REGIONAL DISTRIBUTION OF CIRCULATING MICROSPHERES IN THE FEMUR OF THE RABBIT

P. J. GREGG, D. N. WALDER

From the Departments of Orthopaedic Surgery and Surgery,
University of Newcastle upon Tyne

In an attempt to explain the distribution of lesions of caisson disease of bone in the human femur, the regional distribution of circulating microspheres which had been labelled with scandium-46 was studied in the femur of the rabbit. Microspheres with a diameter of 15 microns were equally distributed between the two ends of the bone and between the upper and lower halves of the shaft. However, microspheres with a diameter of 50 microns congregated in the upper end of the femur and in the lower half of the shaft, the two sites most commonly affected by caisson disease. A large percentage of the microspheres in the shaft, especially the larger spheres, were retained in the marrow. It is suggested that the microcirculation of the marrow may act as a filter and that the nature and distribution of its vessels determine the site of impaction of circulating emboli. This would explain why lesions of the shaft mainly affect the medulla of the bone and not the cortex.

Caisson disease of bone, which affects compressed-air workers and divers, is characterised by regions of necrosis of the bone and marrow of the long bones, which may lead to the development of a severe secondary osteoarthritis of both the hip and the shoulder. Although recent research (Gregg and Walder 1977; Gregg et al. 1977) has provided the possible means for earlier diagnosis, treatment remains unsatisfactory. Prevention is the ideal but is dependent on the identification of the exact cause which unfortunately remains unknown.

Of the several theories of aetiology, the most likely is that of obstruction of the blood vessels to the bone and marrow by circulating emboli which develop during or shortly after decompression. Although it has been suggested that these emboli may be aggregates of red blood cells, platelets (Philip, Schacham and Gowdey 1971) or fat (Jones, Sakovich and Anderson 1974) there is little evidence for this; the subject is well reviewed by Elliott (Elliott, Hallenbeck and Bove 1974; Elliott and Hallenbeck 1975). However, gas bubbles are known to circulate during "safe" decompression (Evans, Barnard and Walder 1972) and there are theoretical grounds at least to explain why they may persist as emboli for long enough to produce necrosis of the bone or marrow. Unfortunately there is no direct evidence for this being the cause of caisson disease. One factor which would appear to throw doubt upon this embolic theory is the distribution of the lesions in the femur (Medical Research Council Decompression Sickness Central Registry, personal communication). The commonest site of lesions, in both compressed-air workers and divers, is the lower femoral shaft whereas the upper femoral shaft is much less often affected (Figs 1 and 2).
When one considers the ends of the femur, the reverse is true, in that the femoral head, particularly in compressed-air workers, is often affected whereas the femoral condyles are probably never affected (Figs 1 and 2). If circulating emboli are the cause of this condition one might expect a more equal distribution of lesions, unless there exists a differential distribution of such emboli.

The aetiology of the avascular necrosis of bone which affects similar regions of the femur in other circumstances, for example in relation to steroid therapy and alcoholism, is also not known but some believe that the cause is fat embolism (Jones 1971, 1974; Cruess, Ross and Crawshaw 1975), although the evidence for this remains uncertain.

For these reasons an experiment was designed to investigate the regional distribution of artificial emboli in the femur.

METHOD

Artificial emboli. Commercially available microspheres were used (Tracer Sephadex, Pharmacia). These microspheres are made of insoluble cross-linked dextran and are available in ampoules ready for labelling with metallic radionuclides. Each ampoule is a combined reaction vessel for labelling and a filter funnel for washing the microspheres. Two sizes of microsphere are available, with diameters of 15 microns and 50 microns respectively. The microspheres do not aggregate or stick to the ampoule or syringe; they are of low density (microspheres are 1.12±0.02 grams per millilitre; red blood cells are 1.098 grams per millilitre) thereby minimising sedimentation artefacts and they have a narrow size distribution. The microspheres were allowed to swell by immersion in a buffered solution, then labelled with scandium-46, which has a half-life of 84 days, fixed and washed.

Introduction of microspheres. Experiments were performed on two groups of animals. In one group 15-micron microspheres were used and 50-micron microspheres in the other. There were 10 adult female New Zealand white rabbits in each group, weighing between 3.2 and 5.3 kilograms. The animals were anaesthetised with intravenous Nembutal (60 milligrams per millilitre) using a dose of approximately 20 to 35 milligrams per kilogram and allowed to breathe spontaneously until they were killed shortly after the introduction of the microspheres. The femoral condyle was opened through a midline incision and the distal aorta and its bifurcation were cleared of adherent tissue. A Longdwell polythene cannula (20 gauge and two inches long) was introduced into the distal aorta so that its tip was just proximal to the bifurcation. The suspension of microspheres was injected into the flowing blood of the distal aorta via this cannula; five millilitres of Nembutal were then injected down the cannula to flush out any remaining microspheres and to kill the animal.

