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EARLY CHANGES OF THE GROWTH ZONE IN RABBIT FOLLOWING ROENTGEN IRRADIATION

By

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It has long been known that local roentgen irradiation in sufficiently large doses in growing animals causes an arrest of growth and bony closure of the epiphyseal plate, resulting in various deformities. Numerous publications have been devoted to this phenomenon, the earliest dating as far back as 1903. Histologic changes of the epiphyseal plate have been demonstrated 2 days after irradiation in a dose of 400 r (Bisgaard and Hunt, 1936). In rats retardation of growth has been produced by doses over 650 r, whereas complete arrest of growth was achieved if the dose exceeded 1335 r (Barr et al., 1943). Careful histologic studies by Hinkel (1943) have provided evidence of changes in the epiphyseal plate and the adjoining cancellous bone in animals examined at the earliest 2 days after irradiation. As far as can be ascertained, no attempts have as yet been made to determine whether changes occur prior to this time.

60 hours after irradiation Hinkel observed changes in the cartilage cells, which appeared swollen, stained differently, and showed a changed nuclear structure. The height of the cartilage columns increased and after a few more days the individual columns contained an increased number of cells. One of the alterations observed in the zone of calcification was a change and partial disappearance of the osteoblasts. They were found freely in the lumen, whereas the bony trabeculae adjoining the epiphyseal cartilage were not covered by osteoblasts. The capillaries, which in addition to other methods were studied by injection of Indian ink, likewise appeared damaged. Hinkel considered his observations to constitute evidence of primary damage to cartilage cells, osteoblasts, and capillaries. As one of the secondary phenomena

following these injuries he regarded the decreased disintegration of cartilage cells in the zone of calcification. This implied that the cartilage cells which were not destroyed, enclosed in calcified matrix might be shifted down to the metaphysis and could after even a short time be observed in the compact bone. Hinkel has also studied the changes apparent at a later stage after irradiation. Detailed information on these observations can be found in his original publication.

Stimulated both by the results of our own earlier studies regarding the effect of papain on epiphyseal cartilage, and by the rapid development of epiphyseal research as a result of the current interest in osteolathyrism, our attention was directed towards other methods of producing damage to the epiphyseal cartilage, such as roentgen irradiation. As earlier authors seem to have disregarded the changes which occur within 2 days of roentgen irradiation of the epiphyseal and metaphyseal areas, we felt justified in devoting a detailed study to these early changes. In addition to histologic examination, our study included a roentgenologic and microradiographic examination.

MATERIAL AND METHODS

The animals selected for the experiments were young white rabbits, weighing from 200 to 400 g. During the roentgen irradiation the rabbits were placed in a lead cylinder with 3 mm thick walls. The right lower foreleg was drawn through a hole in this cylinder and fixed to the outside with tape, after which the hole was covered with lead to prevent any radiation from penetrating into the cylinder. Radiation was administered through a 4 AL filter at 220 kv and at a focus-skin distance of 40 cm. The doses varied from 200 r to 2000 r. The animals were killed by air embolism from 6 hours to 10 days after irradiation. Immediately after death, both forelegs were removed and roentgenographed side by side. As a next step, the radius and ulna from both sides were fixed in 10 % neutral formalin. The radius was then divided lengthwise by means of a circular saw and one of the halves embedded in methylmetacrylate. The other half of the radius and the ulna, following decalcification in formic acid and natrium citrate, were embedded in paraffin and cut into sections of 5 μ thickness. Staining was done with haematoxylin-eosin. The plastic-embedded halves of radius were ground down to various thicknesses, ranging from 800 to 100 μ. A number of specimens were not embedded but immediately cut down with a dental saw into 800–1000 μ thick slices.

Fig. 1a–d.
Radiographs. Left(L) and right(R) forearm. Roentgen irradiation over right forearm.

a. 48 hours after irradiation. (300 r).
On the right side there is a transverse zone with a lower degree of mineralization in the metaphysis close to the epiphyseal cartilage. The change is seen in both radius and ulna.

b. 48 hours after irradiation (1500 r).
Same finding as in a.

c. 96 hours after irradiation (1250 r).
The transverse zone of low mineralization is shifted further down the metaphysis.

d. 10 days after irradiation (1000 r).
No zone of low mineralization is demonstrable. The dense area of the metaphysis close to the epiphyseal cartilage is wider on the right side and has a higher degree of density than on the left side.

