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A STUDY OF THE CAPILLARY BLOOD FLOW THROUGH BONE MARROW BY THE RADIO-ISOTOPE DEPOT CLEARANCE TECHNIQUE

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When the blood flow through the femoral marrow of anaesthetized rabbits was measured directly by the method of venous effluent collection the rate of flow through active erythroid marrow was found to be in the range of 0·2–1·0 ml./g wet weight of marrow/min (Cumming, 1962). Unless an appreciable fraction of the venous blood was coming from bony tissue, which appeared unlikely, this rate of flow represented a very high rate of capillary circulation, as we know of no evidence for the existence of arterio-venous anastomoses in marrow. In order to confirm these findings a method has been developed for the study of marrow blood flow by means of the radio-isotope depot clearance technique. In the absence of significant loss of depot material via the lymphatic system, such techniques are believed to measure the effective capillary circulation (Kety, 1949). A preliminary account of our experiments has already appeared (Brown-Grant & Cumming, 1961).

METHODS

Male rabbits (1·9–3·0 kg body weight) from an inbred colony kept in this Department were used. The animals were kept in individual cages and fed on a pelleted diet (Diet 18B manufactured by Oxo Ltd) and tap water *ad lib.*, together with a small daily supplement of fresh green vegetables.

Anaesthesia was induced by intravenous sodium thiopentone (12·0 mg/kg body weight) and maintained with an ether and oxygen mixture. The anterior surface of the shaft of the femur at the junction of the middle and lower thirds was drilled with a No. 6 rose-headed dental burr so as to expose the endosteum intact in the floor of a saucer-shaped hole of 0·5 cm diameter in the bone. The myeloid cavity of the femoral bone of the rabbit is not traversed by cancellous bone and the cylinder of marrow is suitable for depot injections of small volumes of fluid. Both femora were prepared for injection in the initial stages of each experiment. Figure 1 is a diagram of the apparatus used to inject a depot of 0·02 ml. of carrier-free $Na^{131}I$ in NaCl solution, 0·9 g/100 ml., containing approximately 1·5 μc, and to count the emitted γ-radiation. The bent No. 17 hypodermic needle was connected by fine polythene tubing to a micrometer syringe containing the ^{131}I solution. The syringe was shielded from the injection site by a sheet of lead 9 mm thick. After checking that the tubing and needle were free of air bubbles the needle was pushed through the exposed endosteum and passed along the myeloid cavity in a rostral direction until its tip was about 1 cm from the point of puncture, and then left in position. A lead shielded G–M tube

(20th Century Electronics, G 10 Pb) was mounted on a stand over the femur as shown and arranged so that the centre of the 3·5 × 2·0 cm window in the 9 mm lead shielding was over and a few millimetres above the estimated position of the needle tip. Counting rates were measured with an Ekco type N. 522 rate-meter. Repeated determinations of the clearance of depot injections could be made without altering the position of the needle or of the counter. No injections were made until at least 8 min had elapsed following the insertion of the needle. The time taken for each injection was 8–10 sec. During the first minute after beginning the injection readings of the counting rate were taken every 5 sec with an integrating time constant setting of 1 sec on the rate-meter. In successive minutes readings were taken every 10, 15 and 30 sec with increasing time constants as the rapidity of change of counting rate became less until a new steady background count was reached. This was usually a matter of 5 min or less, but subsequent depot injections were made at 15–20 min intervals in most experiments.

The carrier-free ^{131}I solution used was obtained from the Radio-chemical Centre, Amersham, as was the radio-iodinated human serum albumin used in other experiments described later.

