

INFLUENCE OF SOME PLANT ENZYMES ON EPIPHYSEAL CARTILAGE

By

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According to Thomas 1956, Spicer et al. 1957–1958, McCluskey et al. 1958, both crude papain and inactivated purified papain have a specific effect on the hyaline cartilage of growing rabbits. Our own recent investigations, furthermore, have proved papain to be one of the most effective and rapidly acting agents known to retard the longitudinal growth of different laboratory animals. Within a few hours after even a single injection of papain extensive damage to the epiphyseal cartilage becomes apparent. The intercellular matrix is diminished, there is damage to the cells, and in the border area between epiphysis and metaphysis a necrosis is to be seen. These changes mostly recede after about one week. After repeated injections, however, osseous closure of the epiphyseal lines may ensue.

In 1958 McCluskey and Thomas stated that another plant enzyme, bromelin, if inactivated, has a similar effect on hyaline cartilage. This has led us to investigate the effect of both bromelin and another well known plant enzyme, ficin, on the growth zones of rabbits and mice.

The Biochemistry of Plant Enzymes.

Proteolytic enzymes are widely distributed in many kinds of plant tissue, in particular the latex. *Papain* is obtained from the latex of the tropical tree *carica papaya*. The crude preparation has many practical applications. Papain is now also available in purified doublecrystallized form. Other proteolytic plant enzymes with properties resembling those of papain, and therefore named "papainases", are *ficin* from the latex of the fig, *Ficus carica*, and *bromelin*, obtained from the latex

of the pineapple, *Ananas sativus*. All these plant enzymes act over a wide pH range on proteins and on synthetic peptides. In order to exhibit maximal proteolytic activity toward proteins these enzymes require activation by one of a variety of substances, such as glutathione, cystein, H₂S, and HCN. Crude preparations of these enzymes are always accompanied by sufficient natural activator; the further addition of cystein increases the rate of enzymatic action. Sulfhydryl groups are believed to be essential for enzymic activity. These plant enzymes are inhibited by sulfhydryl inhibitors, iodoacetate, heavy metal salts, H₂O₂, etc.

Investigations by McCluskey et al. (1958) established that neither crude papain which had been activated by cystein, nor purified papain produced any effect on the hyaline cartilage when intravenously injected. On the other hand, both unadulterated crude papain and purified papain inactivated with iodoacetacid had the specified effect. Crude bromelin was found to have no effect unless inactivated with iodoacetacid. The only acceptable explanation of this singular inverse effect is, that these enzymes must be activated locally in the cartilage, whereas initially active enzymes are probably eliminated before they reach the cartilage. Corroboration of this theory was provided by in vitro investigations made by these authors.

Material and Methods of the Present Investigation.

Young white rabbits were used for the experiments. They were 4-5 weeks old and weighed between 250 and 500 g.

Crude bromelin (from the Nutritional Food Co.) was dissolved in physiological saline in 1, 2, and 4 per cent solutions. These were then inactivated with iodoacetacid for 15 minutes at 37° C. according to McCluskey et al., and filtered. Bromelin was also tested without previous inactivation.

Crude ficin (from Mann Research Lab., New York) was used in 1 and 2 per cent solutions and tested both in its original form and after inactivation with iodoacetacid.

The rabbits were injected in the ear veins. The animals were as a rule killed 48 hours after injection, and the front legs of the rabbits were then roentgenographed. Radius and ulna were then removed for histologic examination. All specimens were fixed in 10 per cent formalin, decalcified with equal parts 44 per cent formic acid and 20 per cent natrium citrate (pH 1,9), embedded in paraffin, and stained with hematoxylin-eosin.

RESULTS

Under certain conditions both bromelin and ficin were found to cause roentgenologic and histologic changes of the epiphyseal cartilage similar to those produced by papain, as described in earlier papers. Collapse of the ears of young rabbits, too, could be evoked by these preparations. (Thomas 1956).

