it seems that the common practice of using such figures to compare blood flow between species, or to extrapolate from measurements on a single bone to the whole skeleton, should be regarded with suspicion.

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