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# THE EFFECT OF MOTOR DENERVATION ON MUSCLE AND BONE IN THE RABBIT'S HIND LIMB

*By*

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Motor denervation of a limb leads to atrophy of both muscle and bone. Neither the mechanisms by which this occurs nor the relationship between muscle and bone atrophy are well understood. *Gillespie* (1954) found a positive correlation between the weight of bone of a paralyzed limb and the weight of the whole skinned limb. He concluded that bone atrophy was due to loss of muscular activity. Since the chronological sequence of changes in muscles and bones following motor denervation has not been studied, we undertook to quantitate these changes and by using calcium 47 further to clarify the bone changes.

## MATERIALS AND METHODS

Four-month-old male Chinchilla rabbits averaging two kilograms in weight were kept in individual cages. They consumed about 150 grams daily of Ralston Purina pellets enriched with vitamins A and D. Under Sedosal anesthesia supplemented by one-half per cent novocain locally, left hemilaminectomy of L6 and 7 with unroofing of the upper segment of the sacral canal was performed. The spinal cord and the sixth and seventh lumbar and first sacral nerve roots were exposed (Fig. 1). The dura on the lateral aspect of the spinal cord was incised. Under magnification, the anterior nerve roots were brought to view from under the cord with a nerve hook and sectioned. Severe bleeding, which proved to be a major problem, was reduced by partially suspending the animal from towel clips inserted into a spinus process

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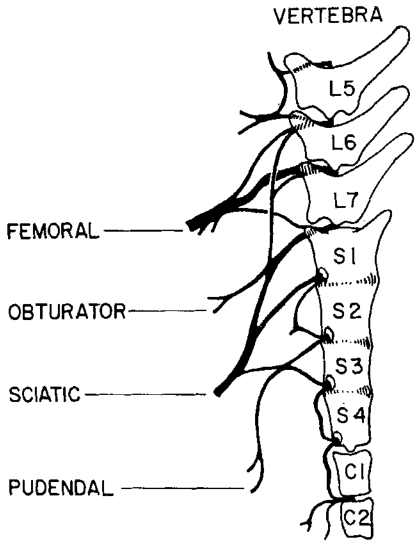


*Fig. 1.*
The lumbosacral plexus from 'The Rabbit in Experimental Physiology' by Harold M. Kaplan, reproduced with the permission of the Scholar's Library, New York.

so as to remove pressure from the abdomen. The operating field was kept dry by suction. Blood loss, estimated at twenty to thirty milliliters, was replaced by injection of saline. On recovery from operation, the paralyzed limb was dragged behind the animal during locomotion and hung down motionless and toneless in contrast to the non-paralyzed right limb when the rabbit was suspended from the skin of the neck.

Post-operative mortality was twenty-five per cent. It was noted that none of the animals had gained weight during the experiment. The rabbits were sacrificed in four groups at ten days, four to six weeks, eight to ten weeks, and twelve to fourteen weeks respectively. Twenty-four hours before sacrifice, each rabbit was intravenously injected with about five microcuries of Ca-47 as $CaCl_2$ with a specific activity of 150 millicuries per gram of calcium. After death, the animals were weighed and the hind legs skinned and disarticulated at the hip joint. Following disarticulation, the skinned limb was weighed and the muscle and soft tissues were cleaned off the bone. The cleaned bone was suspended by fine copper wire in distilled water, degassed in a vacuum chamber for half an hour at five to seven millimeters Hg and reweighed. The volume and density of the bone were then calculated by Archimedes' Principle. The bones were ashed in a muffle furnace at 600° overnight and weighed. The ash was dissolved in 2N HCl and made up to volume in a volumetric flask. Aliquots of this ash, together with a standard, were counted in a well-counter with appropriate adjustments of the base level and window to exclude Sc-47. Results were expressed as fraction of dose per gram of ash. Bone ash weights and muscle weights in the paralyzed limbs were expressed as a fraction of the control limbs.

