Virchows Arch. path. Anat. 336, 209—219 (1963)

From the Orthopedic and Surgical Departments and the Department of Pathology II, University of Uppsala, Sweden

Effect of Cortisone on the Epiphysial Cartilage
A Histologic and Autoradiographic Study

By

A. Hulth and O. Westerborn

With 7 Figures in the Text

(Received August 23, 1962)

The effects of cortisone on connective tissue have long been the subject of keen interest. Several studies — mostly utilizing radioactive sulphate — have shown that cortisone reduces the synthesis of chondroitin sulphate.

In the rat, Follis (1951) found the proliferation of the epiphysial cartilage to be inhibited, and also noted a decrease in osteolysis in the metaphysis. Sissons and Hadfield (1955) showed that repeated injections of cortisone led to retardation of the growth of the long bones (in rabbits and rats) and also observed that the epiphysial cartilage zone became narrower than is normal and had a diminished number of hypertrophic cells, while at the same time the bone trabeculae in the metaphysis became short and thick and fewer in number. They also noted that these trabeculae contained especially highly calcified cartilage matrix.

In view of the wide clinical use of cortisone, it seemed of interest further and with different methods to study the effect of cortisone upon the morphology and function of the epiphysial cartilage.

Papain has been found to have a marked effect on cartilage in different experimental animals (Thomas 1956, Hulth 1958, Westerborn 1961), including the disappearance of chondroitin sulphate from the cartilage matrix, cellular changes, and inhibited endochondral growth. Thomas and his associates (1960) have shown that large doses of vitamin A may lead to cartilaginous changes resembling those brought about by papain.

Therefore the experiments were extended to study the effect of cortisone in combination with papain and with vitamin A with histologic and autoradiographic (following the administration of radioactive sulphate) techniques.

Material and Methods

The experimental animals were young rabbits weighing between 250 and 400 g. Cortisone was administered subcutaneously in a daily dose of 6.5 mg (Cortril, Pharmacia).

Vitamin A (Arovit, Roche) was given in a daily dose corresponding to 300000 i. e. in intramuscular injection.

Papain was given intravenously in a single dose corresponding to 10 mg of crude papain per kilogram of body weight. An 1 per cent filtrated solution of crude papain (Merck) in sodium chloride was used.

Radioactive sulphate ($S^{35} \times$ SjS 2, Radiochemical Centre, Amersham), was administered intravenously in a dose corresponding to one half millicurie per 100 g.

The animals were divided into four groups of nine each, added to which there was the requisite number of untreated controls. As controls for the papain experiments we used a series published earlier (Westerborn 1961).

Group 1: Cortisone alone. The rabbits were sacrificed 24 hours after the final cortisone injection. Three animals were killed after four injections, three after eight, and three after fifteen cortisone injections.

Group 2: Vitamin A alone (control group). The animals were sacrificed three at a time after one, five, and ten days' vitamin A administration respectively.

Group 3: Cortisone in combination with vitamin A. The animals were killed in groups of three after one, five, and ten days' treatment respectively.

Group 4: Cortisone in combination with papain. Cortisone was given for two (6 animals) and five days (3 animals) respectively. Papain was administered in a single dose 6 hours, 48 hours, or 5 days before death to groups of three rabbits, so that the animals which received papain 6 or 48 hours prior to sacrifice had received cortisone for 48 hours, while those given papain 5 days before death had been treated with cortisone for 5 days.

S^{35} was given to all the animals in such a way that in each group of three one rabbit had received the radioactive sulphate 2 hours, one 24 hours, and one 5 days before death.

The animals were killed by air embolism. The distal radius and ulna were immediately removed and fixed in 10 per cent formalin. Decalcification was performed in a solution of equal parts of 20 per cent formic acid and 44 per cent monosodium citrate. The specimens were then embedded in paraffin and sectioned. Two or three sections were stained conventionally with haematoxylin-eosin, after which five sections were prepared for autoradiography with the so-called stripping film technique. Two of these sections were prestained with haematoxylin while three were left unstained. After exposure at 4° C for some 35 days, the films were developed and mounted (cf. ENGFELDT and WESTERBORN 1960a).

Results

Histologic Examination

Group 1. *Cortisone.* In animals given cortisone for four days (Fig. 1b), the changes were most marked in the bone trabeculae of the metaphysis. These were short and thicker than normal and were seen to contain plentiful amounts of calcified cartilage matrix, while at the same time the acidophilic bony seam was abnormally thin. The cartilage cells were negligibly altered or normal. The proliferative cells in the cartilage zone were changed in shape and stained less readily. The cartilage zone was irregular and narrower than is normal owing to a decrease in the number of hypertrophic cells.

