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EXPERIMENTAL RETARDATION OF ENDOCHONDRAL GROWTH BY PAPAIN

By

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The endochondral growth of laboratory animals can be disturbed experimentally in different ways. The response differs, however, with the species used and with the age at which the experimental measures become effective. Young animals fed with diets which are generally or specifically insufficient have retarded growth of length. Endocrine disorders play the most important role. Hypophysectomy leads to prompt cessation of growth and the thickness of the epiphyseal plate decreases (Ray *et al.*, 1954). Surgical or radio-iodine induced thyreoidectomy results in stunting (Crane *et al.*, 1954; Dziewiatkowski 1957; Ray *et al.*, 1954; Silberberg and Silberberg, 1954). Such retardation in skeletal development involves both membranous and endochondral bones. Administration of cortisone in rabbits or rats also results in retardation of growth of the long bones during the time it is given (Sissons and Radfield, 1955). The skeletal lesions produced by injected aminonitriles or by diets containing the seeds of *lathyrus odoratus* (and some other species of *lathyrus*) are very considerable but the endochondral growth is not disturbed (Geiger *et al.*, 1933; Lewis and Esterer, 1943; Ponseti and Shephard, 1954; Selye and Ventura, 1957).

Papain, a drug obtained from *carica papaya*, contains a proteolytic enzyme which, when injected intravenously has a curious effect on the ground substance of the cartilage of young rabbits. L. Thomas (1956) described how as quickly as four hours after injection a rabbit's ears had curled and hung down; this effect was maximal after 24 hours and disappeared after three days. If such injections were repeated several

times and daily doses of cortisone were *not* simultaneously given, then the "curling" failed to appear. According to Thomas the intravenously injected papain results in a very rapid and profound depletion of the intercellular matrix of all cartilage tissue in young rabbits, but not in fully-grown ones. The basophilic staining properties of the cartilage disappear. These effects on the cartilage occur apparently without evidence of systemic illness. Recently Spicer and Bryant (1957) published a histochemical study of the papain effect on rabbit cartilage in which they performed punch biopsies of the ears at varying intervals after injection. About two hours after the injection they found an increase in metachromasia and decrease in acidophilia; the chondrocytes underwent crenation and empty spaces surrounded the shrunken cells; disruption of a part of the elastic fibers also occurred. Four hours after injection there was a complete loss of metachromasia of matrix and increase of acidophilic staining property; PAS-reaction was retained; the chondrocytes re-expanded and filled the lacunae.

According to L. Thomas and Spicer *et al.* the changes occurring after single doses of papain are reversible. In a preliminary report I have described that repeated papain injections produce a more definite and irreversible lesion of the epiphyseal plates, some of which become closed. In the present paper I will give a more detailed account of the experiments performed on young rabbits.

MATERIAL AND METHODS

A filtered, fresh solution of 1–3 per cent of crude papain in sterile water was prepared for every occasion. The filtered solution was clear and had a light yellow colour. The solution was not sterilized because the papain enzymes would then have lost their activity.

Six litters of young rabbits (A.–F.), 21–42 days old (total 34 animals), were used in order to study the effect on cartilage tissue a short time after papain injections. In each litter all but one or two were injected, who were kept as controls. At the beginning of the experiments the rabbits weighed between 160 and 890 g., as shown in the table.

The injections were performed in the ear veins. To test the activity of the papain as well as regulate the dosage, the above-mentioned "curling" effect on the ears was very suitable. It was found that heating the papain solution to 65° C for 15 minutes had no effect on its activity; boiling the solution for the same time, however, completely

destroyed its activity. There was some difficulty in judging the first dose, which was always much less tolerated than the following ones. If stronger doses than 15 mg per kg body weight were given, the mortality was considerable. The "curling" effect in the surviving rabbits, however, was very good. The same effect in rabbits given only 10 mg per kg was a little less obvious. Even with this dosage, however, some of the animals died. This must be due to the fact that these crude papain solutions were not standardized.

In every litter rabbits were sacrificed (air embolus) at different intervals after the onset of the experiments. Both control and papain-treated rabbits were examined roentgenologically at the same time; in the case of living animals, after nembutal anaesthesia. The prone position was always used and the focal distance kept constant. From the sacrified rabbits preparations were made from different epiphyses for histological investigation.

The Immediate Effect of Papain.

Three to four hours after intravenous injection of papain solution the rabbit ears began to curl and hang down as illustrated in Fig. 1. This effect increased during the first 24 hours and disappeared gradually within 2–4 days. The consistency of the ears was felt to be more soft and doughy than normal. The "curling" reappeared after repeated injections given within 10–12 days but to a minor degree. Later repeated injections, however, had no "curling" effect. This is probably due to the fact that the rabbits become immune to papain. Like Tomas, I have found that cortisone in daily doses strengthens and lengthens the effect of papain. Because of the co-existent softening of the tracheal cartilage the rabbits often had stridor for a day or two. In one case I gave papain (20 mg per kg) intraperitoneally. The ears became curled but the effect did not appear for 20 hours.