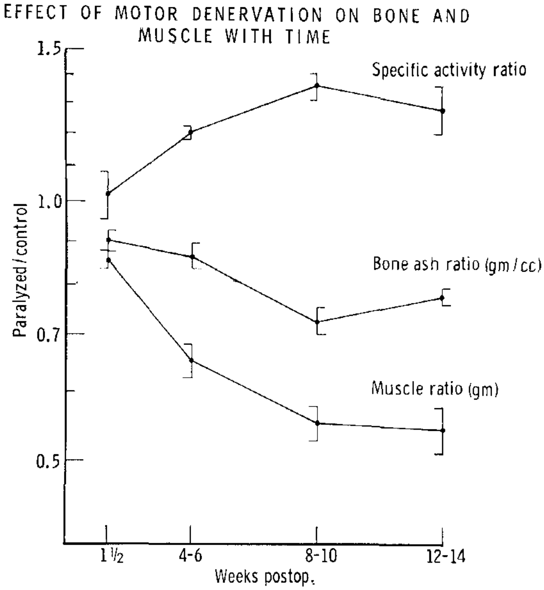


*Fig. 2.*

Muscle weight, bone ash weight and specific activity in the paralyzed limbs are expressed as a fraction of control and plotted against time after operation.

## RESULTS

Significant muscle atrophy was evident by the tenth day post-operatively and progressed steadily until the tenth week, when the weight of the muscles on the paralyzed side averaged 55 per cent of the control side. There was no further loss of weight until the end of the experiment at fourteen weeks (Fig. 2, Table 1).

TABLE 1

*Effect of Motor Denervation on Bone and Muscle with Time.*

| No. of Animals | Duration of Paralysis (wks.) | Muscle Mass per cent of Control $\bar{x}$ $S\bar{x}$ | Femur and Tibia Ash Weight per cent of Control $\bar{x}$ $S\bar{x}$ | CPM/G. Ash per cent of Control $\bar{x}$ $S\bar{x}$ |
|---|---|---|---|---|
| 5 | 1.5 | 85.2 ± 2.6 | 90.2 ± 2.53 | 102 ± 6.7 |
| 14 | 4–6 | 65.2 ± 2.9 | 86.6 ± 3.0 | 120 ± 2.3 |
| 12 | 8–10 | 55.2 ± 2.5 | 72.6 ± 2.6 | 136 ± 4.7 |
| 7 | 12–14 | 54.2 ± 3.3 | 77.5 ± 1.77 | 128 ± 8.2 |

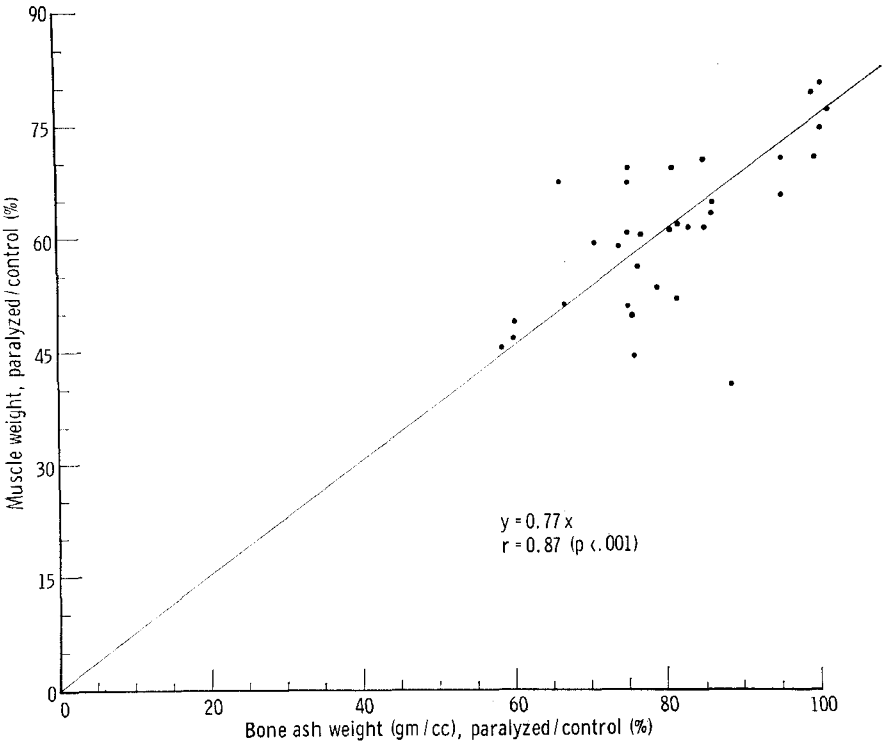


*Fig. 3.*

Muscle weight on the paralyzed side expressed as a percentage of control plotted against bone ash weight on the paralyzed side expressed as a percentage of control. Since the calculated intercept of the regression was not significantly different from zero, the regression line was drawn through the origin.