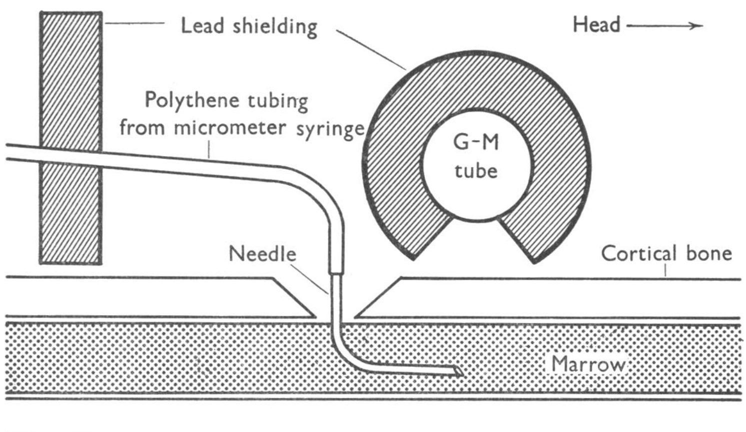

Fig. 1. Diagram to show the arrangement of apparatus finally adopted to measure the clearance of ^{131}I from the femoral marrow.

RESULTS

Preliminary experiments

Experiments were carried out to determine, first, whether the $Na^{131}I$ solution available was suitable for use as a depot material, and secondly, whether it was possible to use the bone marrow of the rabbit's femur as an injection site.

Radio-iodide was preferred to the more usual $^{24}NaCl$ as an injection material as the longer half-life simplified the handling and storage of the stock solutions and obviated the need for corrections for physical decay during the course of experiments lasting 2–3 hr. Hyman, Rosell, Rosen, Sonnenschein & Uvnäs (1959) found that $Na^{131}I$ behaved in the same way as $^{24}NaCl$ when used as a depot injection in the muscles of cats. In experiments on five rabbits, clearance curves similar to those reported by the Swedish workers (half times of from 8 to 20 min) were obtained with the $Na^{131}I$ solution available to us.

In experiments on eight rabbits injections of radio-iodide into the marrow cavity were made by means of a tuberculin syringe. The needle was withdrawn after injection and the G–M tube positioned over the femur. After this, unexpectedly low counting rates indicated that most of the injected material had disappeared. This was confirmed by the observation of a similar counting rate over the opposite, uninjected femur and

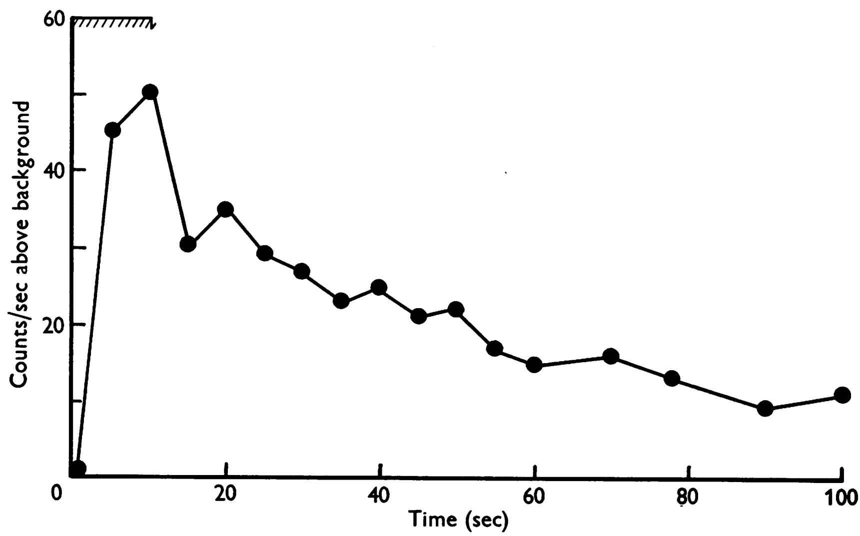

Fig. 2. A typical curve of iodide clearance from marrow; rabbit 1·98 kg. ^{131}I injected over the 0–8 sec period.

a higher counting rate over the thorax. In order to study the clearance during the first 2 min after injection, the technique described under Methods was adopted in subsequent experiments. This was of further advantage because withdrawal and reinsertion of the needle to inject further depots would have caused unnecessary damage and introduced uncertainty as to the exact position of the depot in relation to the G–M tube.