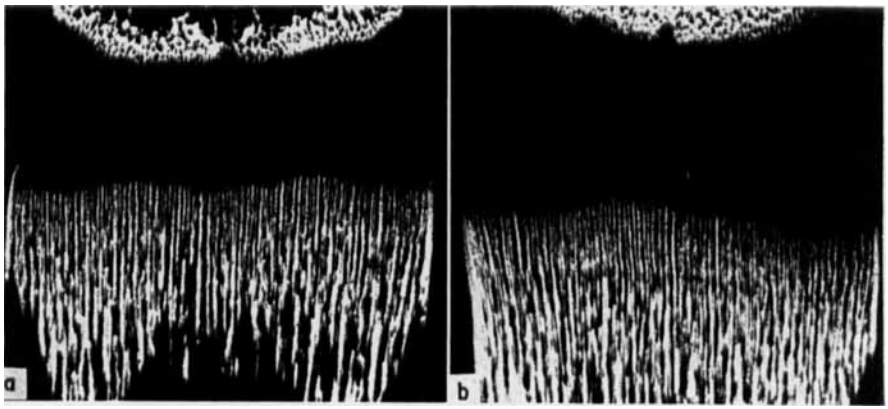

Fig. 2a–b.
Microradiographs. Distal radial epiphyseal cartilage zone.

a. Left side. Untreated. The picture shows normal mineralization.

b. Right side. 48 hours after irradiation (1000 r).
A transverse zone of lower mineralization is demonstrated. The bone spiculae are in this zone broken or thinner than normal.

The ground sections were used for a study of the distribution of mineral salts. The radiation source was a Philips diffraction unit with Cu anode. The specimens were placed directly in contact with extremely fine-grained photographic emulsion. Exposure was made by roentgen rays generated at 24 kv. The pictures obtained in this way were enlarged by photomicrography.

RESULTS

Roentgen examination.

In all cases both forelegs were examined by roentgen immediately after death. Neither 6 hours nor 24 hours after irradiation can any certain changes be detected in the roentgenogram. On examination 48 hours after irradiation, a typical change can be observed in the distal ends of the radius and ulna in all animals exposed to doses of at least 300 r. This change appears as a narrow zone of decreased density across the dense metaphyseal area close to the epiphyseal cartilage, representing a change in the mineralization of the primary cancellous bone (Figs. 1a and b). If the doses are increased to 1500–2000 r, this clear zone becomes wider and less sharply delimitated (Fig 1b). The same change can be observed 96 hours after irradiation, but the zone is then somewhat wider and has shifted further down the metaphysis (Fig. 1c).