Apart from the toxic action of the initial papain dose which sometimes caused death, the treated rabbits appeared completely normal like their respective controls. They ate well and moved and jumped around just like untreated young rabbits. Usually, repeated injections of papain had no effect on the general condition of the rabbit, even though considerably stronger doses were given (25–30 mg per kg). Only in one case did a repeated injection cause death—probably an anaphylactic reaction.

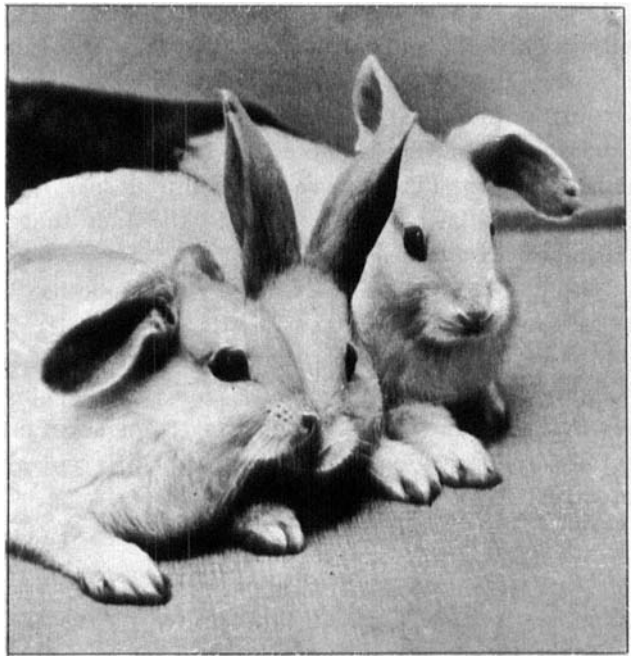

Fig. 1.
Two rabbits injected with papain 2 days earlier and the control. The injected rabbits have curled ears.

The Effect on Weight Increase.

The table records the percentual weight increase for the six litters (A.-F.) of rabbits which were followed-up. Without exception all rabbits given more than a single dose of papain retarded in percentual weight increase when compared with their respective controls. This effect was appreciable eight days after injection (Litter E). The effect was also more significant if papain was initially given with short intervals (2–3 days). The retardation of the weight increase after a series of injections remained for a long time after the cessation of the papain treatment, as seen in the table. Those rabbits receiving only a single dose of papain showed a variable effect on the percentual weight increase, even if the amount given was strong enough to kill some of them. The stature of the papain treated rabbits appeared to be well proportioned, the retarding effect must therefore work on all tissues in a similar way. It must be emphasized that these animals were not at all emaciated,

TABLE I

Effect of Papain on body weight and longitudinal bone length (ulna and pelvis).

Rabbit No.	Number: (duration in days:) of injections. Controls = 0	Initial and end weights in grams	Percentual weight increase	Bone length in mm of	
				ulna	pelvis
		Litter A., 42 Days old at the Beginning.			
		Follow-up period 30 days:			
1	3(7)	630–1045	66	—	45
2	3(7)	600–1040	73	60	55
4	3(7)	570–980	72	54	55
5	0	610–1175	93	64	67
6	0	600–1175	93	67	70
		Follow-up period 42 days:			
2	3(7)	600–1220	103	65	60
3	3(7)	520–1030	98	63	62
6	0	600–1610	168	72	74
		Follow-up period 50 days:			
4	4(30)	570–1210	112	60	64
5	0	610–1640	170	73	76
		Follow-up period 69 days:			
2	3(7)	600–1380	130	72	64
6	0	600–2370	295	80	79
		Litter B., 23 Days Old at the Beginning.			
		Follow-up period 27 days:			
8	0	160–675	320	48	44
9	3(23)	160–460	188	41	36
		Follow-up period 46 days:			
10	3(23)	160–600	275	48	48
14	0	165–890	440	56	58
15	2(18)	185–800	330	49	54
		Litter C., 42 Days Old at the Beginning.			
		Follow-up period 42 days:			
17	1	380–1060	178	61	62
19	1	350–1000	186	58	58
22	1	415–980	136	60	62
23	4(21)	410–840	105	56	57
24	4(21)	375–810	116	55	55
25	4(21)	480–875	108	57	52
26	0	415–1170	182	61	64

TABLE 1 (cont.)

Rabbit No.	Number: (duration in days:) of injections. Controls = 0	Initial and end weights in grams	Percentual weight increase	Bone length in mm of	
				ulna	pelvis
		Litter D., 35 Days Old at the Beginning.			
		Follow-up period 52 days:			
29	1	480–870	81	56	50
34	0	550–1800	240	70	70
		Follow-up period 63 days:			
27	1	610–2020	240	71	80
30	6(58)	660–1270	93	63	62
32	1	620–1520	146	68	72
		Follow-up period 70 days:			
27	1	610–2110	245	72	81
28	3(13)	715–1475	106	69	74
31	8(67)	610–1420	132	68	67
		Litter E., 35 Days Old at the Beginning.			
		Follow-up period 8 days:			
44	2(8)	890–970	9	53	48
45	2(8)	740–800	8	52	49
48	0	850–1025	20	58	53
		Follow-up period 19 days:			
46	5(17)	680–840	23	51	48
47	5(17)	920–1160	26	55	57
48	0	850–1320	55	61	64
		Litter F., 35 Days Old at the Beginning.			
		Follow-up period 30 days:			
57	2(5)	440–660	50	50	–
58	2(10	380–685	80	49	–
59	2(15)	315–585	86	46	–
60	0	320–670	110	51	–

as shown in Fig. 2 which illustrates a papain-treated and a control animal from the same litter with the same initial weight.