The clearance of depot injections of 0·02 ml. (1·5 μc) of iodide solution was studied in 18 experiments on 9 rabbits. The half-time for disappearance of the depot was found graphically to be between 20 and 25 sec; the range of peak rise in counting rate above background was 13–55 counts/sec. Figure 2 shows the result obtained in a typical experiment.

The fall in counting rate over the injection site was extremely rapid. There appeared to us to be three likely explanations for this:

(1) The depot was dispersing rapidly up and down the myeloid cavity of the femoral shaft and thus escaping from the 'field of vision' of the G–M tube.

(2) The iodide was being injected directly into a large vein or venous sinus and was in fact a direct intravascular injection rather than a depot injection.

(3) The iodide was being cleared rapidly by virtue of a very high effective capillary circulation rate.

The experiments described in the next section were carried out to decide between these possibilities.

Validation of the method

It appeared unlikely, in view of the high counting rates over the opposite limb and over the thorax soon after injection, that the first explanation, of physical dispersal up and down the marrow cavity, was the true one. Two experiments were carried out in which the clearance of iodide from marrow was studied before and after death in the same animal. Figure 3 shows the result of one of these experiments. It is clear from the persistence of a high counting rate in the dead animal that the fall in counting rate is not due to physical dispersal but is dependent upon the circulation through the marrow.

The fact that the injected material rapidly entered the general circulation was confirmed in experiments in which a marrow depot clearance was first recorded, and then the G–M tube was placed over the heart and the rise in counting rate measured following a second depot injection. Figure 4 shows how the rapid disappearance from marrow is followed by a rise in the level of activity detected over the heart.

There is, however, an appreciable lag between the injection of ^{131}I and the rise in counting rate over the heart. If the marrow injection were, in fact, intravascular, this delay would be due only to the circulation time from leg to heart. In a further experiment, the rise in counting rate over the heart following two injections into the marrow was determined. The needle was then removed and inserted into the femoral vein of the same side so that the tip lay at the same level as in the marrow and a third injection given. Figure 5 shows how the counting rate over the heart rises earlier when the injection is intravenous than when it is made into the marrow.

Finally, 6 experiments were carried out on different animals in which a 2% (w/v) solution of radio-iodinated albumin (0·02 ml. in 0·9% NaCl

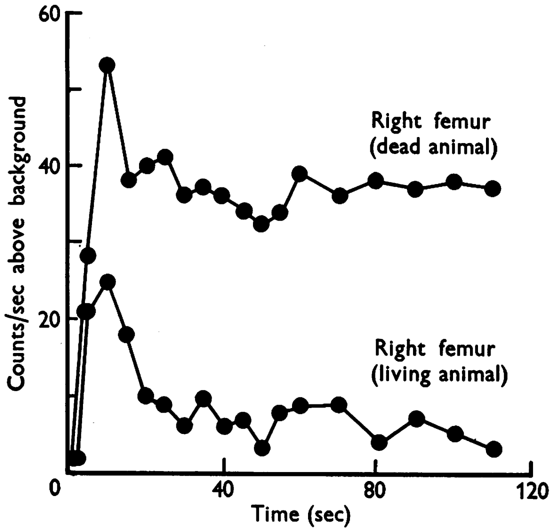

Fig. 3. The lower curve shows the clearance of ^{131}I in life; rabbit 2·2 kg. The upper curve, obtained after killing the animal with intravenous barbiturate, shows the persistence of a much higher counting rate after injection of the same dose of ^{131}I. The fall in counting rate is dependent upon the circulation through the marrow.