The animals given cortisone for eight days (Fig. 1c) showed more pronounced changes. The cartilage zone was distinctly narrower than is usual and the trabeculae in the metaphysis were changed in the manner described above. In several specimens there was a distinct boundary some distance down in the metaphysis between trabeculae closest to the cartilage containing plentiful basophilic matrix and a thin or non-existent bony seam, and thick trabeculae further distally in the metaphysis a strong acidophilic bony seam. In the metaphysis there were also a number of cartilage cells which had not been absorbed in the normal manner. Both the hypertrophic and proliferative chondrocytes were damaged, stained poorly, and often had a pyknotic nucleus.

The cartilage zone showed irregular organization: fairly large regions lacked cells, so that there was more matrix than is normal. The staining properties of the matrix were somewhat weaker than is usual. In the metaphysis, close to the epiphysial cartilage, osteoblasts were seen — many damaged and pyknotic — lying loose in the medullary cavity.

After *15 days' cortisone administration* (Fig. 1d), the changes were even more advanced. Occasional short and thick bone trabeculae bordered directly on the epiphysial cartilage. The transition itself was spiral-shaped and some of the trabeculae were fractured. The capillaries were not of small calibre as is usual but merged to form pools in places, frequently abnormally surrounded by cartilage

cells of abnormal situation in the metaphysis. Otherwise, the cartilage cells showed the same damage as described above. This was also true of the osteoblasts, of which very few were perceptible in these specimens.

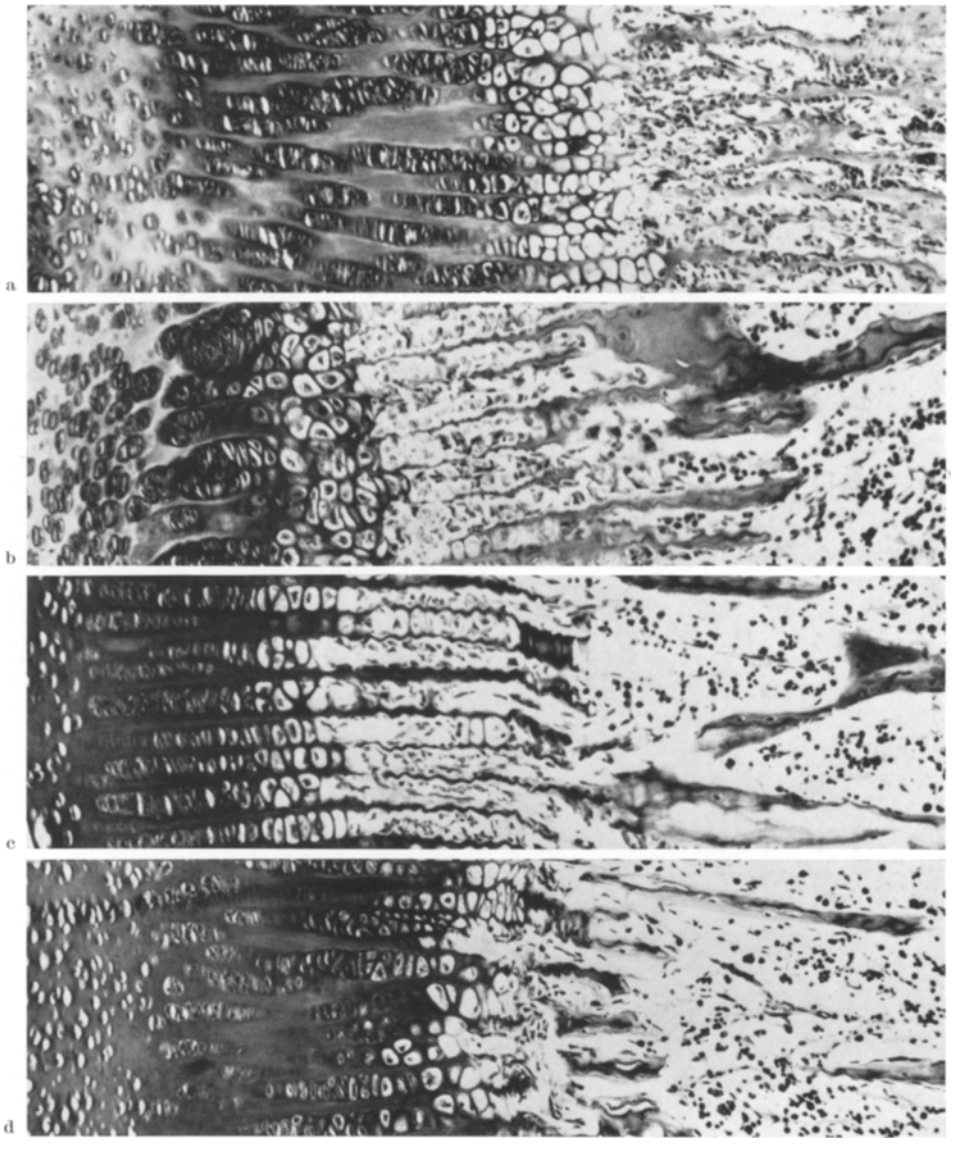
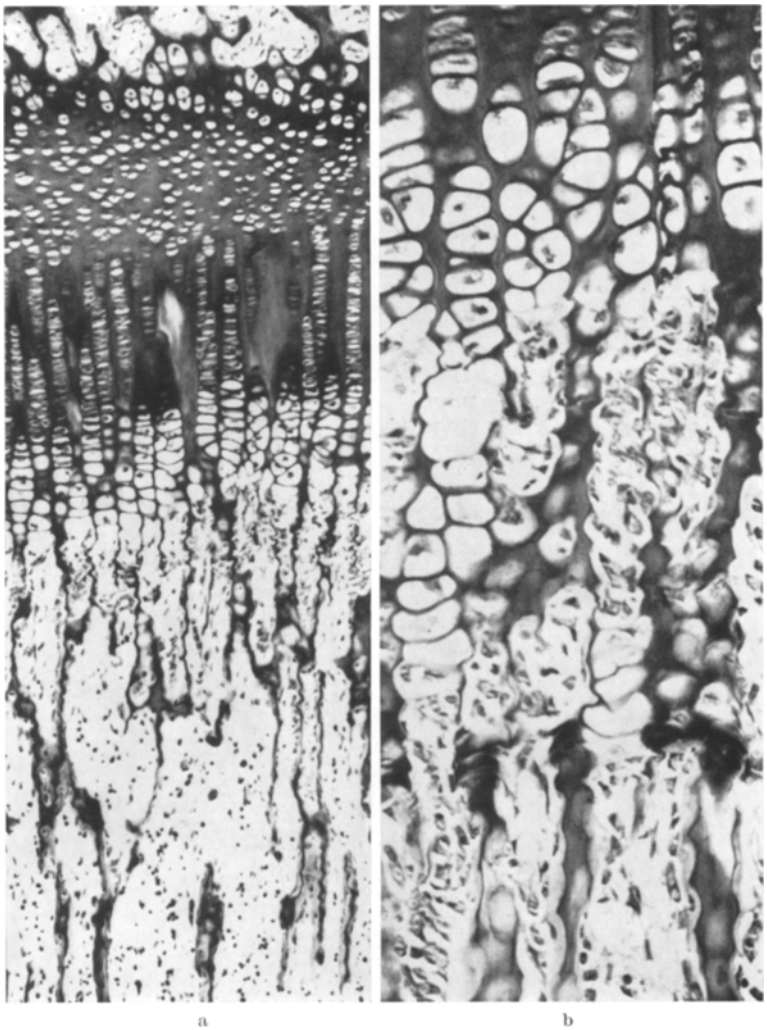