The Effect on the Growth of Length.

The most striking effect of papain administration was the retardation of the endochondral growth, while the membranous growth ap-

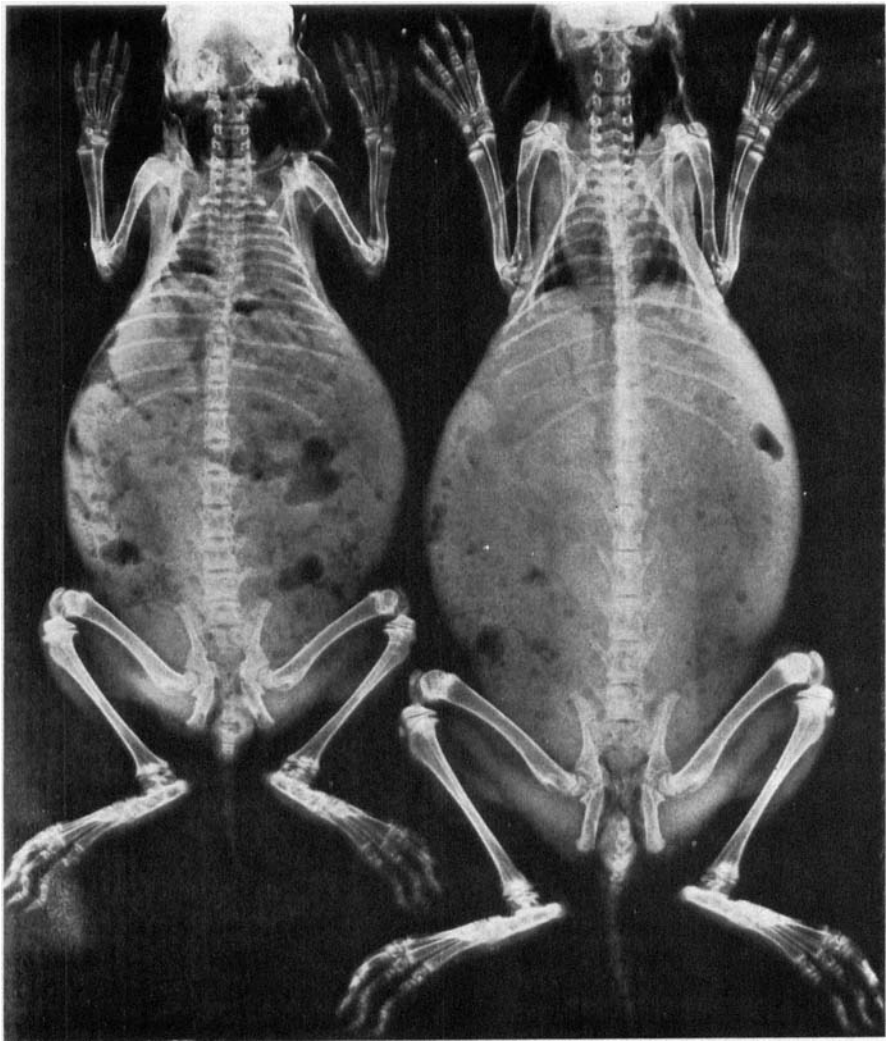

Fig. 2.
The effect of 3 injections of papain during 28 days on the rabbit no. 9 (to the left) and the control rabbit no. 8 (to the right). The entire skeleton is smaller. Some of the epiphyseal plates of the forearm and of the foot are closed.

parently remained intact. Some of the epiphyseal plates were obliterated at once, completely inhibiting the further growth of the bone in question. Fig. 3 shows the effect on the tail vertebrae after two injections and only eight days after the first injection. Most of the epiphyseal plates, however, remained intact at the dosage used, thus per-

Fig. 3.
The tail vertebrae of a rabbit given 2 injections in eight days (to the left). Compared with the normal tail (to the right) it is evident that the growth zones are going to close.

mitting a continued but delayed further longitudinal growth. It appeared that some epiphyseal plates were more susceptible to papain than others and the degree of vulnerability seemed to be connected with the actual growth rate. The wider the epiphyseal plate, the more rapid the longitudinal growth, the less the effect of papain. The epiphyseal zones most regularly closing were: the phalanges, the metatarsal and metacarpal bones, the upper femoral epiphyseal plate, the

