

It appears from the table that 1. Either contracture or tetany was present in all but one case, in which the contracture occurred at the beginning of the excitation of the muscle and was merely transitory. 2. Contracture and tetany were found together in only 6 of the 20 experiments. There appears to be no necessary relationship between them. 3. Contracture did not occur when the ratio of calcium to phosphorus was less than one, except in one case (No. 9). From these and previous observations it would appear that contracture occurs infrequently when the ratio of calcium to phosphorus is less than one, and then only when the calcium is relatively low; on the other hand, contracture was observed in only one case where the concentration of phosphorus was above 8 mg. per 100 cc. serum. (No. 6.) In those experiments in which total protein was estimated (No. 14-21), the range was from 5.9 to 7.3 gm. Values within this range are not considered pathological.³

8099 C

Course of Phosphatase Activity in Healing of Fractured Bone.

WALTER E. WILKINS AND EUGENE M. REGEN. (Introduced by C. S. Robinson.)

From the Departments of Biochemistry and Surgery, Vanderbilt University School of Medicine, Nashville.

Robison^{1, 2, 3} suggested that the enzyme phosphatase may play an important part in the deposition of phosphates in growing bone. Later studies by Robison and his coworkers, Kay⁴ and other authors have furnished much additional evidence in support of this concept. Most of this work has dealt with the growth of bone, and the study of this enzyme in relation to regeneration of bone has been largely neglected. Studies by McKeown and Ostergren⁵ and Kamada⁶

³ McLean and Hastings, *J. Biol. Chem.*, 1935, **103**, 295.

¹ Robison, R., *Biochem. J.*, 1935, **17**, 286.

² Robison, R., *The Significance of Phosphoric Esters in Metabolism*, New York, 1932.

³ Robison, R., *Ergebnisse der Enzymforschung*, 1932, **1**, 280.

⁴ Kay, H. D., *Physiol. Rev.*, 1932, **12**, 384.

⁵ McKeown, R. M., and Ostergren, J. I., *Proc. Soc. Exp. Biol. and Med.*, 1931, **29**, 54.

⁶ Kamada, K., *Fukuoka Acta Med.*, 1932, **25**, 207. Quoted from *Chem. Abst.*, 1932, **26**, 6002.

indicate that phosphatase may play a part in the healing of fractured bone.

The object of the present study was to determine the course of the phosphatase activity of fractured bone throughout the period of healing. The general plan of the experiment was to produce fractures in a large series of uniform animals, to sacrifice them in groups, at different stages throughout the period of repair, and to analyze the bone at the site of fracture for its phosphatase activity.

The estimation of the activity of the enzyme at the site of fracture necessitates the removal of the bone, and in order to study the phosphatase activity at different stages it was necessary to use a series of animals. In order to avoid the influence of growth on the enzyme, old animals were used. This group consisted of 20 female, pedigreed, New Zealand white rabbits, each approximately 2 years of age. We feel that these animals are as nearly identical for experimental purposes as can be obtained. Throughout the experiment the animals were kept at the registered rabbitry from which they were bought.

Under nembutal anesthesia, by open operation, and with aseptic precautions, fractures were produced in both ulnae by cutting with bone forceps near the middle of the shaft. This resulted in extensive comminuted fractures in each case. The wounds were closed with silk and sealed with collodion. Fractures were produced in this manner in 14 animals. No wound infection occurred. Six animals were used to determine the level of the phosphatase activity in normal unfractured foreleg bones.

It had been learned from a few preliminary experiments which had been previously carried out on a small series of similar animals that after fracture there is a rapid rise followed by a prolonged decline in the phosphatase activity at the site of injury. On the basis of these findings the animals were sacrificed in groups of two, 1, 3, 10, 22, 40, 56, and 89 days after fracture as shown in Fig. 1. The bones were removed and thoroughly cleaned, care being taken not to displace any of the soft granulations from those bones which were used during the early stages of healing. The radius and ulna were cleaned without being separated and a section of both of these bones at the site of fracture was removed with a motor saw. Each section was cut as short as possible to include all the fracture. That portion of the radius adjacent to the fracture was included since its removal during the early stages of healing would cause movement at the fracture and loss of tissue fluid. Thus the fracture was left intact until the sample had been weighed and prepared for extraction.

