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EXPERIMENTAL LIMB DISUSE AND BONE BLOOD FLOW

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It is known that blood vessels in immobilized limbs are prone to undergo anatomic changes (*Braibanti* 1958, *Geiser & Trueta* 1958, *Hulth & Olerud* 1960, 1961, *Jaffe* 1930, *Landoff* 1942, *Rieder* 1936, *Semb* 1966, *de Valderrama & Little* 1965). Thus, immobilization of a limb for one to two weeks is sufficient to produce dilatation and tortuosity of arteries, veins, and capillaries in its soft tissues and of capillaries and sinusoids in the bone marrow and bone tissue.

A few haemodynamic studies have been carried out on animal limbs immobilized in plaster. Using electromagnetic flow-meters *Imig et al.* (1953) found two weeks' immobilization of the hindleg of the dog in plaster to increase the blood flow in the limb by about 25 per cent. Shim (1966) used the initial clearance of ^{85}Sr by the bone tissue as a measure of its capillary circulation in an investigation of rabbit and dog limbs immobilized in plaster. He found the capillary blood flow through the bone tissue to be decreased after one to two weeks but to be increased by 50 per cent after two months.

The purpose of the present investigation was threefold, namely to study the circulation in bone tissue during the initial period of immobilization, to follow the effect of continued immobilization on the bone blood flow, and to study any correlation between that flow and the development of disuse osteoporosis.

MATERIAL AND METHODS

One hundred-and-nine rabbits of mixed breed and sex were used. The animals weighed between 2.0 and 3.0 kg. In 94 of the animals the left hind leg was immobilized in plaster for periods of two hours to seven weeks.

The rabbits were then assigned to different groups used in the following studies: (1) acid-base balance in the arterial blood and in bone marrow blood from the right and left tibia (49 treated and 15 untreated controls); (2) oxygen saturation of arterial blood and of bone marrow blood from the right and left tibia (23 animals); (3) blood flow through the right and left tibia and calcaneus (22 animals).

One foreleg in each of six adult dogs of mixed race and sex was immobilized in plaster with the elbow in semiflexion and the radiocarpal joint in 90° volar flexion. The animals were immobilized for 2, 14, and 28 days, respectively, after which the blood flow through the distal part of the radius was studied in both forelegs.

Acid-base balance in arterial and bone marrow blood. Small blood samples were obtained from an artery and bilaterally from the tibial bone marrow cavities of anaesthetized rabbit as described previously (Semb 1966a).

The acid-base status in the blood samples was promptly determined with the micromethod and apparatus (Radiometer, Copenhagen) described by Siggaard-Andersen et al. (1960). In the present investigation pH was measured with equal accuracy as reported by Siggaard-Andersen et al. (1960). In a control group of 15 rabbits the acid-base status was measured in bone marrow blood from the right tibia and from the left. No significant differences were found between the samples from the two sides.

The hind legs of the animals were examined roentgenographically. In addition, transverse sections were cut at corresponding levels from both distal tibial metaphyses. The slices were manually ground down to a thickness of one hundred microns and examined microradiographically.

Oxygen saturation of arterial and bone marrow blood. The influence of plaster immobilization on the blood oxygen saturation was studied in small blood samples from an artery and from the bone marrow of both tibial bones (Semb 1966b). The oxygen saturation (HbO_2) was measured spectrophotometrically (Siggaard-Andersen et al. 1962) with a slight modification. The accuracy of the method was studied on blood from capillary tubes filled with heparinized blood from one and the same sample. S.D. was ± 2.2 and the S.E. ± 0.6 per cent saturation.

Bone blood flow. The initial clearance of intravenously administered ^{85}Sr was used as a measure of the blood flow through the bone tissue.

In the rabbits the capillary bone blood flow was estimated in relative terms in immobilized and contralateral, untreated legs as de•cribed in an earlier paper (Semb 1968). In this study bone marrow blood from the tibial bones was assumed to be predominantly venous. Principally the same technique was used in a study on the effect of immobilization on the bone blood flow through the distal metaphysis of both radial bones of dogs. In this investigation, however, the total bone blood flow was estimated quantitativelly according to Fick's principle (Semb 1966c).