Fig. 1a—d. Distal radius growth zone. Haematoxylin-eosin. 144 ×. a Untreated. Normal growth zone. b Treated with cortisone for 4 days. Irregular and narrow cartilage zone. Short and coarse bone trabeculae in the metaphysis. c Treated with cortisone for 8 days. In the metaphysis several non-resorbed cartilage cells are observed. The amount of osteoblasts is reduced. d Treated with cortisone for 15 days. Irregular cartilage zone. Unresorbed cartilage cells and short irregular and coarse bone trabeculae in the metaphysis. For details see text

Group 2. *Vitamin A.* In our dosage, vitamin A affected the histologic appearances fairly little. One day's administration led to no distinct change, but

the animals which had been given the substance for five days (Fig. 2a) — and especially those treated for ten days — exhibited some changes. These consisted chiefly in shortening and thickening of the bone trabeculae in the metaphysis. Prolonged treatment also appeared in most cases to lead to deepening of the



a b

Fig. 2a u. b. Distal radius growth zone. Haematoxylin-eosin. a 120 ×. Treated with vitamin A for 5 days. Slight irregularity of the organization of cartilage cells. In the metaphysis spiralshaped bone trabeculae. b 250 ×. Treated with cortisone and vitamin A for 5 days. Changes in the growth zone comparable with Fig. 1c and d. In the metaphysis a border is seen between normal bone trabeculae distal and thick trabeculae without normal bony seam close to the cartilage

cartilage zone and an increase in the number of hypertrophic cells. The organization of the cartilage columns was irregular. Otherwise the cartilage cells showed no distinct changes and their staining properties seemed to be unaltered. In this group, too, there were in places non-absorbed chondrocytes down in the metaphysis.