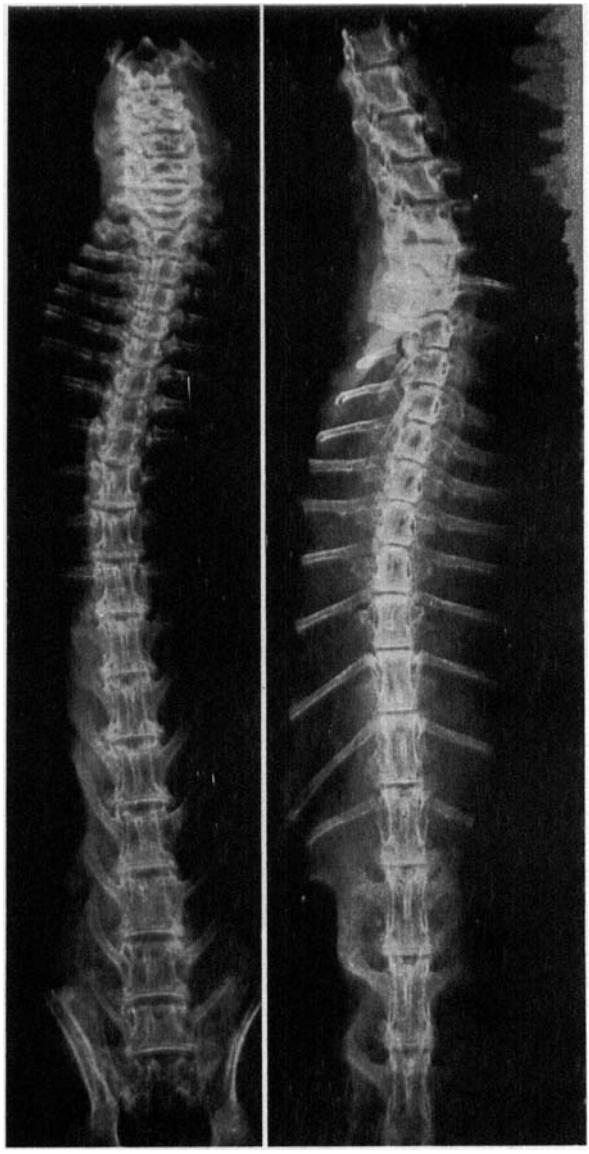

Fig. 4.
Comparison between spines of a papain treated animal (left) and a control rabbit. There is a marked difference in the height between the vertebrae of the preparations.

Fig. 5.
Comparison between the caudal part of the spines of the same rabbits as in Fig. 4. The papain rabbit to the left.

growth zones of the vertebrae of the neck and the tail. Even the inferior epiphyses of radius and ulna, however, became closed in some cases (Fig. 6). Most resistant appeared to be: the inferior femoral and the superior and inferior tibial epiphyseal plates, where premature ossification never occurred.

Radiologically, the papain-treated rabbits all had smaller skeletons than their controls and this difference appeared to remain for a long time after the cessation of papain administration. Figs. 2–6 shows the considerable difference between treated and control rabbits. Fig. 2 re-

Fig. 6.
The forearm of a rabbit injected 5 times during 19 days (to the left) and the control rabbit from the same litter. Closure of the epiphyseal lines of radius and ulna and the phalanges.

presents the papain rabbit no. 9 compared with the control no. 8, both animals having the same initial weight. They were killed at the age of 50 days (experiment period 27 days). Figs. 4 and 5 show the spines of the papain animal no. 4 and the control rabbit no. 5. These rabbits were 93 days old (experiment period 50 days). There is a considerable difference between the height of the vertebrae while the breadth is exactly the same in the two preparations. The pelvic bones of the papain rabbit are significantly shorter. Fig. 6 shows the forearm of an animal injected 5 times during 19 days. In comparison with the control one observes that most of the epiphyseal lines of the phalanges and metacarpal bones, and in addition those of radius and ulna, are going to close.

In the table the lengths of the ulnae and the pelvic bones have been noted. On the roentgenogram of the rabbits lying prone these two bones are in the most suitable position for comparison. The table shows that the rabbits injected two or more times in every case have shorter bones than the controls or those rabbits from the same litter injected only once. This difference is independent of the initial weights. The length of pelvic bones from the individuals of the same litter differs in several cases by more than one centimeter.

HISTOLOGICAL INVESTIGATION

The epiphyseal plates of ulnae from the entire material were subjected to histological investigation. The animals which were killed several weeks after the last papain injection (those in the above-mentioned follow-up) were not so interesting histologically as those killed earlier. In the former either the epiphyseal lines were closed and looked like any ossified epiphyseal line, or the epiphyseal cartilage had recovered after the damaging effect of papain. In the latter, however, it was possible to study the specific action of papain on the growth zones.

The histological investigation here is limited to the morphological changes of the growth zones as the alterations of hyaline cartilage produced by papain have been exhaustively dealt with by Thomas and by Spicer *et al.*

The Effect of Single Doses of Papain.

Two litters of rabbits, all three weeks old, were administered 10 mg of papain per kg; one control rabbit of each litter was not injected. These rabbits were killed 7, 18, 20 and 48 hours, and three and seven days after the injection; the two controls were also killed. The distal part of the ulna was removed, fixed in 10 per cent formalin, decalcified in nitric acid, embedded in paraffin, sectioned at 10 μ, and stained with hematoxylin and eosin.