Bone atrophy showed the same trend as muscle atrophy but was less pronounced. The bone ash weight of the paralyzed side in animals killed ten days post-operatively averaged 90 per cent of the control side (this was highly significant, $P < .001$). The bone ash weight on the paralyzed side of the four to six week group averaged 86 per cent of control side (not significantly different from the ten day group $.5 < P < .6$). Bone ash weight of paralyzed side in the eight to ten week group averaged 72 per cent of the control (significantly different from the four to six week group $P < .01$). There was no significant difference in bone ash weight on the paralyzed side between the animals killed between eight and ten weeks and those killed between twelve to fourteen weeks ($.7 < P < .8$). Correlation between bone and muscle atrophy was highly significant : $r = .87$, $P < .001$. The formula for the

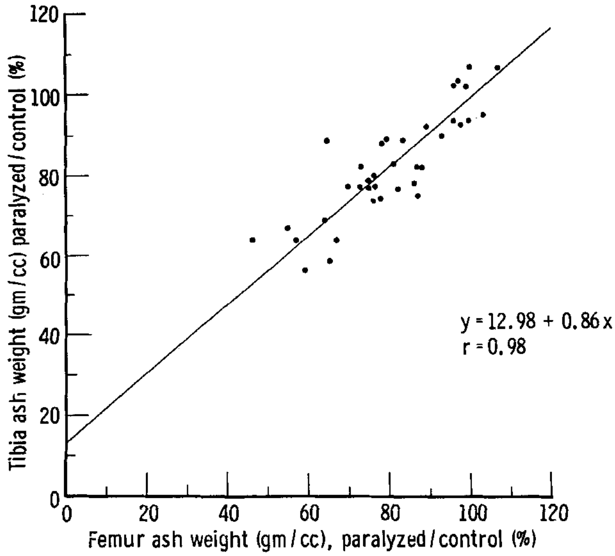


*Fig. 4.*

Tibia ash weight on the paralyzed side is expressed as a percentage of control and plotted against femur ash weight as a percentage of control. The coefficient of correlation and the equation for the regression line are given.

regression of muscle atrophy on bone atrophy was $Y = .77X$, where Y was the weight of muscle from the paralyzed side expressed as a fraction of the control side, and X was the weight of bone ash on the paralyzed side expressed as a fraction of the control side (Fig. 3). The regression of bone ash weight on the paralyzed side expressed as a percentage of bone ash weight on the control side was graphed for tibiae against femora (Fig. 4); $r = .98$, $P < .001$. The equation used in correlating femur and tibia ash loss was $Y = 12.98 + 0.86X$.

Fig. 5 shows the regression of total radioactivity in the bone ash of the paralyzed side compared with the control side, against the weight of bone ash on the paralyzed side as a percentage of the control side, $r = .65$, $P < .001$. As the amount of ash decreased so did the total radioactivity, $Y = 28 + 0.009X$. In Fig. 6, the specific activity in the bone ash on the paralyzed side (Y) was plotted against that on the control side (X). The regression is given by the equation $Y = 1.34\ X$. Fig. 2 and Table 1 show the specific activity ratios between the operated and control bones in the four groups throughout the experiment. Ten days post-operatively the ratio was 1.00, at four to six weeks the ratio was 1.20; at eight to ten weeks 1.36 and twelve to fourteen

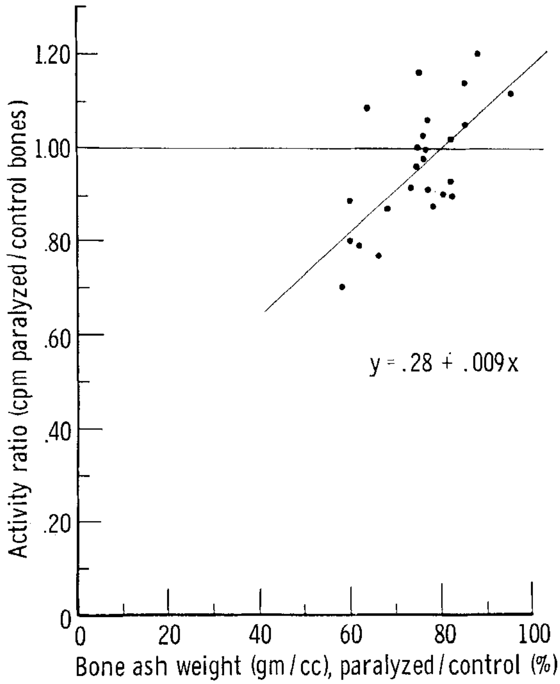


*Fig. 5.*

Radioactivity in the bone ash of the paralyzed side is expressed as a fraction of control and plotted against bone ash on the paralyzed side expressed as a percentage of control.

