The changes in craniofacial growth following papain administration in young mice

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The effect on craniofacial growth after repeated papain administrations was studied in young mice. The length of the upper face and lower face were both seriously affected whereas the length of the neurocranium, the anterior height of the upper and lower face were moderately affected.
The posterior height of the upper face and the height of the neurocranium were not influenced by papain injections.

Key-words: Craniology; growth; papain

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Thomas (1956) was the first to observe collapse of the ears four hours after intravenous injection of a solution of crude papain in a growing rabbit. He also showed that the cartilage throughout the animal's body after papain administration showed loss in basophilic staining reaction.

In subsequent histological and histochemical investigations (*Spicer & Bryant*, 1958) it was found that cartilage, after papain injection rapidly lost its basophilic properties followed by a loss of metachromasia. These findings have recently been shown on nasal septal cartilage in young mice (*Kvinnsland*, 1973). In addition a reduction in alkaline phosphatase enzymatic activity was found together with a negative Alcian Blue — PAS colouring reaction, the last finding indicating a loss of mucopolysaccharides as a result of papain injections.

The effect of papain on the epiphyseal cartilage of the long bones has received special interest. *Hulth* (1958) reported that repeated intravenous injections of papain produced changes in the epiphyseal growth zones throughout the body which could be demonstrated on roentgenological and histological examination. He also found that even a single injection of papain produced changes in the epiphyseal cartilage. Subsequent studies have shown that repeated intravenous injections of papain causes bony closure of all epiphyseal growth zones with dwarfism as a result (*Hulth & Westerborn*, 1959). While most studies have been made on growing rabbits, studies on dogs (*Engfeldt, Hulth & Westerborn*, 1959), and on cats, mice and guinea pigs (*Hulth & Westerborn*, 1959), and on rats (*Merkow & Lalich*, 1961; *Rönning*, 1968, 1971) have shown that papain produced similar

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changes in the cartilagenous growth zones studied in all these different species of young animals.

MATERIALS AND METHODS

A 0.5 per cent crude papain suspension was prepared, buffered to pH 7.0 with 3.6 per cent NaOH, filtered and kept under refrigeration. Twenty mice of the NMRI strain were each given one daily intraperitoneal injection of 0.05 millilitres papain solution from the 10th to the 16th day after birth. Fourteen animals survived the experimental period and were killed on the 30th day after birth. The control material consisted of 12 NMRI strain mice which were killed 30 days after birth. The heads were radiographed with the sagittal plane parallel to the film. An attempt was made to have the teeth in occlusion, if not this was compensated for on the tracings of the radiographs. The central ray was perpendicular to the film, and the focus-film distance was constant. An analysis of growth was carried out. Tracings of the papain administered animals and the controls were superimposed along the palatal plane with a reference line going through the fronto-nasal suture perpendicular to the palatal plane (E line, Fig. 1).

The horizontal dimensions were projected and measured along the palatal plane and the vertical dimensions along the E line.

RESULTS

The length of the neurocranium, O—E. Growth in length of the neurocranium was moderately affected and showed a slight reduction as a result of the papain injections (Table I).

The length of the upper face, E—A. The upper face length showed a significant reduction in the papain injected animals compared to the controls (Table I).

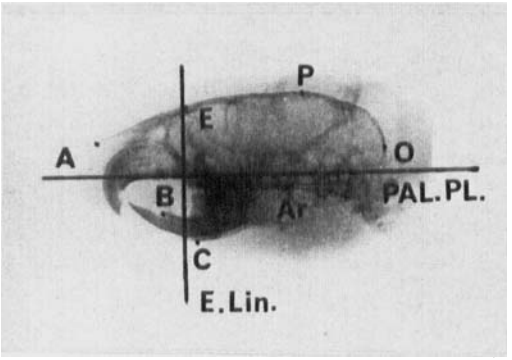

Fig. 1. Reference points and reference lines used on the radiographs. A- the most anterior point on the nasal bone. B- the most anterior bony point on the lower jaw. C- the most caudal point on the lower jaw. E- the superior point on the fronto-nasal suture. Ar- intersection between the posterior contour of the mandibular ramus and the inferior contour of the cranial base. P- the most superior point on the parietal bone. O- the most posterior point on the occipital bone. PAL.PL — Palatal plane, the tangent to the bony palate. E.LIN- E- line; a line through E perpendicular to the palatal plane.