Group 3. *Cortisone plus vitamin A.* Animals given cortisone and vitamin A for five days showed appreciable histologic changes, on the whole corresponding to those described in the cortisone group. A characteristic feature was that the organization of the cartilage was irregular and that the transition of epiphysial cartilage to metaphysis was uneven. Another typical characteristic was the large number of cartilage cells in the metaphysis which had not been absorbed in the manner normal to bone formation. The group given cortisone and vitamin A exhibited more pronounced changes, with a narrow cartilage zone and thick, short bone trabeculae. The hypertrophic cells in particular, and the osteoblasts, showed distinct changes (cf. above). However, in these specimens, too, the staining properties of the cartilage were practically normal (Fig. 2b).

Group 4. *Cortisone plus papain.* The animals given cortisone for two days and papain two or 48 hours before death (Fig. 3a) all exhibited changes wholly characteristic of those associated with papain, consisting in loss of basophilic staining and distinct cellular changes (WESTERBORN 1961). After 48 hours the proliferative cells had recovered somewhat and were again basophilic, lying in a wholly acidophilic matrix. The animals which had undergone a 5-day course of cortisone (Fig. 3b) and had been given papain concurrently with the initial cortisone injection showed more advanced changes than those described earlier in animals given papain alone. In none of the specimens had regeneration of hypertrophic cells definitely started. The proliferative cells were basophilic while the matrix still lacked basophili. The bone trabeculae were short and thick without cartilage remnants, suggesting that no growth had occurred since the start of the experiment. The osteoblasts were of normal appearance but were considerably fewer than is normal and were in some regions wholly absent. The number of cells in the cartilage was less than is usual five days after the injection of papain.

Autoradiographic Examination

The normal specimens corresponded with those described earlier (ENGFELDT and WESTERBORN 1960a). Accordingly, two hours after the sulphate injection there was a strong uptake in the cartilage zone with all the activity situated intracellularly, chiefly in the proliferative but also in the hypertrophic zone (Fig. 4a).

Twenty-four hours after the sulphate injection, activity was seen over all the cells down to the metaphysis and also in the portion of the matrix closest to the cells. Characteristically, the pattern of uptake over the proliferative and hypertrophic cells had the appearance of a ring — that is, the centre of the cell lacked activity (Fig. 5a). Five days after the injection of S^{35} the activity was seen mainly in the cartilage matrix and in the remnants of that tissue enclosed in the bone trabeculae lying closest to the epiphysial cartilage (Fig. 6a).

Group 1. *Cortisone.* The blackening was appreciably less heavy than is usual, and the longer the cortisone therapy had lasted the weaker was the uptake of S^{35}. Two and 24 hours after the S^{35} injection, the uptake in every specimen was more diffuse than is normal, and the characteristic 2-hour picture of distinct intracellular uptake had in part changed (Figs. 4b and 5b). Twenty-four hours after the S^{35} injection most of the activity was diffusely distributed within cell columns and matrix. At the same time the ring-pattern mentioned above was absent and the cells seemed not, as is normal, to have freed their centres from activity