The earliest changes seemed to appear in the resting cartilage nearest the epiphysis and in the proliferating zone, where the cells are arranged in longitudinal rows. Fig. 7 shows, from left to right, the area of proliferating cells from the ulna of a) a normal rabbit, b) a rabbit killed seven hours after, and c) a rabbit killed 20 hours after papain. The effect produced by papain is striking. In b) the chondrocytes appear to have swollen. They have a slightly stainable endoplasma con-

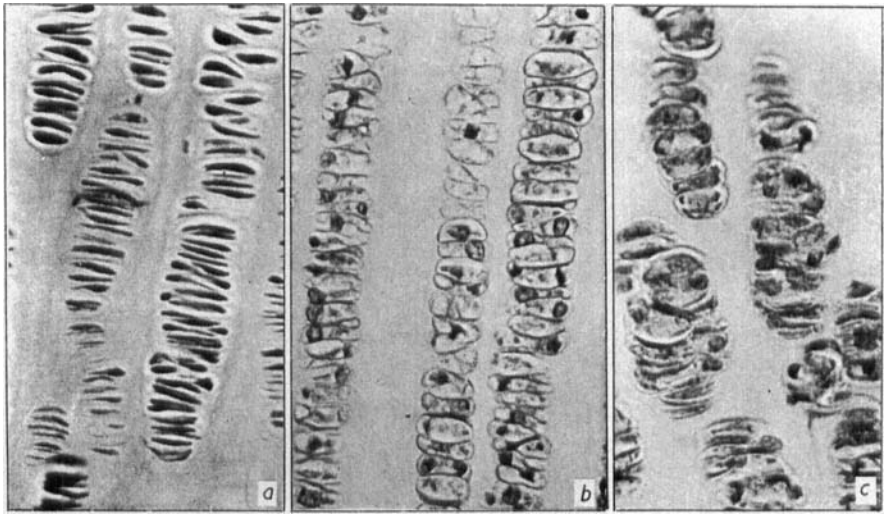

Fig. 7.

a: Normal proliferating cells.—*b*: The proliferating cells as they appear 7 hours after a papain injection. The endoplasma stains badly, the nuclei are roundish with clumping of chromatin.—*c*: The changes 20 hours after papain injection. Swelling of the cells. The stain of the endoplasma is more distinct.

taining vacuoles. The nuclei which normally are wedge-shaped and stain very darkly, after papain become roundish, almost vesicular, stain poorly except some clumps of chromatin. From 20 hours onwards there is some disarrangement of the rows of cells, which begin to cluster. Gradually the histological changes mentioned take place even in the cartilage cells close up to the metaphysis. From about 20 hours after papain, changes of the large hypertrophic or degenerating cells begin to occur, and 48 hours after papain the changes become manifest. There is also a disorder of the new bone formation. Fig. 8 a is a survey of the entire width of the growth zone of the ulna from a normal rabbit. Figs. 8 b and 8 c show the alterations 48 hours and 72 hours after papain, respectively. The width of the proliferating zone is gradually diminishing. Forty-eight hours after papain the cells of the proliferating zone begin to recover and their nuclei stain again. The hypertrophic cells near the metaphysis which are about to be destroyed by ingrowing vascular buds, have normally a square or polygonal form, but in the slides from 48 and 72 hours after papain they have assumed roundish or flat form, are of smaller size and contain vacuoles. Normally there are ingrowing capillaries from the metaphysis into every