RESULTS

Four of the rabbits in group 1 (acid-base balance) were excluded owing to technical mishaps in association with analysis of the blood samples. Analyses of blood from the other animals gave results suggesting certain systematic changes.

Figure 1 gives the differences between the pH values in the blood from immobilized and from normal bone after various periods of

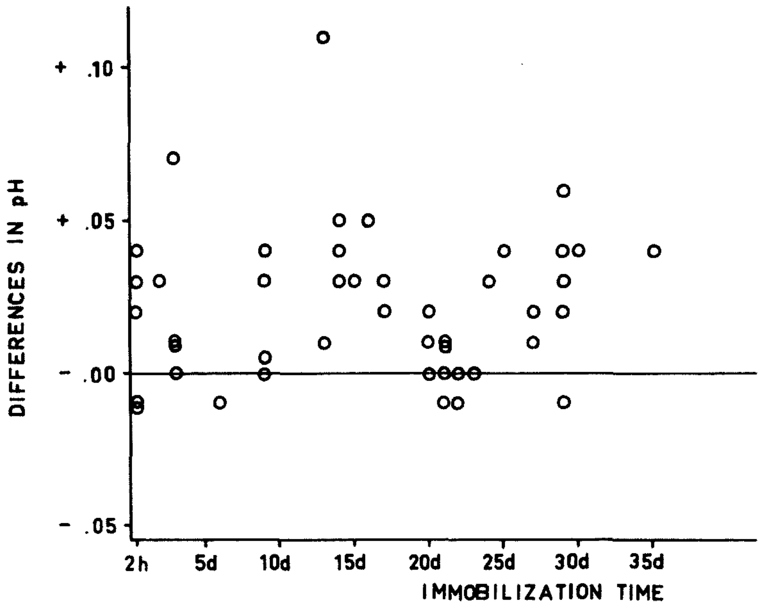

Figure 1. pH changes in intramedullary blood from legs immobilized for various periods.

immobilization. Irrespective of the duration of treatment the pH was somewhat higher on the treated side. On the average, the pH was 0.022 units higher on this side. The difference was statistically significant ($P < 0.001$).

In arterial blood the average pH was 7.37 (S.D. ± 0.042; S.E. ± 0.007). The corresponding values for bone marrow blood from the immobilized and untreated side were 7.36 (S.D. ± 0.062; S.E. ± 0.009) and 7.34 (S.D. ± 0.062; S.E. ± 0.009) respectively.

Figure 2 gives the relative differences between P_{CO_2} in marrow blood from the treated and from the untreated limb. The values were constantly lower on the immobilized side. The difference was statistically significant ($P < 0.001$). The correlation coefficient was 0.138, suggesting no connection with immobilization time. The mean P_{CO_2} was 40.7 (S.D. ± 10.8; S.E. ± 1.9) and 43.8 (S.D. ± 12.1; S.E. ± 2.1) mm Hg in bone marrow blood from the treated and untreated side, respectively.

The standard bicarbonate level in the marrow blood in one leg did not differ significantly from that in the other. The mean values were

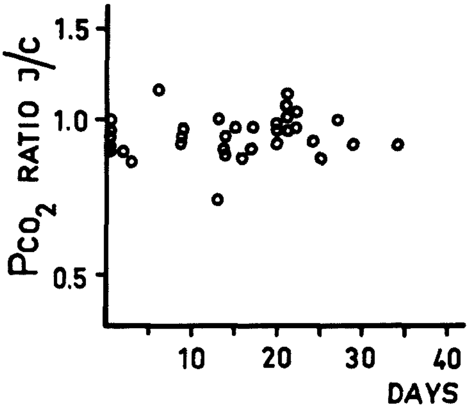

Figure 2. Ratios (in a logarithmic scale) between P_{CO_2} in intramedullary blood from legs immobilized for various periods and from control legs.

22.0 (S.D. ± 3.1; S.E. ± 0.5) and 21.8 (S.D. ± 3.1; S.E. ± 0.5) mE/l in blood from immobilized and normal leg, respectively.