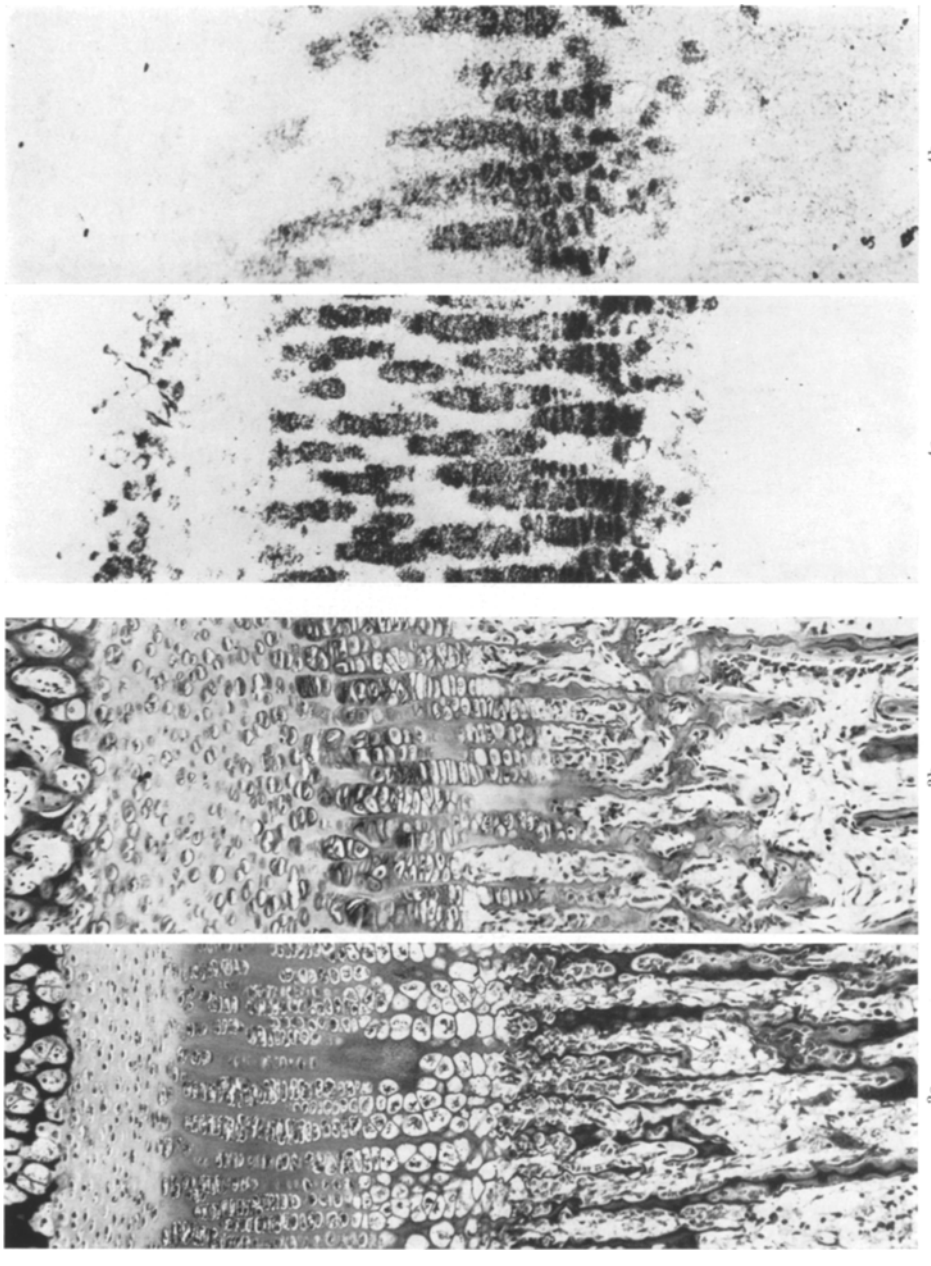
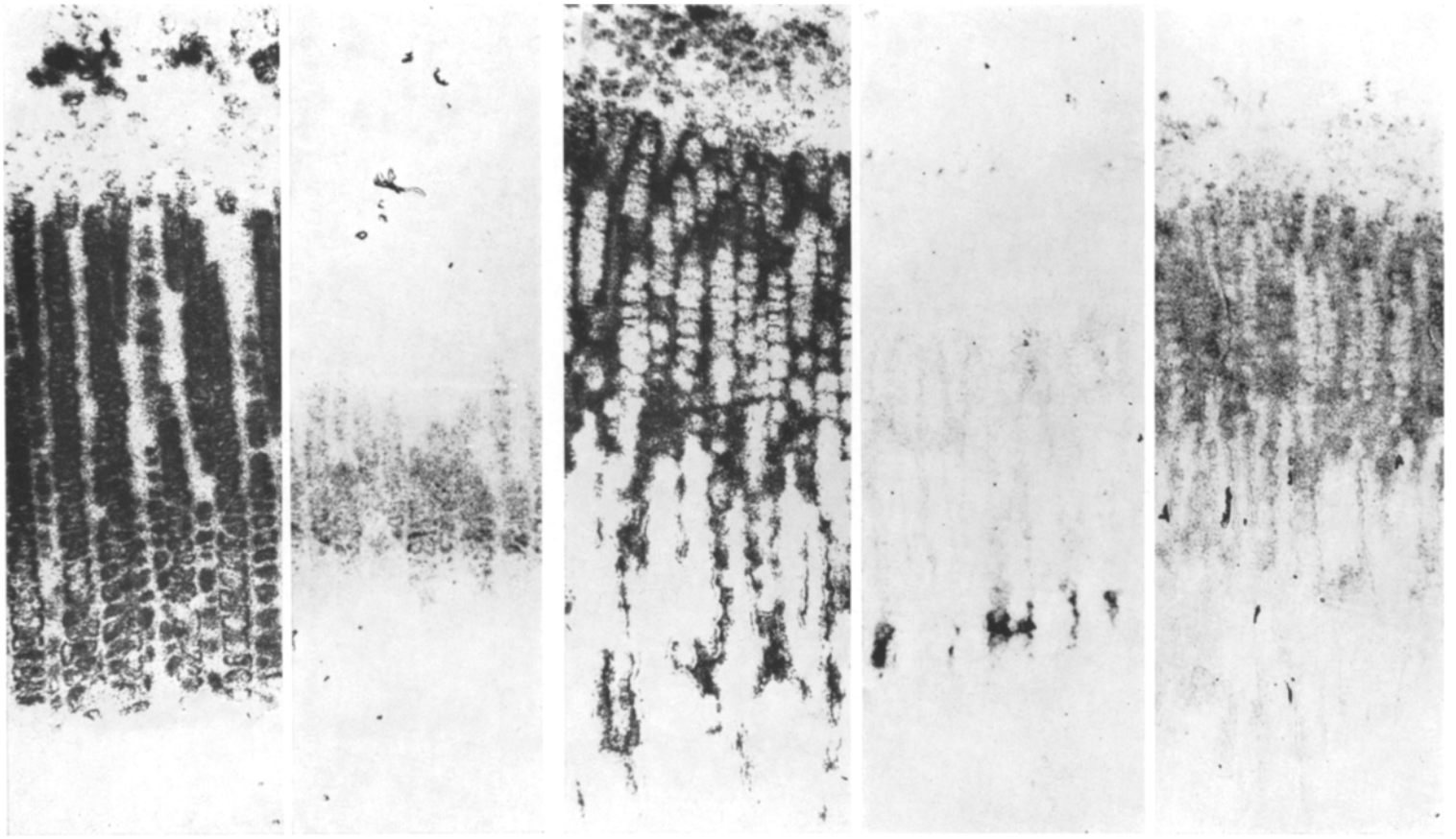