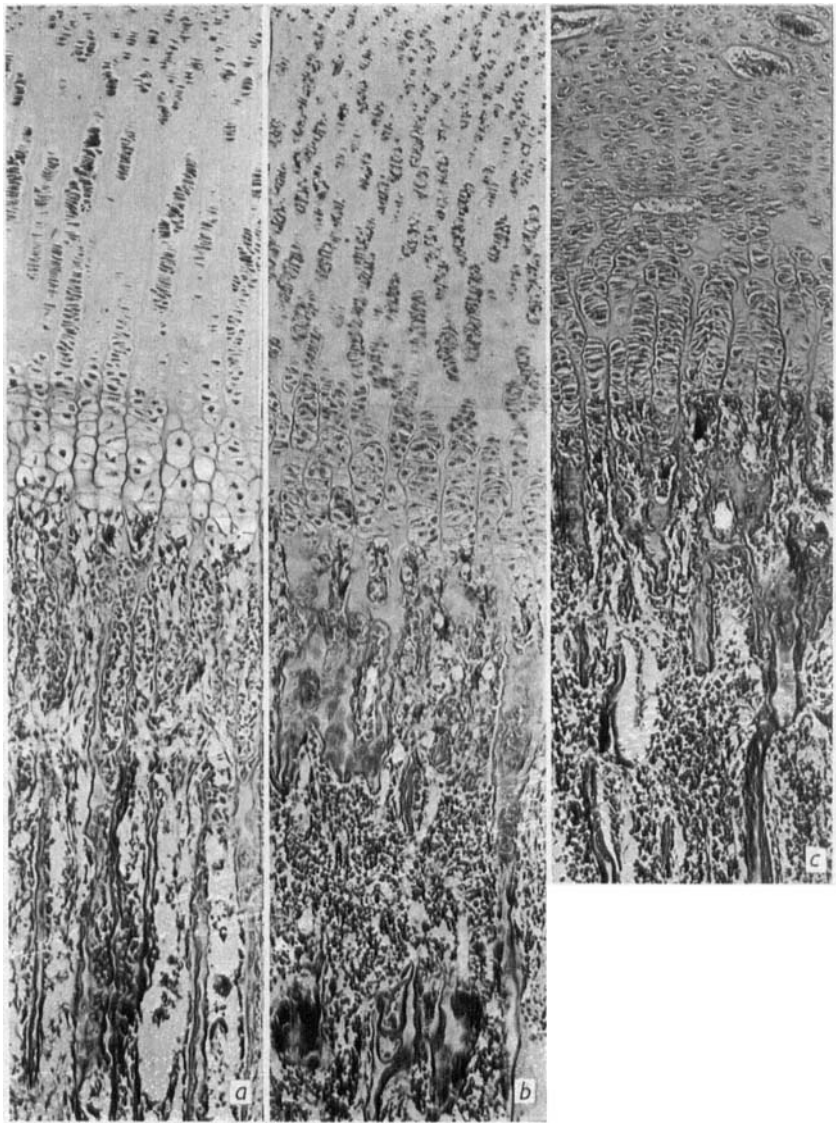

Fig. 8.

a: Normal epiphyseal cartilage and metaphysis.—*b*: Papain changes 48 hours after injection. The proliferating cells begin to be restored. Small and vacuolized hypertrophic cells. Clumsy bone trabeculae.—*c*: The changes 72 hours after injection. Short and ramified bone trabeculae. The entire zone of growth is narrowed.

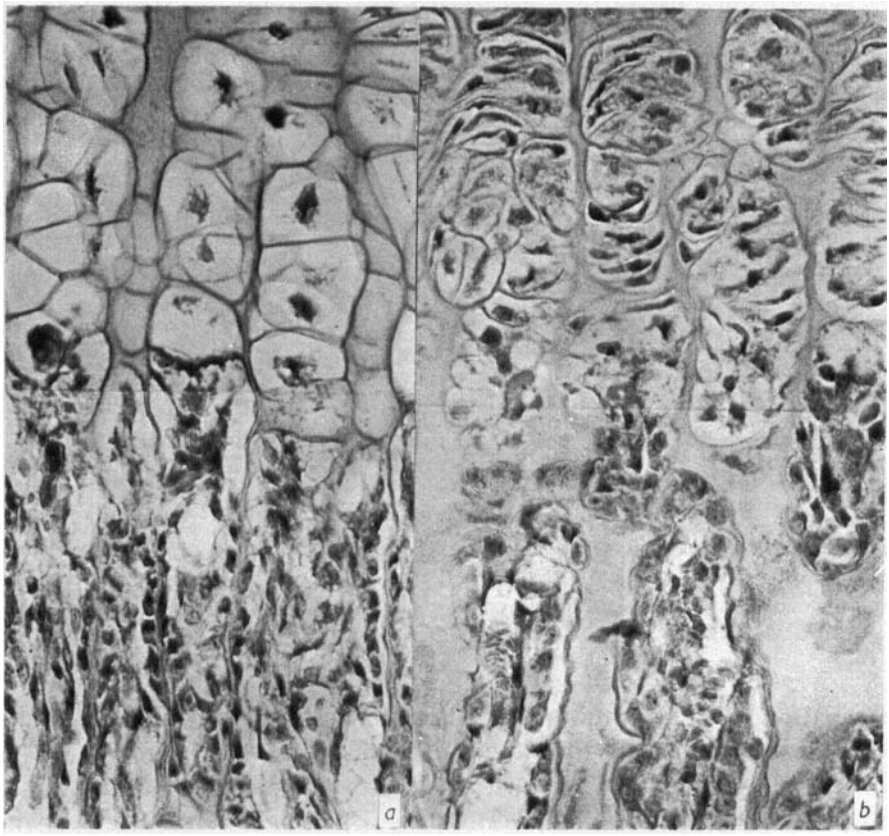

Fig. 9.

a: A normal metaphyseal region. Vascular buds grow into every row of hypertrophic cells which disappear. Very thin bone trabeculae.—*b*: The changes of the same region 48 hours after a papain injection. Shrinking of the hypertrophic cells. Very broad trabeculae which consist largely of calcified cartilage matrix. Swelling of the osteoblasts which have an altered nuclear structure.

row of degenerating cartilage cells which open and disappear, whilst the cartilaginous matrix between the cell columns remains, forming the core of the bone trabeculae. In Figs. 8 b and 8 c it is evident that some of the cartilage cell rows are not going to be removed by invading vessels. The trabeculae are much more clumpy and shorter than is normal. The breadth of the separate trabeculum is about 6–7 times larger than the normal trabeculae and consists largely of calcified cartilaginous matrix. The trabeculae ramify with one another; this does not occur normally. Fig. 9 shows in a higher magnification the altered