Figure 3 gives the relative differences in oxygen saturation between bone marrow blood from the treated and untreated tibia in relation to the duration of immobilization. For all periods of immobilization the oxygen saturation was higher in blood from the immobilized side. The average difference in saturation was 8.2 per cent. The difference

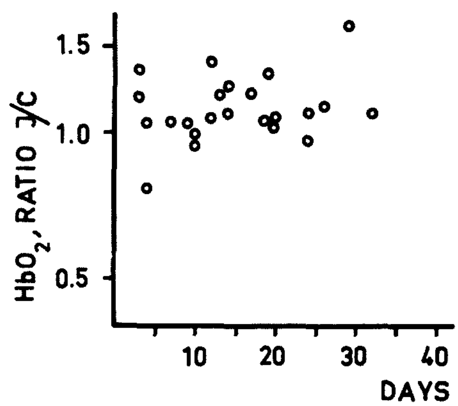

Figure 3. Ratios (in a logarithmic scale) between HbO_2 in intramedullary blood from legs immobilized for various periods and from control legs.

was significant ($P < 0.001$) but there was no connection between changes in HbO_2 and immobilization time (correlation coefficient 0.259). The average oxygen saturation in the arterial blood was 88.2 (S.D. ± 12.1; S.E. ± 2.5), in marrow blood from the immobilized leg 79.5 (S.D. ± 10.4; S.E. ± 2.2) and from the control leg 71.5 (S.D. ± 10.0; S.E. ± 2.1) per cent.

The uptake of ^{85}Sr by bone tissue, an indicator of the capillary blood flow, was distinctly affected by the treatment and varied with the duration of the immobilization.

Figure 4 gives the average difference in uptake between the treated and untreated side of rabbits after different periods. In the very early stage (after 2 hours) the uptake of ^{85}Sr decreased considerably. The differences were then significant for the different parts of the bone ($P < 0.001$). The uptake, however, soon became normal again. Already after one day the values for the uptake on the treated side were almost the same as on the untreated. The uptake by the bone tissue on the immobilized side then increased successively. The transition to an increase occurred some time between the first and seventh

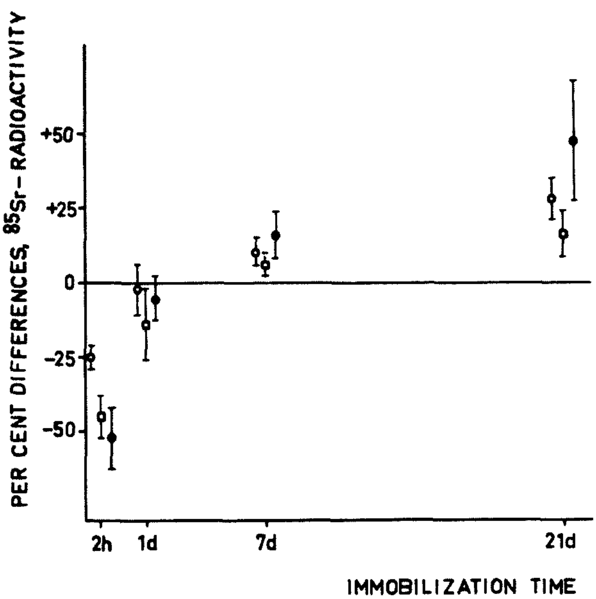

Figure 4. Differences (in per cent) between ^{85}Sr radioactivity in bone of different sources from immobilized and control legs as a function of time. Standard error of the mean. Bone sources: (○) proximal tibia; (□) distal tibia; (●) calcaneus.