Preparation of femora and measurement of radioactivity. Both femora were excised and cleaned of all soft tissues. Their external surfaces were thoroughly washed with tap water to minimise contamination with extra-osseous microspheres. Each femur was placed in a plastic container before measuring the amount of radioactivity present in the whole bone. Then the femur was sectioned transversely immediately below the lesser trochanter, immediately above the femoral condyles and at approximately the midpoint of the shaft. This gave four sections of bone (Fig. 3) each of which was placed in a small plastic tube before measuring the amount of radioactivity in it. Finally, both the upper and lower halves of the shaft were placed in a single tube and the amount of radioactivity present was measured. The segments were then sectioned longitudinally and marrow was removed manually. These marrow fragments were placed in another plastic tube and the amount of radioactivity present in the marrow was measured.

The radioactivity present in each sample was measured by placing it in the centre of a gamma counter designed for bulky samples. This consisted of two crystals of sodium iodide, each measuring five inches in diameter and two inches thick, facing each other across a space of 25 centimetres. The entire counter was shielded with lead three inches thick. The gain of the system was maintained throughout the experiment by routine calibration. A background count was performed before and after each session of counting. Each measurement, including that of the background, was made by measuring the time needed to accumulate 20,000 counts: this normalises the coefficient of variation of all sample counts at 0.7 per cent.

![Fig. 3](image)

Three lines of section of the rabbit femur giving four segments for measurement: a, upper metaphysis; b, upper half of shaft; c, lower half of shaft; and d, femoral condyles.

Other measurements and calculations. For each animal approximately half the contents of each ampoule containing the microspheres was drawn up into a graduated syringe and the total volume made up to one millilitre with Ficoll 70 solution. The suspension was then ejected into a small glass vial and the weight of the vial plus microspheres ascertained on an analytical balance with an automatic pre-weighing system and digital read-out to 0.1 milligram (Sartorius 2842). When the microspheres had been drawn up for injection the vial was re-weighed so that the weight of the microsphere suspension injected could be calculated (W). Before these weighings were performed, a small quantity of the suspension was removed and ejected into a small plastic tube which had previously been weighed empty. The weight of this "standard" was calculated and the amount of radioactivity present was measured from which the counts per second per gram of microsphere suspension could be calculated (S). After injecting the microspheres into the distal aorta and flushing the cannula with Nembutal, the cannula and syringe which had contained the microspheres were removed and placed in a plastic container so that the amount of radioactivity remaining in the syringe and cannula could be measured (D). The actual amount of radioactivity (in counts per second) of the microspheres injected (C) was calculated as follows: 

\[ C = (W \times S) - D. \]

Knowing the total amount of radioactivity present in each femur, the percentage of the injected dose present could be calculated. After the radioactivity present in each of the four segments of each femur had been measured, each segment was weighed in the fresh state so that the radioactivity could then be expressed as counts per second per gram.
RESULTS

The percentage of injected radioactivity in the femora of each group of animals and a statistical comparison of left and right femora, using the Student paired *t* test, is shown in Table I. A comparison of the two groups using the Student unpaired *t* test is shown in Table II. When the 15-micron microspheres were used the percentage of the injected radioactivity present in each femur ranged from 0.18 to 1.51 and there was no significant difference between the percentage present in the left femur and the percentage in the right. When the 50-micron microspheres were used the percentage of the injected radioactivity present in each femur ranged from 0.17 to 1.61 and again there was no significant difference between the left and right femora. Nor did the size of microspheres cause a significant difference in the percentage of radioactivity present in each femur.

The distribution of radioactivity counts between the upper metaphysis and the femoral condyles, and the upper and lower halves of the shaft for each group of animals is shown in Tables III and IV, together with a statistical comparison using the Student paired *t* test. When using 15-micron microspheres the amount of radioactivity in the upper metaphysial region did not differ significantly from that in the femoral condyles, nor did the amount in the upper half of the shaft differ significantly from that in the lower half. This was true for both the left and the right femora. When using 50-micron microspheres, a greater amount of radioactivity was found in the upper metaphysial region than in the femoral condyles of left femora; however, in the right, the amount in these two regions did not differ significantly. In both left and right femora there was a significantly greater amount of radioactivity in the lower half of the femoral shaft than in the upper half.

The radioactivity found in the marrow expressed as a percentage of that present in the shaft is shown in Table V, with a statistical comparison between the two groups (the Student unpaired *t* test). With the 15-micron microspheres the percentage in the marrow ranged from 53.99 to 90.49 while with the 50-micron microspheres...
the range was 78.70 to 97.27. Thus, the percentage of
total radioactivity in the marrow was significantly less
with the smaller microspheres.