weeks 1.28. The difference between the first and second groups was significant ($P < .05$). The difference between the four to six week and the eight to ten week groups was significant ($P < .02$). There was no statistical difference between the eight to ten week and the twelve to fourteen week groups ($P > .2$).

## DISCUSSION

*Armstrong* (1946) measuring bone ash weight, showed that the percentage difference between the two limbs in normal rats is statistically insignificant. *Slack* (1954) showed that there is less than two percent difference in weight of the left and right normal hind limbs of rats and concluded that muscle wasting could be most conveniently expressed as a percentage of the control limb. *Bohr & Sørensen* (1950) showed that there is equal radioactive uptake in the right and left femurs of

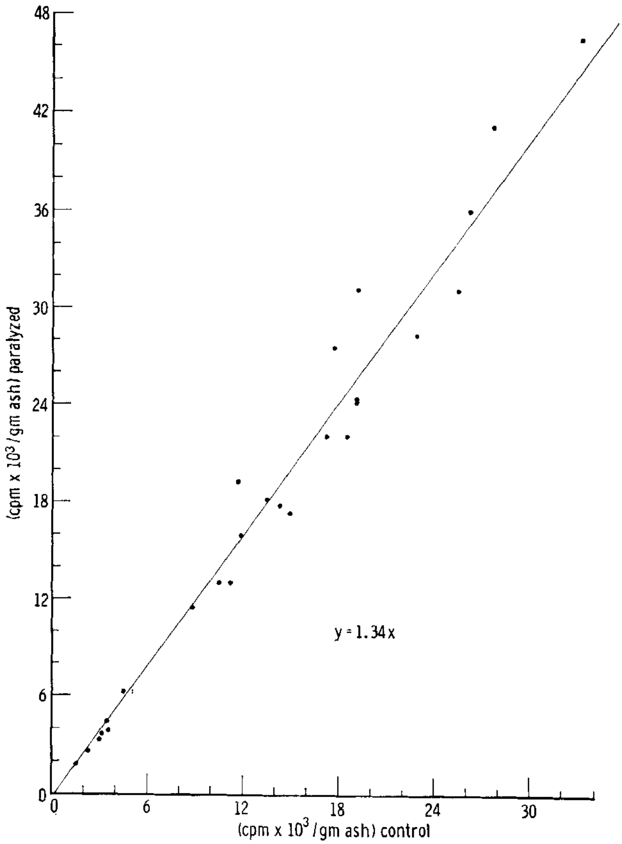


*Fig. 6.*

Specific activity in bone ash on the paralyzed side is plotted against specific activity in bone ash on the control side. The equation for the regression line is given.

rabbits. These findings seemed to justify the use of the right normal limb as a control for the left paralyzed one. The results show that muscle atrophy begins immediately after motor denervation and continues for ten weeks after which it stops. Bone atrophy also is significant at ten days and then continues but to a lesser degree as long as muscle atrophy is going on. This finding, together with the high correlation between muscle loss and bone ash loss in a paralyzed limb, suggests that muscle atrophy determines the extent of bone ash loss. *Gillespie* (1954) found a similar correlation in his denervated rabbits. *Geiser & Trueta* (1958) sectioned the Achilles tendon or immobilized rabbit hind limbs and concluded that lack of muscular action is the most important factor in inducing loss of bone.

If bone ash loss is proportional to the extent of muscle paralysis providing there is complete motor denervation, the extent of paralysis in both the thigh and the leg muscles would be expected to be equal and atrophy of femur and tibia would be similar. Fig. 4 demonstrates that there is a high correlation between bone ash loss in the femora and the tibiae in the paralyzed limbs, but that atrophy in the tibia is greater than that in the femur and starts earlier, implying more complete denervation of the distal muscles.