Bromelin: Regardless of whether it had been inactivated with iodoacetacid or not, administration of a 1 or 2 per cent bromelin solution in a dose of 10–20 mg. per Kg. of body weight—a dose which in the tests with papain had proved uniformly effective—did not produce any curling of the ears, nor could any clear changes be found in the roentgenograms or in the histologic specimens. All these changes did appear, however, when the dose was increased to 30–40 mg. of a 4 per cent solution per Kg. of body weight. The preparation proved equally efficacious whether iodoacetacid was added or not. As early as 48 hours after a single dose of bromelin the roentgenograms of the rabbits' front legs showed a distinct narrowing of the distal epiphyseal lines of radius and ulna, with a decrease of roentgen density in the metaphysis indicating an impaired development of bone trabeculae from the growth zones. (Fig. 1 a-b).

Histologic examination 48 hours after injection of the increased dose disclosed extensive damage to the epiphyseal lines. (Fig. 3 a-b). The entire epiphysal cartilage had become narrowed, while in the proliferative zone a higher density and more irregular formation of the cells could be observed. The zone of hypertrophic cells was collapsed and partly necrotic. The bone trabeculae adjoining the epiphyseal cartilage are short, broad, and deformed, showing a clear deviation from the normal pattern. The basophilic properties had decreased noticeably as well.

Ficin: Administration of ficin which had not been inactivated with iodoacetacid, in dose of approximately 10–20 mg. per Kg. of body weight did not seem to cause curling of the ears, but in this respect the effect of the preparation was not easily gauged on account of the strong local reactions in the ears resulting from ficin injections. Both histologically and roentgenologically, however, the deleterious effect of a single injection could be clearly discerned after 48 hours. After repeated treatment the changes had progressed even further. The effect appeared to be identical with that resulting from papain treatment. When a 1 or 2 per cent ficin solution had been inactivated with iodoacetacid



Fig. 1.

according to the method indicated in the foregoing, a dose of 10 mg. per Kg. of body weight produced a distinct curling of the ears. The roentgenogram (Fig. 2 a-b.) clearly illustrates the effect of ficin in narrowing the epiphyseal cartilage. The histologic changes (Fig. 4) ensued after a single injection of ficin that had not been inactivated and although not so distinct, they are nevertheless easily discernible if compared with the normal specimen.

DISCUSSION

The aim of the present investigation has been to establish that the effect of papain on the epiphyseal cartilage, as described in some of our earlier papers, can equally well be produced by other proteolytic plant enzymes, more specifically bromelin and ficin. The effect does indeed appear to be identical as regards curling of rabbit ears, roent-