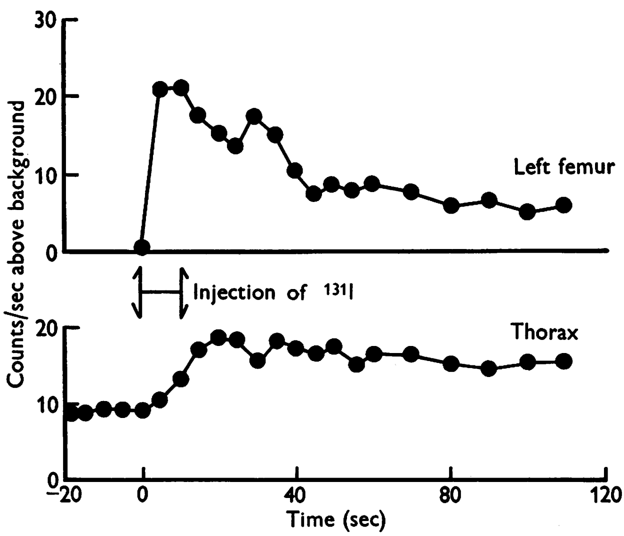

Fig. 4. To show a marrow clearance curve and to illustrate how the counting rate over the heart rises rapidly as the depot is cleared from the marrow: rabbit 2·28 kg.

solution) was injected into the marrow. Figure 6 shows the result of one of these experiments compared with the result of an experiment in which radio-iodide was injected. The rate of clearance of albumin is negligible compared with that of iodide. If the injections had been given

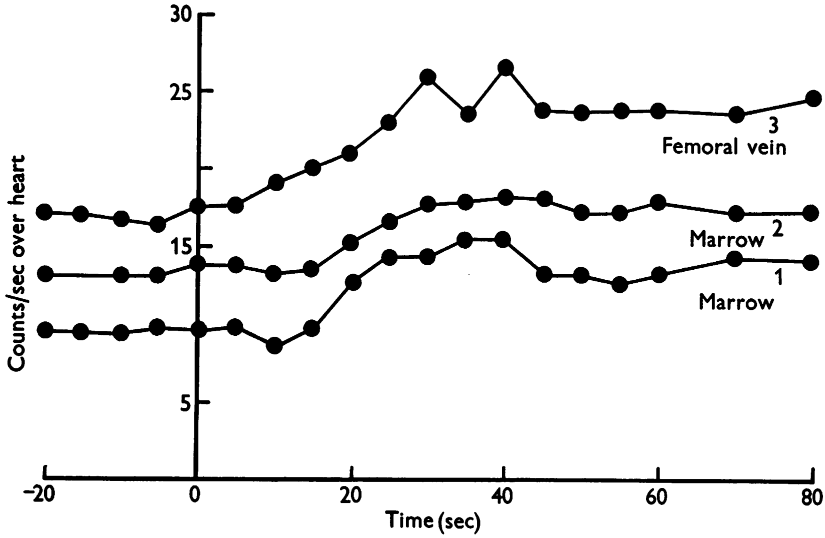

Fig. 5. To illustrate the difference in delay in rise of counting rate over the heart following injections of ^{131}I into femoral marrow (1 and 2) and into the femoral vein on the same side (3); rabbit 2·3 kg. Each injection was given at 0 sec and the tip of the needle was the same distance from the heart in each case.