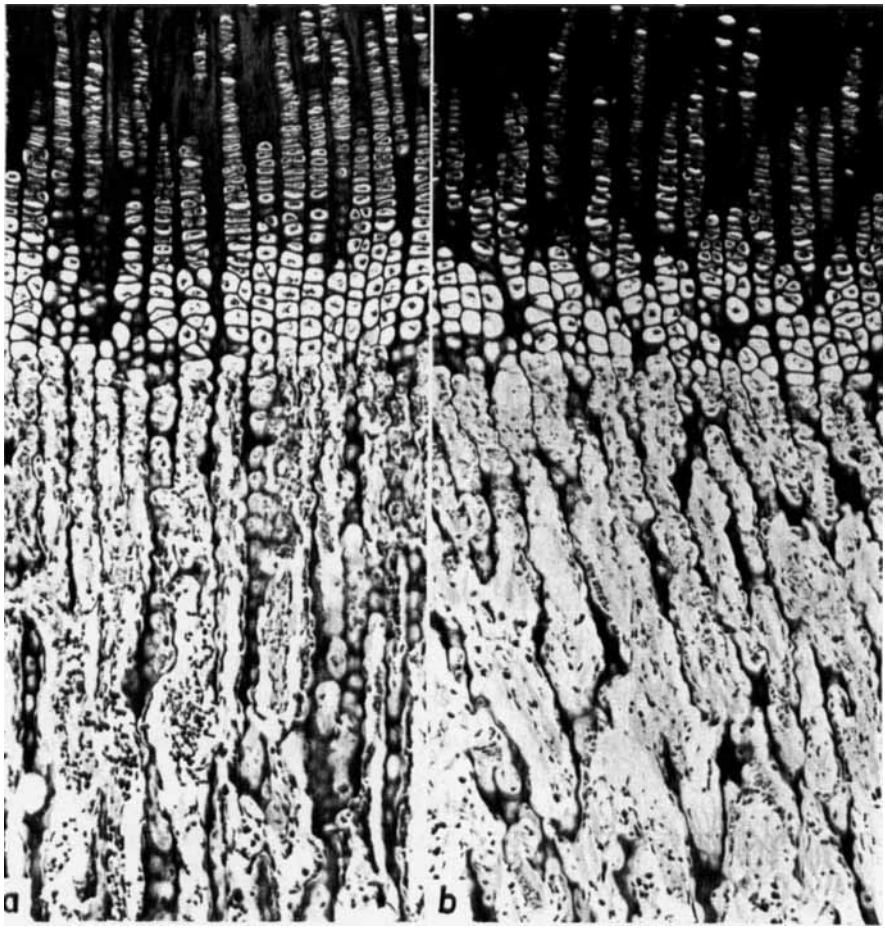

Fig. 3a–b.
Distal radial epiphyseal cartilage zone. Haematoxylin-eosin. 115 ×.

a. Left side. Untreated. Normal histologic picture.

b. Right side. 6 hours after irradiation (1000 r).
There are no changes in the epiphyseal cartilage. In the primary spongiosa there is a very low number of osteoblasts.

If more time is allowed to pass after irradiation, the clearer zone disappears and instead the metaphyseal area of the exposed limb is found to have a higher degree of density over a larger area in comparison with the unexposed side (Fig. 1d).

Fig. 4a–b.
Distal radial metaphysis close to the epiphyseal cartilage.
Haematoxylin-eosin. abt. 1000 ×.

a. Left side. Untreated. Normal osteoblasts situated close to the bone spiculae.

b. Right side. 6 hours after irradiation (1000 r).
The osteoblasts are not normal. The structure of the nucleus is more diffuse and there is a higher granulation of the cytoplasm. The osteoblasts are not in the normal way situated close to the bone spiculae.

Microradiographic examination.

For a more detailed investigation into the nature of the clearer zone described above, a study has been made of microradiographed sections.

The zone of decreased density can be identified in the microradiogram provided the sections used are not too thin. If the specimens are ground down to 100 μ, no certain changes can be detected in the microradiograms, whereas they are on the other hand quite unmistakable in specimens of 250–800 μ thickness.

The change is here, as in the roentgenograms, apparent as a fairly sharply delimitated zone of decreased density straight across the bony trabeculae (Figs. 2a and b). The individual bony trabeculae within the area affected by the change appear to be interrupted or have become hour-glass shaped.

