PAPAIN INDUCED CHANGES IN CARTILAGE

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Introduction

THE effects of the systemic administration of crude papain to young rabbits were first described by Thomas (1956), who noted that the most obvious change was a bilateral collapse of the ears. This effect was lost within a few days. Subsequently Hulth and Westerborn (1959) and Merkow and Lalich (1961), who used crude papain in single and repeated doses, and in different species of animals, not only confirmed but also elaborated on the effects of its administration.

After finding that crystalline papain failed to produce collapse of rabbit ears, even when injected in large amounts, McClusky and Thomas (1958) observed that if this enzyme preparation was inactivated before administration by the addition of a thiol-combining agent such as iodo-acetamide, the typical collapse did occur. The inactivated enzyme, however, did not produce this effect *in vitro*.

This study was undertaken to determine the effects of local and systemic injections of inactivated crystalline papain in rabbits and rats, with a view to its possible clinical application in the early treatment of bilateral cleft palates.

Materials and Methods

Crystalline papain protease, obtained from British Drug Houses and which was prepared according to the method of Kimmel and Smith, was diluted with a phosphate buffer pH 7.0* to make a 1.6 per cent solution of papain. An equal volume of 0.1M iodo-acetamide was added and the resultant solution was incubated for 30 minutes at 37.0°C.

For the initial experiment 18 young male and female rabbits were selected, varying in weight from 400 to 1,400 gms. The first three were used to confirm the typical effects of inactivated crystalline papain on their ears; 0.8 mg. was administered intraperitoneally. Under general anaesthesia approximately 0.4 mg. of the drug was injected into one ear of each of the remaining 15. To avoid the large blood vessels the subcutaneous injections were given on the inner aspect of the ears. The solution was deposited in a horizontal plane across the ear about an inch from the tip. As soon as the injections were completed, three of these rabbits were sacrificed by an overdose of anaesthetic and placed in an incubator at 37.0°C.

In the second experiment there were 32 young albino rats ranging in age from 11 to 15 days. Twenty-five received a single intraperitoneal injection of 0.8 mg. of the inactivated papain solution. They were sacrificed at daily intervals up to one week, and thereafter weekly up to 19 weeks. Seven litter mates served as controls. These were sacrificed daily during the first week. The livers, kidneys and spleens were removed, fixed in formalin and prepared for histological examination. The ears, skulls and femora were also removed and fixed in formalin. The skulls and femora were decalcified. All specimens were embedded in wax and stained with haematoxylin and eosin, and toluidine blue.

Results

The first three rabbits to receive the intraperitoneal papain showed the typical collapse of the ears, which indicated that

*24 c.c. of a solution containing 2.388 gm. Na_2HPO_4 per 100 ml. were added to 16 c.c. of a solution containing 0.907 gm. KH_2PO_4 per 100 ml. to make up the buffer.

FIG. 1.—Drooping of ear at injection site on sacrificed animal after two hours of incubation.

this solution of inactivated crystalline papain and the solutions prepared by other workers did not differ in action.

In all the rabbits which received an injection of inactivated papain into an ear, there was a change in the cartilage adjacent to the site of the injection; this was manifested by a drooping of the ear.

In those rabbits sacrificed and incubated immediately after the injection, the drooping was demonstrable after two hours and no change was observed in the uninjected ears (Fig. 1).

In the 12 live rabbits the drooping took slightly longer to become evident (Fig. 2) and during the first 24 hours it was accompanied by a progressive inflammatory reaction in the injected ear; oedema was most marked in the anterior border. There was also a progressive inability on the part of the rabbit to raise or move the injected ear (Fig. 3). After 24 hours this feature was noticeable only in the smaller animals. The uninjected ears showed no change throughout the experiment (Figs. 2 and 3).

A varying degree of scab formation occurred over the injected area on both inner and outer aspects. Recovery was more rapid in the large animals and eventually the only sign that remained at the injection site was a slight thickening of the cartilage.

Histological examination of the livers, kidneys and spleens of the rats which received an intraperitoneal injection of the inactivated papain revealed no apparent pathological change. Most sections of the cartilage, the ears, the nasal septum and femoral epiphysis showed that the cartilage had been affected. The greatest effect was seen in the specimens sacrificed two days after injection. Changes in metachromasia were used as an indication of change in cartilage matrix.

DISCUSSION

If the resilient nature of cartilage can be temporarily altered and if it can recover its properties after being distorted mechanically while its matrix is softened, it is likely that treatment of the disproportionate antero-posterior, and to a lesser extent the vertical growth of the

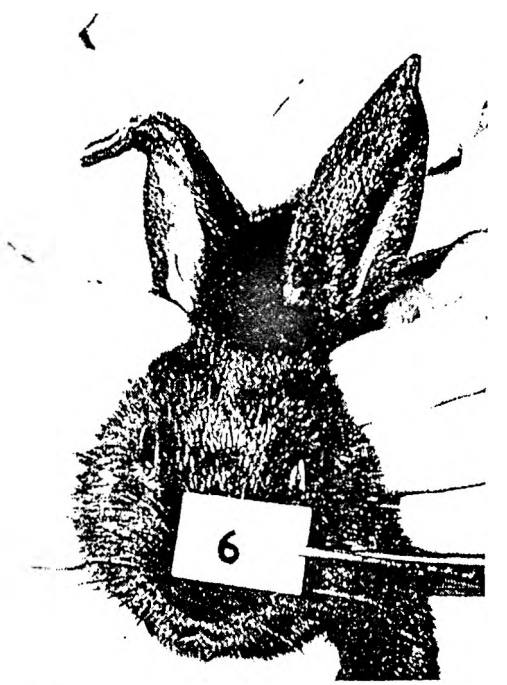

FIG. 2. Drooping of ear at injection site on sacrificed animal after two hours of incubation.

FIG. 3.—Inability to raise the affected ear 24 hours after injection of papain. Note upright uninjected ear.

nasal septum, in bilateral cleft palates can be aided by the local injection of papain.

The systemic administration of papain on cartilage and its subsequent effects on skeletal morphology has received a great deal of attention, but few clinical uses in its local administration have been suggested. The most important of these is the injection of chymopapain into pathologically affected intervertebral discs for their dissolution. Smith and Brown (1967) treated 75 patients in this manner and found no untoward reactions attributable to the enzyme.

Although no long-term detrimental effects after a single intraperitoneal injection of papain have been described—and our rat experiments confirmed this—the drug nevertheless has side-effects. Our experimental animals which had a large enough intraperitoneal dose to produce demonstrable cartilage changes also showed a decreased activity during the 24 hours following the injection.

We assumed that a smaller dose introduced locally would decrease side-effects. Thus if the desired effect can be obtained at a specific site in cartilage by a dose less than that required by interperitoneal injection, the general reaction will be reduced. This may well be achieved by local administration.

The results obtained by injecting the inactivated papain solution adjacent to the ear cartilage tend to confirm that the desired local effect can be achieved with minimal side-effects.

An unusual finding was that a similar effect was produced in the animals sacrificed immediately after the injection and then incubated at 37.0°C.

SUMMARY

Some effects of a solution of crystalline papain, inactivated by iodo-acetamide, on the cartilage of rabbits and rats are reported.

Subcutaneous injections of the papain solution into one ear of each of 15 rabbits produced a reversible drooping in that ear alone.

A single intraperitoneal injection of papain solution into 25 rats produced effects on their cartilages but no pathological changes in their livers, spleens or kidneys could be determined histologically.

It is suggested that the local administration of inactivated papain may aid in the treatment of bilateral cleft palates.

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