Fig. 3a u. b. Distal radius growth zone. Haematoxylin-eosin. 144 ×. a Treated with cortisone for 2 days and given papain 6 hours before death. Loss of basophilic stainability in the cartilage matrix and severe damage of cartilage cells. Same changes as in animals treated only with papain. b Treated with cortisone for 5 days and given papain 5 days before death. Narrow and irregular cartilage zone with large and active hypertrophic cells and acidophilic cartilage matrix. In the metaphysis there are coarse and short irregular bone trabeculae including acidophilic matrix rests

Fig. 4a u. b. Distal radius growth zone. Autoradiogram. Unstained. 144 ×. 2 hours after injection of S^{35}. a Untreated. Normal autoradiogram. See text. b Treated with cortisone for 4 days. Diminished uptake of S^{35}. Irregular cell columns. Many cells have not taken up S^{35}. Even the cartilage matrix is labelled



5a 5b 6a 6b 6c

Fig. 5a u. b. Distal radius growth zone. Autoradiogram. Unstained. 144 ×. 24 hours after injection of S^{35}. a Untreated. Normal autoradiogram. See text. b Treated with cortisone for 8 days. Narrow cartilage zone. Diminished uptake of S^{35} and unnormally diffuse labelling. See text

Fig. 6a—c. Distal radius growth zone. Autoradiogram. Unstained. 144 ×. 5 days after injection of S^{35}. a Untreated. Normal autoradiogram. See text. b Treated with cortisone for 4 days. Weak uptake of sulphate in the cartilage zone, and in the bone trabeculae. Distal in the metaphysis a transverse band of strong labelled matrix rests is seen. c Treated with cortisone for 8 days. Weak and diffuse labelling of the cartilage zone. The bone trabeculae labelled with S^{35} are very short. No band of strong labelled matrix rests is seen in the metaphysis as in b. See text

(Fig. 5b). In the experiments in which the rabbits had been given S^{35} five days prior to sacrifice, the height of bone trabeculae containing blackened matrix was appreciably less than is normal, and after 15 days' cortisone administration there was no uptake in the metaphysis (Fig. 6b, a, d, c). The animals given cortisone for 4 days and the radioactive sulphate 5 days before death exhibited a band of greatly blackened matrix farthest distally in the metaphysis (Fig. 6b); this contrasted against the extremely weak uptake of the cartilage zone and other parts