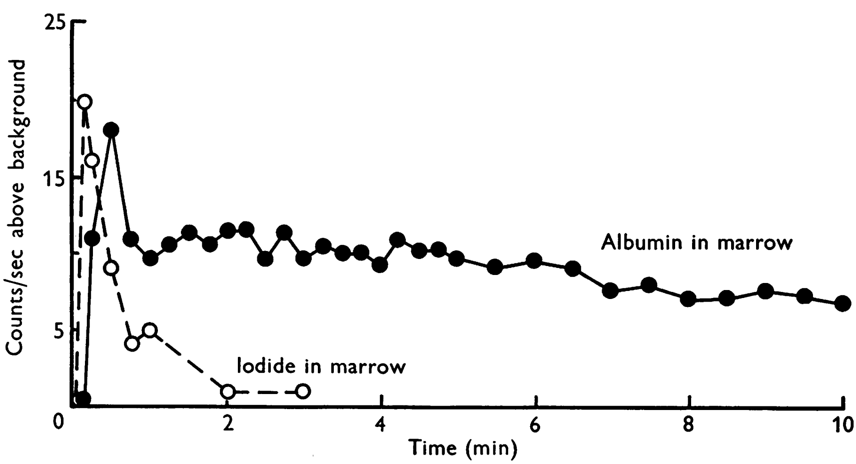

Fig. 6. The clearance of iodide from marrow compared with the clearance of radio-iodinated human albumin: ●——● rabbit 2·2 kg; ○– – –○ rabbit 1·9 kg. The striking difference (note that the time scale is in minutes in this figure) indicates that the depot injections could not have been intra-vascular.

directly into a blood vessel, then albumin would have been expected to disappear as rapidly as iodide.

It seems probable, then, that the third explanation proposed is correct and that the very rapid clearance of iodide from bone marrow indicates a high rate of effective capillary circulation.

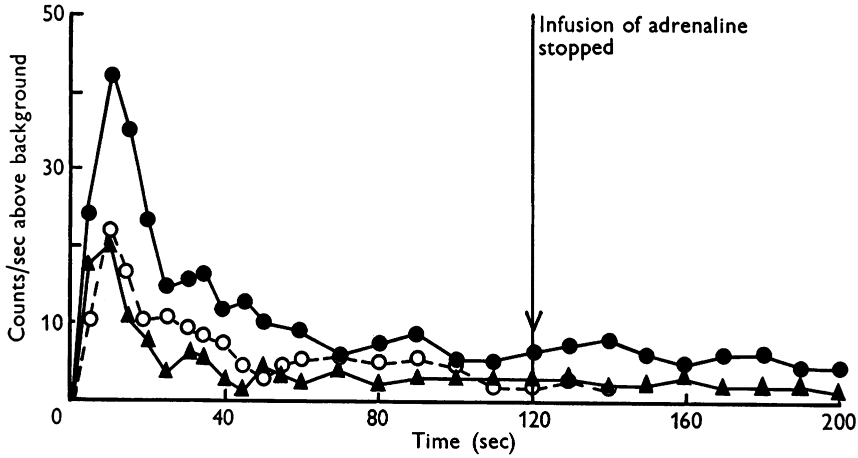

Fig. 7. The effect of an infusion of adrenaline on the clearance of radio-iodide from the marrow. Note the higher peak and the slower fall when ^{131}I is injected during the infusion of adrenaline (2·5 μg/kg/min). ○ – – ○ First control; ●——● during adrenaline infusion; ▲——▲ post-adrenaline control.

TABLE 1. The effect of adrenaline infusion on the clearance of ^{131}I from femoral bone-marrow. Peak counts/sec above background counting rate usually at 10–15 sec after injection. Half-time of disappearance estimated graphically over first half-life of curve without correction for recirculation of ^{131}I

	Dose of adrenaline (μg/kg/min)	Control		During adrenaline infusion		Control	
		Peak counts	Half-time (sec)	Peak counts	Half-time (sec)	Peak counts	Half-time (sec)
1	2·5	26	15–20	42	20–25	16	10–15
2	2·0	30	35–40	40	35–40	30	25–30
3	5·0	53	10–15	55	15–20	44	10–15
4	5·0	50	35–40	86	45–50	54	40–45
5	20·0	22	20–25	25	35–40	21	20–25

The effect of adrenaline on the clearance of ^{131}I *from bone marrow*

The effect of a continuous intravenous infusion of adrenaline (2–20 μg/kg body weight) upon the rate of clearance of radio-iodide from marrow was examined in five rabbits. In each case a control experiment was first carried out; 90 sec before a second injection of ^{131}I, the infusion of adrenaline was begun and continued for a further 2 min. Finally, 15–20 min after stopping the adrenaline infusion, a third control experiment was performed.

