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 (1959) 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 2.368 gm. Na₂HPO₄ per 100 ml. were added to 16 c.c. of a solution containing 0.907 gm. KH₂PO₄ per 100 ml. to make up the buffer.
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In the 1 2  live rabbits the drooping

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was accompanied by a progressive in-

flammatory reaction in the injected ear;

oedema was most marked in the anterior

border. There was also a progressive in­

ability on lie 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

cars 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 re­

ceived 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 car­
tilage 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 re­
cover its properties after being distorted

mechanically while its matrix is softened,

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

Fig. 2. Drooping of ear at injection site on
sacrificed animal after two hours of
 incubation.
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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**References**