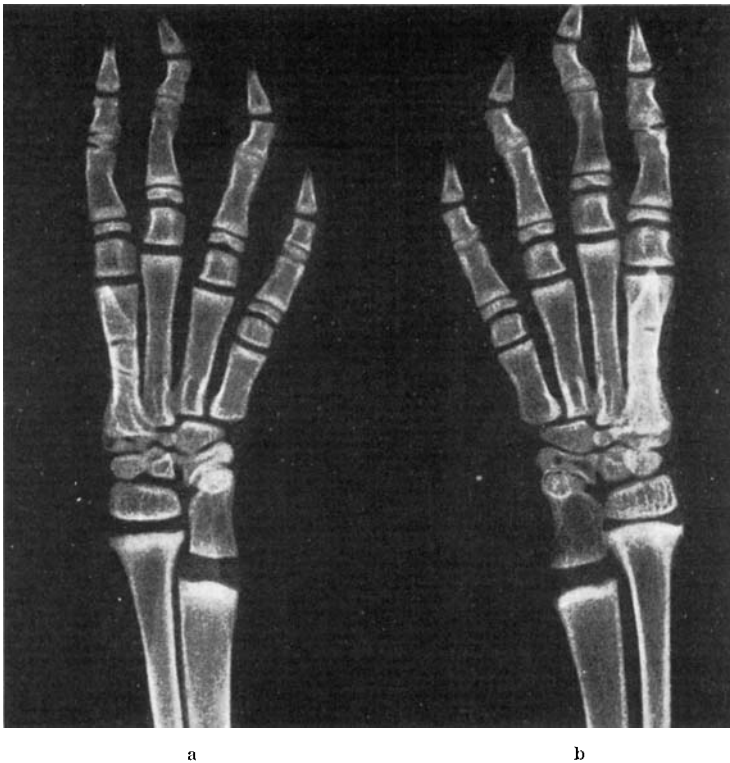


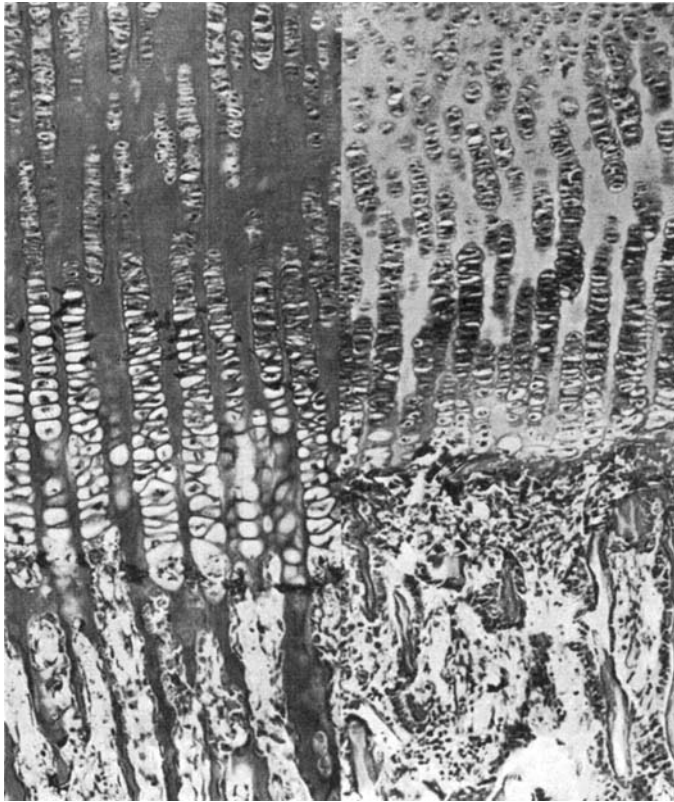
Fig. 2.

Figs. 1 a and b; 2 a and b.

Roentgen pictures of the forepaws of a bromelin-treated rabbit (1 b) and ficin-treated rabbit (2 b) compared with resp. controls of the same litters (1 a and 2 a). Both bromelin and ficin in single doses produce 48 hours after injection a marked narrowing of the ulnar and radial epiphyseal lines and a diminishing of the zone of metaphyseal trabeculae.

genologic narrowing of the epiphyseal lines, and strong histologic changes of both the epiphyseal cartilage and the metaphyseal trabeculae.

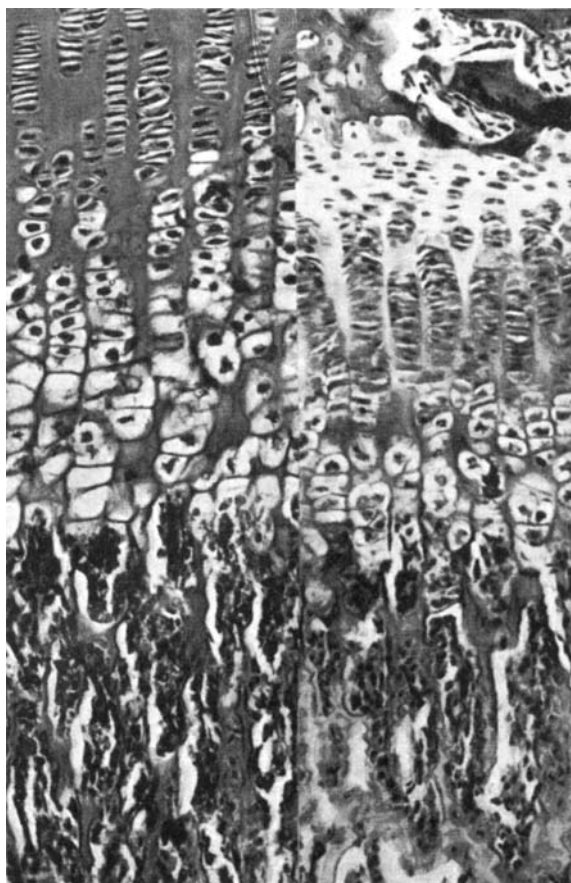
Several authors (Tsaltas, 1958, Bryant et al., 1958, Spicer and Bryant, 1958) have pointed out that papain injections in growing rabbits cause a decrease of chondroitin sulphate in the hyaline cartilage and at the same time an increase of chondroitin sulphate in the blood and the urine. It is generally believed that the papain action upon the proteins binding the chondroitin sulphate in the matrix causes a release of this chondroitin sulphate.