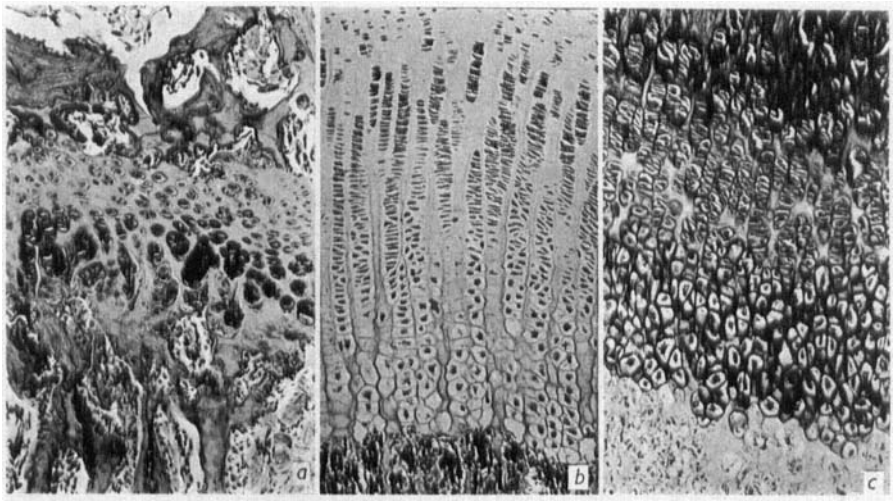

Fig. 10.

b: A normal ulnar epiphyseal cartilage.—*a* and *c* represents the effect of two papain injections on two animals of the same litter.—*a* is a epiphyseal line which is almost closed. The epiphyseal cartilage on *c* has an excess of atypical cartilage cells and the matrix is fibrillated. No bone formation occurs.

formation of bone trabeculae 48 hours after papain. The appearance of the osteoblast seems also to be changed, as they have swelled, have a different nuclear structure with clumping of the chromatin, similar to the chondrocytes of the proliferating zone seven hours after papain.

A week after papain most of the abovementioned changes have disappeared and the cartilage plate is almost restored. There is still, however, some irregularity of the rows of the proliferating cells, and the epiphyseal plate is still narrowed.

The Effect of Repeated Injections of Papain.

The changes after a single dose of papain described above are mainly reversible. If papain is given repeatedly at short intervals, the damage of the epiphyseal plate becomes more profound and some of the plates obliterate. The effect varies however, in different epiphyseal plates and in different individuals of the same litter, treated in the same way. Probably the phase of maturation of the epiphyseal cartilage in question plays some role. Fig. 10 illustrates the effect of two doses of papain on two individuals of the same litter. Both animals received two injections and were killed seven days after the onset of the experiment. The

Fig. 11.
The upper part of Fig. 10 c in a higher magnification. The matrix is fibrillated.

ulnar epiphyseal plates have reacted differently. That in Fig. 10 a is almost closed and there are only dispersed gatherings of cartilage cells left. The other cartilage on Fig. 10 c is not narrowed, but is nevertheless damaged to a very high degree. The matrix, which stains in an intense basophilic colour, has fibrillated areas. The endoplasma of the irregular formed cells is acidophilic; the cells usually arranged in rows, are clustered together. There is no formation of new bone. See also Fig. 11.

DISCUSSION

Crude papain injected intravenously in young rabbits brings about a profound but reversible damage on their cartilage tissue, as shown by Thomas and by Spicer *et al.* These authors used rather big rabbits weighing about 1 kg. In the experiment reported here I have used very young rabbits (3–5 weeks old) and weighing from 200 to 600 g. This fact may explain why the above-mentioned authors observed no papain effect on the endochondral growth of the bones. The effect seems to be

greater in younger rabbits. If papain is injected repeatedly with short intervals (as in litter A, where three injections were given in one week) a rétardation of the percentual increase of weight occurs which remains for several weeks. The entire skeleton becomes smaller as regards the length of the separate bones whilst their breadth is the same as in the controls. Some epiphyseal lines ossify prematurely. If the dosage of papain is spread out over a month or more the growth-delaying effect becomes less distinct (as in litter D), probably because of an acquired immunity.

The cause of the delayed longitudinal growth becomes clear by studying the visible histological changes in the epiphyseal cartilages. As early as a few hours after a papain injection there is a swelling of the proliferating cells. The cells contain vacuoles. The nuclei are poorly stainable and have clumped chromatin. From 24 hours after injection these alterations proceed to the hypertrophic cells in the vicinity of the metaphysis. Two days after injection even the ossification of the cartilage is highly affected. At this time the changes in the proliferating zone begin to be restored. If repeated injections are given within short intervals severe damage of the epiphyseal cartilage occurs. A visible fibrillation of the intercellular substance—as if the mucopolysaccharides had been washed off—may occur. In other cases the epiphyseal cartilage becomes ossified and then appears as a full-grown epiphyseal line.

There are many difficulties in interpreting the effect of papain on the cartilage tissue. Its very rapid action makes an enzymatic action probable. As papain contains proteolytic enzymes, it is very probable that it attacks the proteins of the cartilaginous matrix. Another proteolytic enzyme, trypsine (Trypure Novo), injected in sublethal doses in rabbits, had no similar effect on rabbit ears, which become curled after papain (Hulth & Westerborn). This agrees with an investigation by Blumberg and Ogston (1956), who found that papain attacks the protein component of the hyaluronic acid complex of ox synovial fluid, leading to removal of protein from the polysaccharide. Chymotrypsin and trypsin, however, had no such action. Papain, therefore, must contain some agent specific to the polysaccharide-protein complex of the cartilage tissue. The apparent effect of papain on the cartilage cells may be secondary to a damage of the proteins of the matrix. This procedure will be subject to a more close study with aid of different histochemical methods in a forthcoming paper (Hulth and Westerborn).