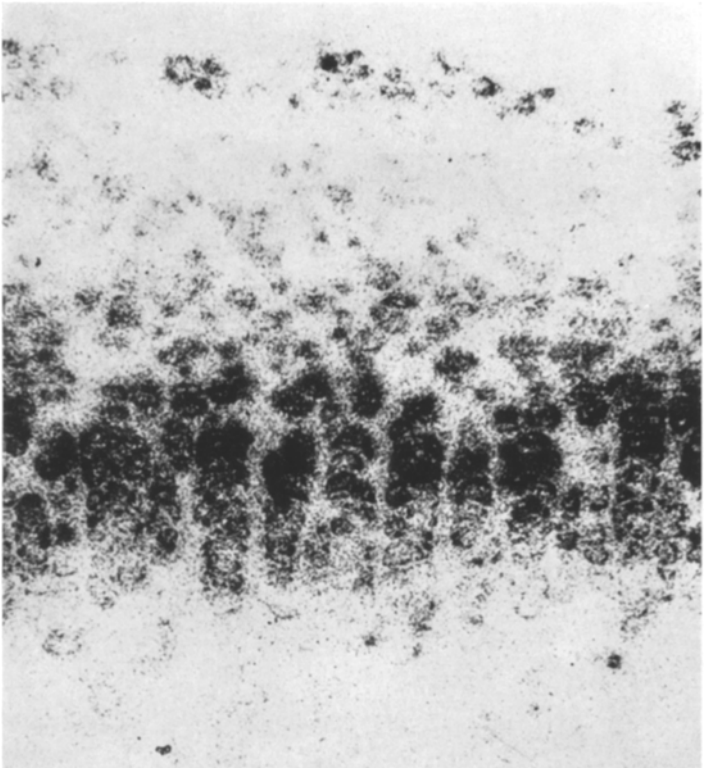


Fig. 7. Distal radius growth zone. Autoradiogram. Unstained. 250 ×. 2 hours after injection of S^{35}. Treated with cortisone for 5 days, and given papain 5 days before death. Narrow and irregular cartilage zone. Diffuse labelling with very strong labelled proliferative and hypertrophic cartilage cells. See Fig. 3b and text

of the metaphysis. If sulphate was injected five days after the start of cortisone-treatment and the animals killed five days after sulphate injection this band of blackened matrix distally in the metaphysis was not observed (Fig. 6c).

Group 2. *Vitamin A.* The autoradiographic examination revealed no deviations of note from the normal picture. Animals given S^{35} five days before death and treated with vitamin A for ten days had bone trabeculae in which the height of the blackened region of the matrix was appreciably less than is normal.

Group 3. *Cortisone plus vitamin A.* The autoradiographic findings in this group corresponded on the whole with those in Group 1. Blackening was appreciably weaker than normal in all specimens. The non-absorbed cartilage cells in the metaphysis had not taken up any activity.

Group 4. *Cortisone plus papain.* In specimens from animals given papain 6 or 48 hours before death and cortisone for two days, the autoradiographic appearances corresponded with those described earlier in rabbits treated solely with papain (ENGFELDT and WESTERBORN 1960b): six hours after the papain injection there was diffuse blackening of the cartilage zone even in specimens from animals which had received the radioactive sulphate only two hours before death, and the proliferative cells in every instance appeared in almost normal measure to take up activity 48 hours after the papain injection. In specimens from animals given papain five days prior to sacrifice and cortisone throughout that period, the cells present in the proliferative zone were seen to function; two hours after the sulphate injection these cells showed an uptake which was strong although somewhat more diffuse than usual (Fig. 7). The hypertrophic cells, when still present in the cartilage, were not seen to show activity.

Discussion

The *effect of cortisone* upon epiphysial cartilage described earlier, consisting in narrowing of the epiphysial cartilage zone resulting chiefly from a reduction in the number of hypertrophic cells, and diminished growth in the long axis, was confirmed in the present experiments. We found this in part to be due to the cartilage cells being damaged by the cortisone therapy. Another feature characteristic of the damage to the epiphysial cartilage is that the absorption of the hypertrophic cells is not as effective as normal, but that a number of cells are seen down in the metaphysis. This may be attributable to an impairment in the absorptive faculty of the metaphysial capillaries, or perhaps to the cell damage mentioned above. The experiments also showed that the osteoblasts in the metaphysis are fewer than usual and often damaged. This, in its turn, may account for the bone trabeculae nearest to the cartilage zone consisting chiefly in calcified cartilage matrix with an extremely thin bony seam or none at all.

In the *cortisone plus vitamin A series,* there were no noteworthy changes over and above those observed to follow cortisone administration alone. This seems somewhat remarkable in view of the severe changes described by THOMAS and his associates (1960) in experiments with vitamin A alone. In our control experiments with the vitamin alone, the changes in the epiphysial cartilage were relatively slight despite the fact that we used the same dosage as the American investigators; we were, then, not able to verify their results in our experiments. However, a point which may or may not be of significance is that in our study we used younger and smaller experimental animals. (Growth may have been quicker in our rabbits.)

The *combination of cortisone and papain* led to severe histologic changes which, 6 and 48 hours respectively after the papain injection, corresponded with those described following the administration of papain alone. If continued, the cortisone administration appears to prevent the restitution of the cartilage which occurs after treatment with papain only. It was of especial interest to note that the numbers of proliferative and hypertrophic cells in the animals treated with cortisone were considerably reduced as compared with animals given papain alone. However, the cells which were present seemed to regain their usual faculty of producing basophilic matrix (Fig. 7).