a *Figs. 3 a and 3 b.* b

Microphotograph of the ulnar epiphyseal cartilage of a bromelin-treated rabbit (3 b) and the control of the same litter (3 a). Narrowing of the epiphyseal line, compression and partly necrosis of the zone of the hypertrophic cells, irregular bone trabecles and disappearance of the basophilia are the most marked changes already, 48 hours after injection. Haematoxylin-eosin. Magn, 160 \times .

Considering that the biochemical properties of bromelin and ficin closely resemble those of papain, it seems very likely that these enzymes act upon the epiphyseal cartilage in a similar way. A trait common in the three enzymes is that they are effective as crude preparations. The purified papain preparation can, however, be made effective by inactivation with iodoacetic acid. This suggests the presence of some substance in the growing cartilage which activates these enzymes, all three of which are dependent on sulfhydryl groups for their proteolytic activity. It must furthermore be assumed that these enzymes have a special



a *Figs. 4 a and 4 b.* b

Microphotograph of the ulnar epiphyseal cartilage of a ficin-treated rabbit (4 b) and the control of the same litter (4 a). There is narrowing of the epiphyseal line and broadening of the bone trabecles. Haematoxylin-eosin. Magn. 240 \times .

affinity for the protein bindings of chondroitin sulphate, to be able to explain the selective effect of these plant enzymes on the growing cartilage, and in particular the epiphyseal cartilage.

SUMMARY

Bromelin and ficin are papain-like plant enzymes with similar biochemical properties. Our investigation has shown that the effect of these enzymes, when intravenously injected, on the epiphyseal cartilage

in young rabbits is identical with that of papain. Within 48 hours of a single intravenous injection of these preparations a narrowing of the epiphyseal lines can be discerned in the roentgenogram. Histologic examination shows a diminished area of the matrix as well as deformation of both chondrocytes and trabeculae.

RESUME

La bromeline et la ficine sont des enzymes de plantes ressemblant à la papaïne avec des propriétés biochimiques similaires. Notre enquête a montré que l'effet de ces enzymes, injectés intraveineusement, sur le cartilage épiphysaire de jeunes lapins est le même que celui de la papaïne. Dans les 48 heures qui suivent une seule injection intraveineuse de ces préparations, on peut discerner sur le radiogramme un rétrécissement des lignes épiphysaires. Un examen histologique montre une diminution de l'extension de la matrix ainsi qu'une déformation des chondrocytes aussi bien que des trabécules.

ZUSAMMENFASSUNG

Bromelin und Ficin sind Papain-ähnliche Pflanzenenzyme mit gleichartigen biochemischen Eigenschaften. Unsere Untersuchung hat gezeigt, dass die Wirkung dieser Enzyme auf den Epiphysenknorpel junger Kaninchen bei intravenöser Verabreichung identisch mit der des Papains ist. Innerhalb von 48 Stunden nach einer einzigen intravenösen Injektion dieser Präparate kann man eine Verschmälerung der Epiphysenlinien im Röntgenbilde erkennen. Die histologische Untersuchung zeigt sowohl eine Abnahme der Ausdehnung der Matrix als auch eine Deformierung der Chondrozyten und Trabeculæ.

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