Figure 7 shows the result of one such series of experiments. In each case there was a higher peak above background following the injection of ^{131}I during adrenaline infusion and the rate of fall in counting rate was slower than the mean of the preceding and subsequent control values (Table 1).

DISCUSSION

The clearance of $Na^{131}I$ from the femoral marrow of the rabbit is very rapid in comparison with the clearance from other tissues. The half-time for clearance of radio-iodide from resting skeletal muscle of the rabbit, under our experimental conditions, is between 8 and 20 min. This is close to the value obtained by Hyman *et al.* (1959) for the skeletal muscles of the cat. Depot injections in the submucosal layer of the stomachs of anaesthetized dogs are cleared with a half-time of 3–5 min (J. D. Cumming, A. L. Haigh and E. H. L. Harries, unpublished). In contrast, half-times of the order of 20–30 sec were observed consistently in rabbit femoral marrow. These high clearance rates are not peculiar to iodide. In experiments on three rabbits similar values were obtained for the clearance of depot injections of $^{24}NaCl$. From the results obtained in experiments in which $Na^{131}I$ was injected intravenously and in experiments in which radio-iodinated albumin was injected, we do not consider that the rapid clearance of iodide from marrow is an artifact, but that this is the result of a very high effective capillary circulation.

We have not attempted to express the results obtained by this technique in absolute terms. The crucial assumption in any such attempt would be that the isotope is uniformly distributed through a known volume at zero time. The rate of flow, in terms of this assumed volume, could then be calculated from a knowledge of the half-time of the exponential curve for the disappearance of isotope by treating the system as an open single-compartment model (Veall & Vetter, 1958). We do not feel that we can justifiably make any guess as to the volume or uniformity of distribution, although, of course, the upper limit of volume is defined by the finite marrow mass. It can be said, however, that the rapid clearance is certainly compatible with the high average rate of flow (0·5 ml./g wet weight/min) determined by direct measurement (Cumming, 1962).

Sympathomimetic amines are known to cause vasoconstriction in the bone marrow (Cumming, 1962). In experiments in which adrenaline was infused intravenously, the peak count above background was increased and the rate of clearance reduced, as compared with control experiments carried out before and after the infusion, in four out of five experiments. In the fifth experiment, when the rate of infusion of adrenaline was the lowest in the series (2 μg/kg/min), the peak count was raised but no effect on the rate of clearance was seen. These results support the view that this technique may afford a method at least for the study of gross changes in the blood flow of this important, but somewhat inaccessible, tissue in other species.

SUMMARY

1. A method is described for studying the clearance of a depot injection of radio-iodide from the femoral bone-marrow of the anaesthetized rabbit.

2. The clearance is rapid; the mean half-time of disappearance is 20–30 sec.

3. Experiments are described to support the view that this rapid clearance is not an artifact but represents a very high effective capillary circulation rate.

4. Adrenaline reduces the capillary blood flow through the marrow.

5. These findings confirm and support the results obtained by direct measurement of venous outflow.

We should like to express our thanks to Professor H. P. Gilding under whose supervision these experiments were carried out for his encouragement and for his valuable advice.

REFERENCES

Brown-Grant, K. & Cumming, J. D. (1961). Blood flow through bone marrow studied by the isotope depot clearance method. *J. Physiol.* **158**, 30–31*P*.

Cumming, J. D. (1962). A study of blood flow through bone marrow by a method of venous effluent collection. *J. Physiol.* **162**, 13–20.

Hyman, C., Rosell, S., Rosen, A., Sonnenschein, R. R. & Uvnäs, B. (1959). Effects of alterations of total muscular blood flow on local tissue clearance of radio-iodide in the cat. *Acta physiol. scand.* **46**, 358–374.

Kety, S. S. (1949). Measurement of regional circulation by the local clearance of radioactive sodium. *Amer. Heart J.* **38**, 321–328.

Veall, N. & Vetter, H. (1958). *Radioisotope Techniques in Clinical Research and Diagnosis*, 1st ed. Chap. 10. London: Butterworth.