Autoradiographic examination revealed appreciable deviations from normal. The uptake of radioactive sulphate was considerably reduced during cortisone administration, which agrees with the supposed retarding effect of cortisone upon the synthesis of chondroitin sulphate. The cellular damage mentioned in the foregoing is exemplified by the lost ability of the proliferative and hypertrophic chondrocytes in the cartilage columns of normal, distinct, intracellular uptake. Twenty-four hours after the S^{35} injection, the chondrocytes should normally show activity, so that a ring-pattern is seen, while at the same time the radioactivity invades the matrix nearest to the cells. In the cortisone experiments this ring-pattern failed to materialize, and the diffuse blackening seen over the matrix and cells in our view confirmed the cellular damage. The experiments also verified the definite reduction in growth during cortisone administration, by measurement of the length of the S^{35}-marked bone trabeculae. In animals sacrificed five days after the radioactive sulphate injection, which had received cortisone only during those five days, the sulphate taken up by the cartilage before the cortisone had had time to act was seen to produce heavy probably normal blackening furthest distally in the bone trabeculae, while otherwise the marking was weaker and more diffuse than is normal. In animals treated more than 5 days with cortisone this zone of heavy blackening was absent (Fig. 6c).

The papain experiments showed the small number of proliferative cells in the severely damaged cartilage still to function normally with a heavy sulphate uptake despite current cortisone administration. Nor did the cortisone appear to impede the strongly increased function described in the proliferative cells restituted or newly formed on the second to fourth day after the injection of papain in animals treated with that agent alone.

Summary

Cortisone leads to damage of the epiphysial cartilage cells of young rabbits, and also of the osteoblasts in the metaphysis, which brings about a change in the appearance of the bone trabeculae in the metaphysis; growth and the faculty of the cartilage cells to produce chondroitin sulphate is reduced, as demonstrated by autoradiography. Combined treatment with vitamin A and cortisone leads to the same changes as cortisone alone. In combination with papain cortisone, during the first few days at any event, does not influence the effect of papain on the cartilage. Prolonged cortisone administration impedes the restitution of the papain-induced changes; the cartilage cells however regain their faculty of chondroitinsulphate synthesis despite the administration of cortisone as shown by autoradiography.

Die Wirkung von Cortison auf den Epiphysenknorpel. Histologische und autoradiographische Untersuchungen.

Zusammenfassung

Cortison führt bei jungen Kaninchen zu einer Schädigung der Zellen des Epiphysenknorpels und der Osteoblasten in der Metaphyse, so daß die knöchernen Trabekel in der Metaphyse ein verändertes Aussehen zeigen; das Wachstum ist deutlich vermindert, ebenso die Fähigkeit der Knorpelzellen, Chondroitinsulfat

zu bilden, wie man autoradiographisch nachweisen kann. Kombinierte Behandlung der Tiere mit Vitamin A und Cortison führt zu denselben Veränderungen wie Cortison allein. Mit Papain kombiniert, beeinflußt Cortison wenigstens während der ersten Tage nicht die Wirkung des Papains auf den Knorpel; längere Cortisonzufuhr hindert die Wiederherstellung nach den durch Papain verursachten Veränderungen. Wie die Autoradiographie zeigt, gewinnen jedoch die Knorpelzellen trotz der Cortisongaben ihre Fähigkeit zurück, Chondroitinsulfat zu synthetisieren.

References

Boström, H., and E. Odeblad: The influence of cortisone upon the sulphate exchange of chondroitinsulphuric acid. Arkiv kemi (Stockh.) **6**, 39 (1953).

Engfeldt, B., and O. Westerborn: An autoradiographic study of the epiphyseal cartilage in normal rabbits after administration of radiosulphate. Acta path. microbiol. scand. **49**, 73 (1960a).

— — An autoradiographic study of the epiphyseal cartilage in papaintreated rabbits after administration of radiosulphate. Acta path. microbiol. scand. **49**, 55 (1960b).

Follis jr., R. H.: Effect of cortisone on growing bone of the rat. Proc. Soc. exp. Biol. (N.Y.) **76**, 722 (1951).

Hulth, A.: Retardation of the endochondral growth by papain. Acta orthop. scand. **28**, 1 (1958).

Layton, L. L.: Cortisone inhibition of mucopolysaccharide synthesis in the intact rat. Arch. Biochem. **32**, 224 (1951).

McCluskey, R. T., and L. Thomas: The removal of cartilage in vivo by papain. Amer. J. Path. **35**, 819 (1959).

Sissons, H. A., and G. J. Hadfield: The influence of cortisone on the structure and growth of bone. J. Anat. (Lond.) **89**, 69 (1955).

Thomas, L.: Reversible collapse of rabbits ears after intravenous papain and prevention of recovery by cortisone. J. exp. Med. **104**, 245 (1956).

— R. T. McCluskey, J. L. Potter and G. Weissman: Comparison of the effects of papain and vitamin A on cartilage. J. exp. Med. **111**, 705 (1960).

Westerborn, O.: The effect of papain on epiphyseal cartilage. Acta chir. scand., Suppl. **270**, 1 (1961).

Dr. A. Hulth,
Orthopädische Abteilung, Akademiska Sjukhuset, Uppsala (Sweden)