DISCUSSION
Although it is generally believed that caisson disease of
bone results from obstruction of the blood vessels
supplying the bone and marrow by circulating emboli,
whatever their true nature may be, there is as yet no
direct evidence for this hypothesis. One objection to it is
that lesions have been observed only in certain parts of
the affected bones. It was felt therefore that any
evidence that emboli were distributed in a similar

Table V. Radioactivity in the marrow cavity as a percentage of that
in the femoral shaft

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<tr>
<th></th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
<th>SE of difference</th>
<th>P (2-tail)</th>
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<tbody>
<tr>
<td>15-micron</td>
<td>20</td>
<td>70.61</td>
<td>11.00</td>
<td>2.732</td>
<td>0.001*</td>
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<td>microspheres</td>
<td></td>
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<tr>
<td>50-micron</td>
<td>20</td>
<td>86.98</td>
<td>4.67</td>
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<td>microspheres</td>
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*Cochran's approximation

manner would constitute further evidence, albeit
indirect, in support of this embolic hypothesis. Such an
experiment is clearly not practicable in human subjects
and one must be careful about extrapolating from
animal experiments. However, the blood supply of the
rabbit femur (Brookes and Harrison 1957) is very
similar to that of the human femur (Rogers and
Gladstone 1950; Laing 1953; Truea and Harrison
1953), and it has been shown (Cox 1974) that the rabbit
femur is susceptible to necrosis of the bone and marrow
after the injection of glass microspheres into the
circulation of the hind limb.

The microspheres were injected into the distal aorta
to allow adequate mixing with flowing blood before they
reached the hind limb. This injection is much simpler to
perform than an intracardiac injection and ensures the
maximal possible concentration of radioactivity in both
hind limbs, thus producing the highest possible count.
The fact that there was no significant difference between
the percentage of the injected dose present in the left
and right femora when using either size of microsphere
would indicate that mixing was adequate.

Assuming that the 15-micron microspheres are
trapped in the circulation just proximal to the capillary
bed and that the amount of radioactivity present in each
segment is proportional to the number of microspheres
present, it would appear that they were equally
distributed between the upper metaphy whole region and
the femoral condyles and between the upper and lower
halves of the femoral shaft. If one assumes that the tissue
distribution of labelled microspheres, which do not cross
the capillary bed, is proportional to the blood flow to the
tissue (Rudolph and Heymann 1967; Buckberg et al.
1971) then it would appear that blood flow to the upper
metaphy whole region is the same as that to the femoral
condyles and also that the blood flow to the upper half of
the shaft is the same as to the lower half.

Considering the results obtained using 50-micron
microspheres a different picture emerges. A signifi-
cantly greater number of microspheres appeared to be
present in the upper metaphy whole region than in the
femoral condyles although this was only statistically
significant for the left femur. This anomaly cannot be
explained and in neither compressed-air workers nor
divers do lesions appear to be more prevalent in one
femur than in the other (Figs 1 and 2). There was also a
greater number of 50-micron microspheres present in
the lower half than in the upper half of the shaft and this
was noticed in both left and right femora.

Approximately 70 per cent of the 15-micron
microspheres and 87 per cent of the 50-micron
microspheres that were present in the shaft were in the
marrow. The latter percentage is somewhat different
from the 41 per cent observed by Wootton (1974) using
\(^5\)Cr-labelled microparticles ranging in size from 40 to
50 microns, but those particles may have been of rather
irregular shape. Since there is no reason to believe that
the group of rabbits injected with 15-micron micro-
spheres is different from that injected with 50-micron
microspheres it is suggested that microspheres making
their way to the cortex of the femur, via the nutrient
artery to the bone, have to pass through the microcircu-
lation of the marrow which acts as a filter. It would
appear that while some microspheres of 15 microns can
pass this filter, fewer of the larger microspheres are able
to do so. It may be that the nature and distribution of the
microvessels determine the site of obstruction of the
50-micron microspheres.

Thus when we consider the distribution of 15-
micron microspheres there is no clear correlation
between blood flow and the sites of predilection for bone
lesions; but when we consider the distribution of the
50-micron microspheres, which some believe are about
the size of the gas-bubble emboli which may circulate
during decompression (Walder, unpublished observa-
tions), there is a suggestion that they impact at sites
known to be vulnerable to necrosis of the bone and
marrow, that is the upper metaphy whole and the lower
shaft.

These findings have another important implication.
The assumption that circulating microspheres are
distributed in proportion to blood flow has been used as
the basis of the arteriolar blockade technique for
measuring blood flow in many tissues including bone.
However, the difference in results obtained using
different sizes of microspheres is important because it
shows that the size of the microsphere is critical in
determining the distribution. This is a factor which has, in the past, not always been taken into account by some workers when interpreting results of blood-flow measurement when using this technique in bone.

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REFERENCES