*Heaney* (1962) studied accretion rates in patients suffering from acute poliomyelitis. He found a higher accretion rate in disuse osteoporosis than in controls. *Wendeberg* (1961) in his studies of isotope uptake in fracture of the tibia in man, showed that the accretion rate increased both at the fracture site and in the adjacent limb bones as well. These patients were immobilized in plaster casts for many weeks following their fractures. These findings are in agreement with our own observations of a higher activity ratio in the paralyzed limbs compared with the controls; moreover, we had the benefit of ashing the bones and measuring directly the specific activity. Fig. 5 shows that the activity per bone is related to the amount of bone ash. When bone ash decreases below eighty per cent of control, the total radioactivity is less in the atrophic bones although the specific activity is higher. The implication is that if there were considerable bone atrophy, external counting would show a lower count in the paralyzed limb compared with the control, even though the accretion rate was higher on the paralyzed side, as it is in our animals. The data suggest that bone formation rate is higher during motor denervation. Since we know that bone atrophy is occurring at this time, it follows that bone resorption must be higher still. These findings amplify and confirm the emerging concept that whether bone formation may be normal or higher than normal in localized or generalized osteoporosis, increased bone resorption is the dominating mechanism that results in bone atrophy: *Bauer* (1964), *Heaney* (1964), *Nordin et al.* (1963), *Dymling* (1964), *Eisenberg & Gordan* (1961), *Frost* (1963). Moreover, although statistically significant bone atrophy has occurred by ten days, the accretion rate has not yet changed. The latter rises appreciably at three to four weeks, reaching a maximum value at eight to ten weeks, and remains constant thereafter. It may be inferred that increased bone resorption starts almost immediately after motor denervation and is followed about three weeks later by a progressive increase in accretion rate, until at ten weeks accretion and resorption have reached a new steady state and are equal. It is apparent that normal bone mass is only partly dependent on muscle function. This is consistent with clinical findings that osteoporosis is usually found soon after a limb is immobilized but does not usually progress indefinitely.

## SUMMARY

Unilateral motor denervation of rabbit hind limbs was performed via hemilaminectomy. The muscle weight and bone ash weight on the paralyzed side was expressed as a fraction of that on the control side. Total radioactivity as well as specific activity ratios of bone ash were also determined.

Muscle and bone atrophy started immediately, continued for ten weeks and then stopped. Bone accretion did not change until sometime between ten days and four weeks when it increased by about thirty per cent.

The findings suggest that muscle atrophy leads to bone atrophy. The mechanism of the latter is by increased bone resorption followed by increased accretion until equilibrium is reached and the atrophy stops.

## RESUME

Une dénervation motrice unilatérale des membres postérieurc chez les lapins a été pratiquée par hémilaminectomie. Le poids du muscle et de la cendre de l'os du côté de contrôle. La radioactivité totale aussi bien que le rapport de l'activité spécifique de la cendre de l'os ont également été déterminés.

L'atrophie du muscle et de l'os fut observée immédiatement. Elle continua pendant dix semaines et s'arrêta. L'accrétion de l'os n'a pas changé entre approximativement 10 jours et 4 semaines où elle a augmenté d'environ 30 pour cent.

Ces trouvailles suggèrent que l'atrophie du muscle mène à l'atrophie de l'os. Le mécanisme de cette dernière est suivi, en cas de résorption accrue de l'os, par une accrétion accrue jusqu'à ce que l'équilibre soit atteint et que l'atrophie s'arrête.

## ZUSAMMENFASSUNG

Einseitige motorische Denervation der rückwärtigen Gliedmassen von Kaninchen wurde auf dem Wege einer halbseitigen Laminektomie ausgeführt. Das Muskelgewicht und Knochenaschegewicht der gelähm-

ten Seite wurde als Fraktion des Gewichtes der Kontrolseite ausgedrückt. Gesamtradioaktivitet als auch spezifisches Aktivititsverhältnis der Knochenasche wurde ebenfalls bestimmt.

Muskel- und Knochenatrophie begann unmittelbar, setzte zehn Wochen hindurch fort und hörte dann auf. Der Knochenzuwachs veränderte sich nicht bis zwischen zehn Tagen und vier Wochen und nahm dann ungefähr dreissig Prozent zu.

Diese Befunde machen es warhscheinlich, dass Muskelatrophie zu Knochenatrophie führt. Der Vorgang der letzteren besteht in einer Zunahme der Knochenaufsaugung, die von einem gesteigerten Knochenansatz gefolgt is bis ein Gleichwicht erreicht wird und die Atrophie aufhört.

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