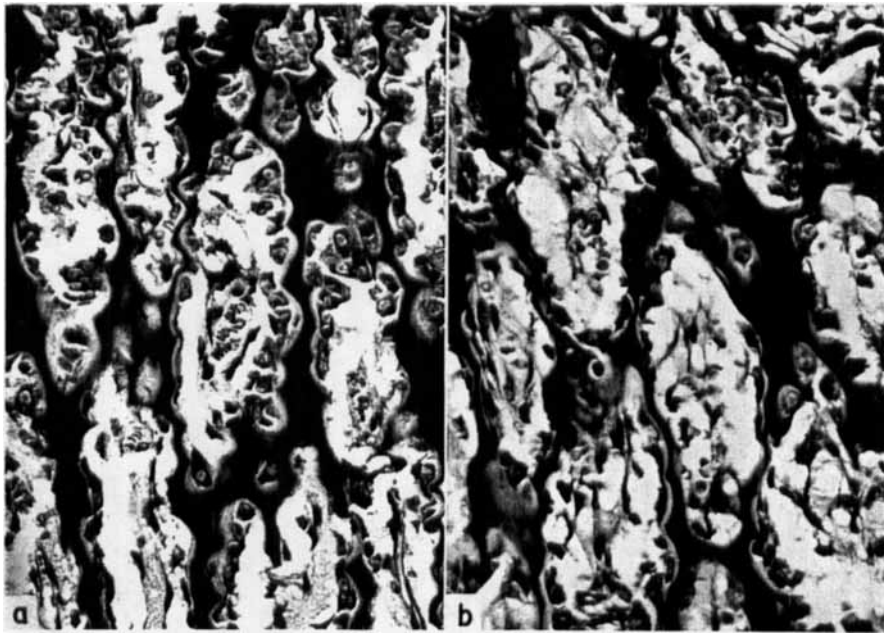

Fig. 5a–b.
Distal radial metphysis close to the epiphyseal cartilage. Haematoxylin-eosin. abt. 190 ×.

a. Left side. Untreated. Normal histologic picture.

b. Right side. 24 hours after irradiation (1000 r).
The number of osteoblasts is lower than on the untreated side. The nuclei of the osteoblasts are mostly pycnotic.

Histologic examination.

The histologic examination has primarily been concentrated upon the changes that can be observed within 48 hours of irradiation. In addition, in order to have a basis of comparison with earlier investigations, the changes apparent 10 days after exposure are reported.

The results presented below are based on examination of two litters, one of which was given doses of 500 r, the other of 1000 r.

6 hours after irradiation.

No changes are apparent in either the cells or the matrix of the epiphyseal cartilage. Marked changes can be observed in the part of the primary cancellous bone immediately adjoining the epiphyseal car-

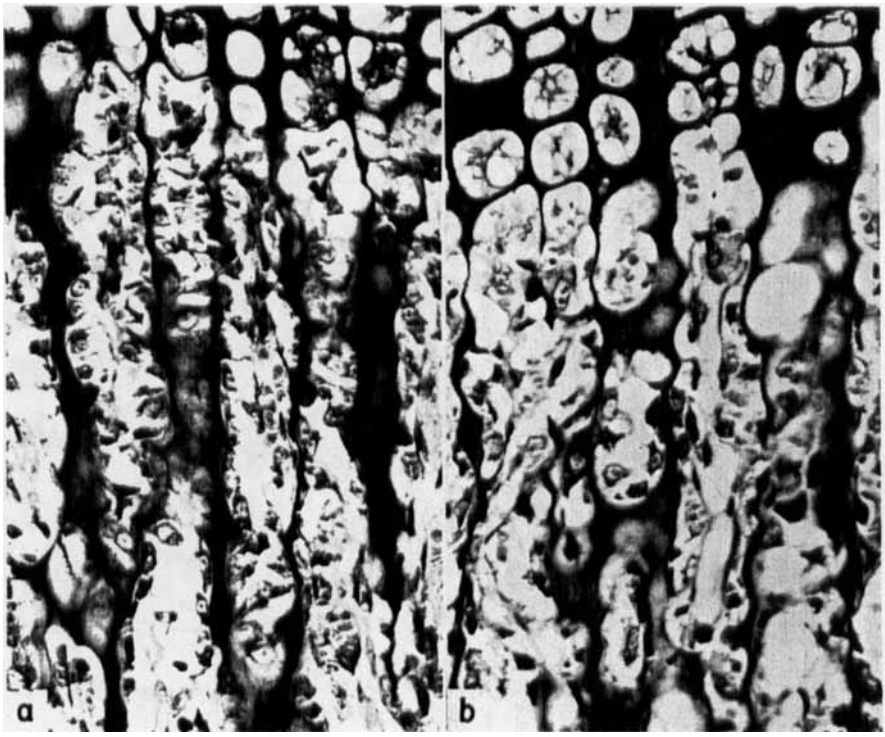

Fig. 6a–b.
Distal radial epiphyseal-metaphyseal zone. Haematoxylin-eosin. abt. 200 ×.

a. Left side. Untreated. Normal number of osteoblasts and other cells. The bone spiculae are without breaks.

b. Right side 48 hours after irradiation. The number of osteoblasts and other cells is diminished. The osteoblasts seem to be dead and are situated free in the cavities. The bone spiculae show several breaks.

tilage. On the exposed side the number of osteoblasts is considerably reduced. The same observation applies to the other cells found in the marrow cavities of the cancellous bone (Figs. 3a and b). The remaining osteoblasts appear damaged. Their nuclear structure is less regular than normal and their cytoplasm shows an increased granulation (Figs. 4a and b). The osteoblasts are furthermore largely found detached from the bony trabeculae, which normally does not occur. On the damaged side they are found freely scattered in the lumen, which in addition contains detrital matter. It would seem that the changes in the osteoblasts decrease with increased distance to the epiphyseal cartilage.