Table I. *Comparison between experimental variables in papain injected and control animes*

	Papain injected animals n=14		Controls n=12			
	$\bar{x}$	SD	$\bar{x}$	SD	t	
O-E	13.1	0.40	13.8	0.25	3.28	**
E-A	5.6	0.25	6.7	0.25	7.15	***
O-B	14.8	0.41	16.8	0.48	7.99	***
Ar-B	8.1	0.37	9.0	0.28	4.97	***
Ar-O	7.0	0.31	7.0	0.28	0.0	
P-palatal plane	5.1	0.25	5.1	0.24	0.17	
E-palatal plane	4.3	0.25	4.4	0.18	0.76	
A-palatal plane	2.6	0.37	3.1	0.18	3.07	*
C-palatal plane	3.9	0.20	4.3	0.25	2.75	*

* = 1 %>p>0.5 %
** = 0.5 %>p>0.1 %
*** = 0.1 %>p

Lower jaw prognathism, O—B, Ar—B and Ar—O. Lower jaw prognathism was also significantly reduced in the experimental animals compared to the controls. Reduced lower jaw prognathism can be the result of a shortened lower jaw, a posterior positioned lower jaw or a posterior rotation of the lower jaw. In the experimental animals it seems that the reduced lower jaw prognathism is a result of a shortened lower jaw, as the measurement Ar—B was shorter in the experimental group than in the controls and as no posterior positioning (Ar—O) of the lower jaw was found on the radiographs (Table I).

The height of the neurocranium, P-palatal plane. The height of the neurocranium was not affected by papain administration.

The posterior height of the upper face, E-palatal plane. The posterior height of the upper face does not seem to be influenced by papain injection (Table I).

The anterior height of the upper face, A-palatal plane. The anterior height of the upper face was moderately affected in the experimental animals and showed a small but significant reduction as a result of papain injection (Table I).

The height of the lower face, C-palatal plane. The height of the lower face was also moderately affected in the experimental animals and showed a small but nevertheless significant reduction as a result of papain administration (Table I).

DISCUSSION

Previous studies (*Thomas*, 1956; *Hulth & Westerborn*, 1959; *Merkow & Lalich*, 1961) have shown that papain administration in laboratory animals leads to reduced growth of the cartilagenous zones investigated. *Rönning* (1968) found that papain injections induced a considerable reduction in the cranial and mandibular lengths during and for some time after the treatment in young rats. The present study, however, revealed a moderate reduction in cranial length, but a significant reduction in upper facial length in young mice. The difference in these findings may be explained by the division of the total cranial length in a facial and neurocranial part, in the present study. Otherwise the present study agrees with earlier investigations showing that papain has a specific action on cartilage causing a reduction in growth.

The moderate effect papain had on the increase in length of the neurocranium tends to agree with *Koski* (1968) who throws doubt on the spheno-occipital synchondrosis as an important growth centre.

Papain injection had a marked effect on the growth of the upper facial skeleton causing a significant reduction in length and a more moderate reduction in anterior height. This tends to indicate the importance of the cartilagenous nasal septum as an important factor in normal facial growth. The posterior height of the upper face was not affected by papain injections, this was somehow expected as this area, the fronto-nasal suture is probably as much part of the neurocranium as of the face, and as such is possibly more under the influence of the growth of the brain than of the cartilagenous nasal septum.

Lower jaw prognathism was significantly affected as a result of a shortened mandible. This would tend to show that normal growth of the condylar cartilage is a prerequisite for normal mandibular development.

Previous and present studies on the effect of papain on cartilage does not, however, either prove or disprove that the cartilagenous zones affected are primary

growth centres, but it proves fairly conclusively that when growth of cartilage is impaired by chemicals such as papain or by pathological conditions as achondroplasia, normal growth is not achieved.

If it is assumed that cartilage in the various craniofacial areas are primary growth centres, then the effect on growth after papain administration can be explained by the specific action of papain on cartilage, namely an arrest in the normal growth pattern. If, on the other hand, these same centres are regarded as passive zones of cartilage merely acting as filling-in areas as the various bony components, and cavities (cranial, orbital, nasal and oral) increase in size, then the effect on growth after papain injection must be explained by an arrest in the filling-in capability of cartilage.

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