The papain effect on the cartilage is not similar to that of the *lathyrus* factor. Feeding of young rats with *lathyrus* seeds or injecting different aminonitriles, attacks all mesenchymal tissues, causing skeletal deformities, broadening of the epiphyseal cartilages, slipping of the epiphyses and producing dissecting aneurysms of aorta. In lathyrism there is an increase in number and size of the cartilage cells which become clustered. The cartilaginous matrix appears to disintegrate in the plane between the zone of proliferating cells and the calcified cartilage. The lesions produced by papain, however, seem to be limited to the cartilage tissue and the endochondral bone building and this effect is rather different to those produced by the *lathyrus* factor.

SUMMARY

Crude papain, a drug obtained from *carica papaya,* when injected intravenously in young rabbits results in a retardation of the longitudinal growth of bones, but does not attack their appositional growth. There is also a diminished percentual weight increase.

Papain acts upon all cartilage tissue of young rabbits but not on that of full grown ones. This paper describes the effect on the epiphyseal plates. As demonstrated in the histological investigation papain first attacks the resting and proliferating zones of the epiphyseal cartilage where the chondrocytes within a day undergo swelling and vacuolization, whilst the nuclei become roundish and acquire an altered chromatine structure. Within two days after a papain injection an altered bone formation occurs with very broad and short bone trabeculae. Even the osteoblasts swell and get a changed nuclear structure with clumping of chromatin. After repeated injections of papain the changes in the epiphyseal cartilages can be irreversible, some of the epiphyseal lines becoming obliterated. Other epiphyseal lines get a fibrillated matrix and an excess of atypical chondrocytes. It is probable that papain, as a proteolytic enzyme, attacks the proteins of the mucopolysaccharide-protein complex in the cartilaginous matrix.

RESUME

La papaïne crue, drogue extraite du carica papaya, lorsqu'elle est injectée intraveineusement chez les jeunes lapins provoque un retardement dans la croissance longitudinale des os, mais n'attaque pas leur

croissance appositionnelle. Il y a aussi une diminution proportionnelle de l'augmentation du poids.

La papaïne agit sur tout les tissus cartilagineux des jeunes lapins, mais non chez les individus adultes. Il est rendu compte dans ce travail de son effet sur la substance épiphysaire. Comme l'a démontré l'examen histologique, la papaïne attaque en premier lieu les zones du cartilage épiphysaire en formation et en prolifération dans les chondrocytes desquelles il se produit en moins d'un jour une enflure et une vacuolisation, les noyaux s'arrondissant et prenant une structure chromatique altérée. Dans les deux jours qui suivent uns injection de papaïne, la formation osseuse s'altère, les trabécules devenant très larges et courts. Les ostéoblastes eux-mêmes enflent et présentent une structure nucléaire modifiée avec accolement des chromatines. Après des injections répétées de papaïne, les modifications des cartilages épiphysaires peuvent devenir irrévocables, certaines des lignes épiphysaires peuvent s'effacer. Dans d'autres parties épiphysaires, la cartilagéine devient fibrillaire et on constate un accroissement de chondrocytes atypiques. Il est probable que la papaïne est un enzyme protéolytique qui attaque les protéines du complexe mucopolysaccharide-protéine de la cartilagéine.

ZUSAMMENFASSUNG

Ungereinigtes Papain, eine Droge, die von der *Carica papaya* erhalten wird, bewirkt bei intravenöser Verabreichung eine Verlangsamung des Längenwachstums der Knochen von jungen Kaninchen, stört aber nicht das appositionelle Wachstum. Man findet auch eine Verringerung der perzentuellen Gewichtszunahme.

Papain beeinflusst alles Knorpelgewebe von jungen Kaninchen, hat aber keinerlei Wirkung auf dasjenige von voll Erwachsenen. Diese Arbeit beschreibt den Effekt auf die Epiphysenfugen. Wie mittels der histologischen Untersuchung gezeigt wird greift das Papain zuerst die ruhenden und die proliferierenden Zonen des Epiphysenknorpels an, in welchen die Chondryten innerhalb eines Tages Schwellung und Vakuolisierung erleiden, während die Kerne rundlich werden und eine Veränderung der Chromatinstruktur aufweisen. Innerhalb von zwei Tagen nach der Injektion von Papain geschieht eine Veränderung in der Knochenbildung indem sehr breite und kurze Knochentrabekel entstehen. Selbst die Osteoblasten schwellen und weisen eine Veränderung der Kernstruktur mit Zusammenballung des Chromatins auf. Nach wiederholten Einspritzungen mit Papain können die Veränderun-

gen am Epiphysenknorpel irreversibel werden, indem sich ein Teil der Epiphysenfugen schliessen. Andere Epiphysenfugen erhalten eine fibrilierte Grundsubstanz und einen Überfluss an atypischen Chondrozyten. Es ist wahrscheinlich, dass Papain, als ein proteolytisches Enzym, die Proteine der mucopolysaccharid-protein Verbindung in der Knorpelgrundsubstanz angreift.

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