The illustrations presented here (Figs. 3 and 4) refer to animals exposed to radiation of 1000 r. The animals irradiated with 500 r show similar and equally severe changes.

24 hours after irradiation.

At this stage the changes resemble those described in the foregoing, but they have to some extent increased in severity. There are still no certain changes to be detected in the cartilage cells. The cavities of the cancellous bone on the exposed side contain a considerably lower number of cells and capillaries. The red blood corpuscles appear to lie free instead of being enclosed by capillary endothelium. The changes in the osteoblasts are clearly visible in Fig. 5b on comparison with Fig. 5a. On the exposed side (Fig. 5b) these cells are smaller and shrunken. To some extent they occur freely in the cavities of the cancellous bone where they lie apparently dead. The primary trabeculae in the metaphysis, furthermore, appear narrower than on the unexposed side, mainly due to the fact that no normal bone deposit has taken place. This appears clearly from Figs. 5a and 5b; in Fig. 5a a relatively wide acidophilic bone seam can be seen on both sides of calcified cartilage matrix. In several places on the exposed side breaks appear in the trabeculae, in far larger number than is ever seen in normal specimens.

48 hours after irradiation.

There are still no certain changes to be detected in the cartilage tissue. There remains an unmistakable and large insufficiency of cells in the marrow cavities. The cytoplasm of the osteoblasts is shrunken, while their nuclei are more or less pycnotic. The cells lie largely detached from the trabeculae. Hematopoietic cells are lacking entirely and it is difficult to find any capillaries on the exposed side (Figs. 6a and b).

10 days after irradiation.

At this stage the epiphyseal cartilage is seen to have undergone a marked change (Fig. 7a). The proliferating cartilage cells, which normally are arranged in regular columns, are now concentrated in dense, irregular clusters, separated by disintegrated matrix. Even the hypertrophic cells down in the zone of calcification have collected in clusters. The epiphyseal-metaphyseal border, which normally is

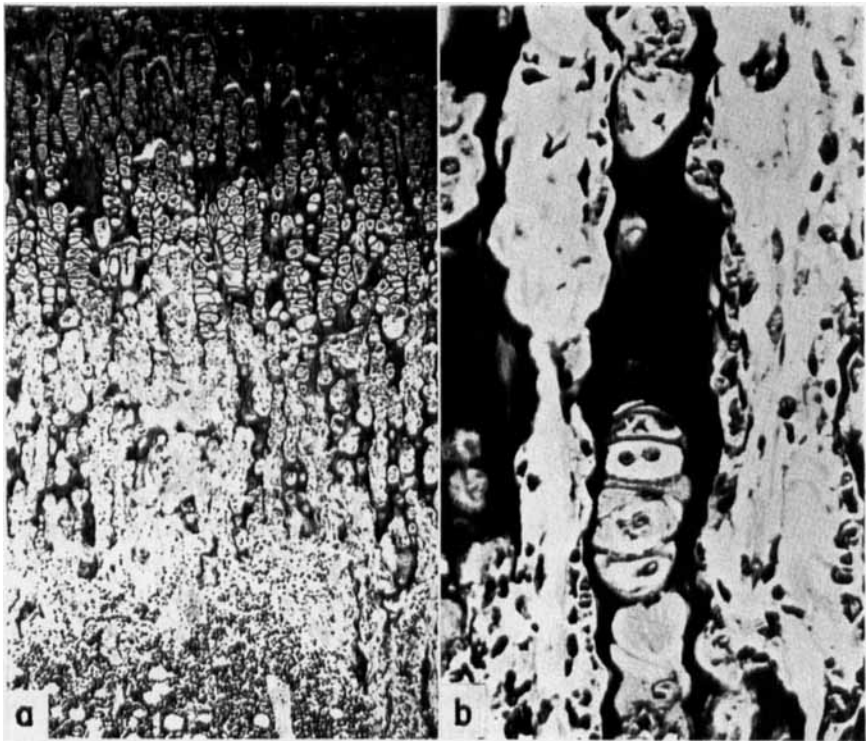

Fig. 7a–b.
Distal radial epiphyseal-metaphyseal zone. Haematoxylin-eosin.
Right side. 10 days after irradiation (1000 r).