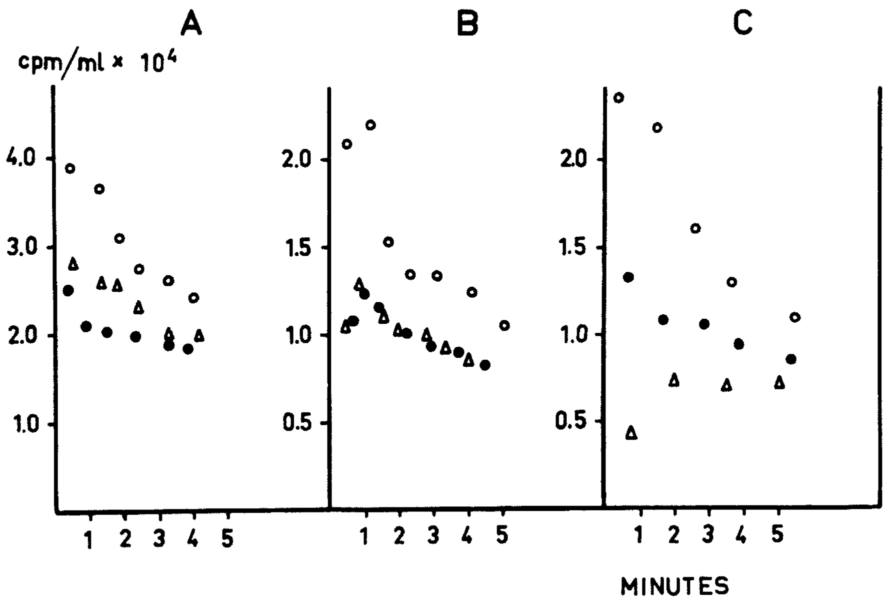

Figure 5. ^{85}Sr radioactivity in arterial blood (○) and in bone marrow blood from immobilized (●) and control (△) legs during the period of measurement. Periods of immobilization: 2 hours (A); 7 days (B); and 21 days (C).

day. After 21 days, the uptake was distinctly increased. The difference was, however, significant only for the proximal part of the tibia ($P < 0.05$). The effect of immobilization on the uptake of ^{85}Sr appears to be strongest in the calcaneus (Figure 4).

The average extraction by bone of ^{85}Sr from perfusing blood was increased initially but decreased after 21 days on the immobilized side (Figure 5). This means that the decrease of bone blood flow after two hours, indicated by the decreased uptake of ^{85}Sr in bone, should be still more accentuated. After 21 days, however, the blood flow should be still more increased than was shown by the uptake data.

In the dogs the total blood flow through the distal radial metaphysis after 4 weeks was 50 per cent higher on the immobilized side. After two and fourteen days, respectively, no disturbance in the circulation could be demonstrated. The blood flow was estimated at about 25 millilitres/min/100 gram bone tissue on the untreated side.

After 10 days' immobilization X-ray showed signs of atrophy of the tibia and calcaneus: the corticalis was thinner and the spongiosa in the epiphysis and metaphysis was more transparent. Microradiography of transverse sections of the distal tibia obtained after the same interval showed increased porosity, suggesting incipient osteoporosis on the immobilized side.

DISCUSSION

Immobilization of a limb in plaster results in atrophy of its muscles and bone tissue. It has long been assumed that vascular and haemodynamic changes play an important role in the development of bone atrophy. The most widely accepted concept is that disuse of the limb muscles impairs venous return with venous stasis and acidosis as a result, and that the acid environment presumably stimulates dissolution of minerals from the bone and leads to osteoporosis (*Lenggenhager* 1956, *Nordin* 1960, *Rieder* 1936). Not until recently have attempts been made to study the haemodynamic changes in the actual bone tissue. Using an isotope-clearance technique *Shim* (1966) found a certain decrease in the blood flow through bone in rabbit limbs, immobilized for two weeks in plaster. After two months' immobilization, however, he found a relative increase in the bone blood flow.

In the present investigation, based on the initial clearance of ^{85}Sr from the plasma, a significant decrease was found in the bone blood flow both proximally and distally in the tibia and in the calcaneus of rabbits immediately after immobilization of the limbs in plaster. The blood flow, however, soon became normal, presumably within a day, and then increased as a consequence of the immobilization. A similar effect on the flow of blood through bone was demonstrated in dogs, four weeks' immobilization in plaster resulting in a roughly 50 per cent increase of the flow.

The results of blood gas analysis can be explained in two fundamentally different ways. They may thus reflect a changed bone metabolism in the immobilized limb with reduction of both the consumption of oxygen and the production of carbon dioxide, in other words, decreased aerobic metabolism. On the other hand, the results may reflect a relative increase of the flow of blood through the bone marrow. Judging from the findings in the present investigation, all that can be said about the metabolism is that the blood flow is increased to a level above that necessary for the oxygen requirements of the surrounding tissue.