The 2 samples from each animal were pooled for analysis. Similar sections were taken from normal unfractured shaft bones of 2 of the control animals. Phosphatase determinations were made as described in a previous study.⁷

The results of these analyses and the gross condition of the fractures at the time of removal are shown in Fig. 1. Each column

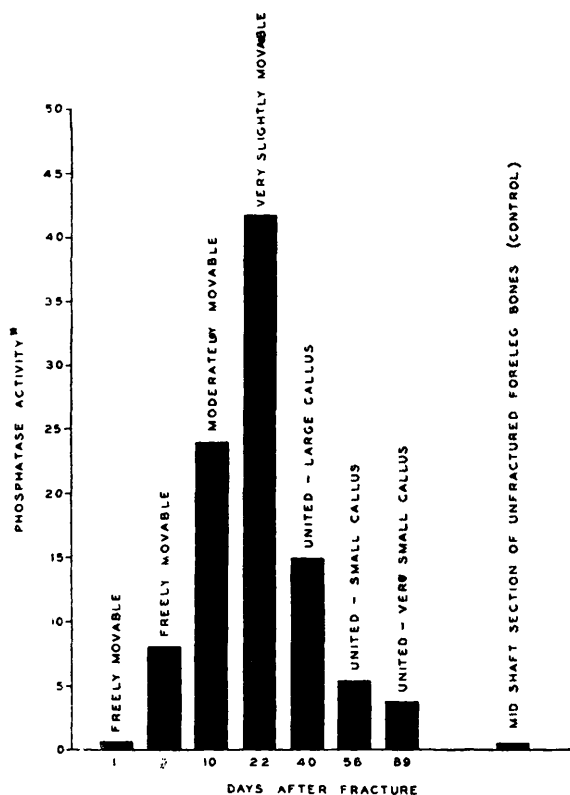


FIG. 1.

Course of Phosphatase Activity in Healing of Fractured Adult Rabbit Bone.

Each column represents average value of 4 fractures (2 from each of 2 animals), sacrificed on the days indicated. Condition of fractures in respect to mobility is indicated above each column. Last column represents average activity of unfractured shaft bone of the control animals.

⁷ Wilkins, W. E., and Regen, E. M., *Radiology*, 1934, **22**, 674

* The phosphatase activity is expressed as the number of milligrams of inorganic phosphorus liberated per hour by the enzyme contained in the entire water extract of the sample from a substrate of sodium-Beta-glycerol-phosphate buffered with glycine-NaOH, at a pH of 8.8 and a temperature of 38°C. The activity of the enzyme is calculated on the basis of the entire water extract rather than per unit of volume, since this is a better index of the "amount" of phosphatase present in the healing tissues.

represents the average value of 4 fractures (2 from each of 2 animals). One day after fracture the phosphatase activity was still within the range of that of normal unfractured shaft bone of the control animals. Observation on the third, tenth and twenty-second days showed a rapid rise in the activity of the enzyme. Since only one determination was made between the tenth and fortieth days, it is obvious that the value obtained on the twenty-second day does not necessarily represent the highest point reached. Observations on the fortieth and fifty-sixth days showed a marked drop in phosphatase activity. But even after 89 days it had not returned to the normal level. It is likely that the true peak occurred near the twenty-second day since values obtained 12 days before and 18 days after the twenty-second day are considerably lower. In any case the period of decline was much more prolonged than the period of increase.

Plasma phosphatase determinations were made on some of the animals at the time they were sacrificed. During that part of the experiment when the phosphatase activity at the fracture site was highest, there was an increase in the plasma phosphatase by comparison with the controls. However, in both groups the activity was quite low and the significance of the changes is questionable.

It was not planned originally to study the possible generalized effect of fracture on uninjured portions of the skeleton, but in 2 of the experimental animals, those sacrificed on the fifty-sixth day, phosphatase determinations were made on the remaining portions of the foreleg bones, after the section including the fracture had been removed. In each case it was within the range of normal unfractured whole foreleg bone. This suggests that the increase in the enzyme activity is more or less limited to the site of injury. Further studies on this problem are in progress.

Summary. Following fracture of adult rabbit bone there is a rapid rise in the phosphatase activity at the site of bone injury which reaches a peak at some time near the twenty-second day and drops gradually as repair progresses.