In view of the initially reduced bone blood flow one might expect local acidosis. But no such acidosis was found in the bone marrow blood. This, however, does not exclude the possibility of acidosis in the actual bone tissue. It is possible that the vessels in the marrow cavity form collaterals soon after immobilization to compensate the impaired venous return due to loss of the "muscle pump". There is experimental evidence that this can occur on proximal obstruction of venous drainage. Thus it has been shown that contrast media and tracer substances injected into the distal veins of laboratory animals are transported via vessels in the bone marrow to a level proximal to a venous obstruction (*Cuthbertson et al.* 1965, *McPherson et al.* 1961).

It is thus probable that arterialization of bone marrow blood found on the immobilized side in the early stage of plaster immobilization is a result of a shunting of blood in proximal direction via vessels in bone tissue and bone marrow. Abundant vascular communications exist between bone and surrounding soft tissues in the metaphyseal region, which facilitates a collateral return via the marrow cavity.

No signs of osteoporosis in rabbits could be demonstrated until after the limb had been immobilized for about 10 days. This development of bone atrophy was accompanied by an increased bone blood flow. The accelerated resorption of bone resulting in osteoporosis of disuse is therefore, if anything, a consequence of an increased, rather than of impaired, circulation. Of interest in this connection are the investigations by *Goldhaber* (1963), who showed in tissue culture that bone resorption is favoured by an optimal oxygen concentration. Thus resorption of mouse calvaria was increased rapidly when the oxygen concentrations was increased from 10 to 20 per cent. Resorption was found to be most marked at 30 per cent oxygen concentration. *Goldhaber* (1965), however, also showed that resorption of bone tissue in culture also required factors other than optimal oxygen concentration. Recently it has been reported that intact parathyroid and thyroid function is necessary for the development of osteoporosis of disuse in dog limbs immobilized in plaster (*Burkhart & Jowsey* 1967). The increased blood flow through the bone can, however, as a consequence of increased oxygen tension in the tissue create optimal conditions for a local effect of factors promoting resorption such as the parathormone.

It is, however, possible that disuse osteoporosis develops via an initial stage of dissolution of the minerals as a consequence of impaired local bone blood flow with tissue acidosis. Such a course has also been suggested by *Bohatirchuk* (1966). He bases his opinion on historadiographic investigation of bone specimens from *inter alia* immobilized rabbit limbs.

Sundén (1967) paralysed the muscles of one hind leg of rabbits by division of the motor nerve roots. He measured the heat clearance in the proximal tibial epiphysis and used this as a qualitative measure of the local blood flow. Already after one day he found the blood flow on the treated side to be increased. The increase was largest after six days. Immobilization in plaster and division of the motor nerve roots of a limb thus appear to influence the bone blood flow in principally the same way. Both measures result in muscular inactivity. It is therefore possible that the precipitating factor is inactivation of the musculature as pointed out previously by, *inter alias, Whedon* (1960).

SUMMARY

The effect of immobilization of the limbs on the bone blood flow was studied experimentally in rabbits and dogs. One limb served as a control. After various periods of immobilization bone marrow blood from the limbs was examined regarding its acid-base balance and oxygen saturation. In addition, the relative changes in the bone marrow blood flow were measured indirectly as the initial uptake of intravenously injected ^{85}Sr by bone tissue with correction for variations in extractions of the isotope from perfusing plasma.

Irrespective of the type of treatment the pH and the oxygen saturation were increased and the carbon dioxide tension was decreased in the bone marrow blood from the immobilized limb. Initially the bone blood flow was decreased but became normal again during the first week and then increased.

The underlying mechanism of the increase in the bone blood flow is obscure. It may be due to an increased collateral return of blood via vessels in the bone marrow and bone tissue as a compensation for the loss of the "muscle pump". Bone resorption does not appear to be favoured by impaired bone blood flow due to stasis. On the contrary, resorption appears to increase with the blood flow through the bone.

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