a. abt. 75 ×. The epiphyseal cartilage is severely damaged. There is irregular organization of the cartilage with the cells clustered and with defibrillated matrix. The marrow cells have increased in number.

b. abt. 225 ×. Shifted down in the metaphysis there are cartilage cells which are not disintegrated in the normal process of the endochondral growth.

straight and even, is now extremely irregular. The stainability of the epiphyseal cartilage appears to be unchanged.

In the zone of primary cancellous bone (Figs. 7a and b) the normally regular, straight and close-ranked bone spiculae are lacking. The trabeculae are more sparsely distributed, shorter than normal, and irregularly shaped, sometimes spirally distorted. The number of osteoblasts and other cells remains considerably reduced in the zone of primary cancellous bone. The hematopoietic cells in the free marrow cavity appear to have increased in number again.

It is particularly interesting to observe surviving cartilage cells within the trabeculae in various places fairly far down in the metaphysis; these cells have not been destroyed as usual during the process of calcification (Fig. 7b).

DISCUSSION

Owing to a large number of both histologic and roentgenologic publications on the subject, the detrimental effect of roentgen irradiation on epiphyseal cartilage has long been known. Earlier studies have been concerned with the changes apparent in the growth zone at a fairly late stage after exposure and, in addition, with the retardation or complete arrest of endochondral growth produced by irradiation.

In the present investigation we have been able to demonstrate that from 2 to 4 days after irradiation of the foreleg in rabbits in doses varying from 300 r to 2000 r, roentgenologic evidence can be found of changes in the mineralization of the part of the ulnar and radial metaphysis adjoining the epiphyseal cartilage. In order to find an explanation of this phenomenon, the exposed limbs were subjected to a microradiographic and histologic examination. A transverse zone of decreased density visible in the roentgenograms was in the microradiograms represented as an interruption of hour-glosslike narrowing of the individual bony trabeculae. The histologic examination provided as early as 6 hours after exposure evidence of primary damage to the osteoblasts in the area adjoining the epiphyseal cartilage. The number of osteoblasts was reduced and to some extent they had become detached from the bony trabeculae and were found free in the lumen. Twenty-four hours after irradiation the osteoblasts appeared for the greater part necrotic. In addition, a change in the capillaries in the marrow cavities of the cancellous bone could be demonstrated. The normal deposit of osteoblasts on the bone spiculae did in many places not take place, which meant that the bony trabeculae were narrower than normal and here and there showed breaks. The epiphyseal cartilage itself did not appear to have undergone any major changes during the first 48 hours after exposure, but 10 days after irradiation it showed severe alterations. From the fact that fairly far down in the metaphysis surviving cartilage cells were found enclosed in the bony trabeculae it appeared that growth to some extent had continued during these 10 days.

Our investigations indicate that the effect of the irradiation is con-

centrated upon the osteoblasts immediately adjoining the epiphyseal cartilage, and possibly simultaneously affects the capillaries present in this area. The osteoblasts at a further distance from the epiphyseal cartilage appear less damaged and are probably less sensitive to the radiation. This may be connected with the fact that the osteoblasts closest to the cartilage are most active. The altered mineralization can probably be explained as a secondary effect following upon the damage to the osteobasts. A secondary phenomenon to the capillary damage described above is furthermore undoubtedly the failure of certain cartilage cells in the zone of calcification to disintegrate as usual; instead the intact cells are shifted down with the cartilage matrix to the metaphysis in the course of the growth process. It seems that the damage to the epiphyseal cartilage itself does not materialize until from 48 hours to 10 days after irradiation, which probably explains the continued growth during the course of the experiment. This agrees with, for instance, Regen's and Wilkins' (1936) findings from roentgen absorption measurements of exposed growing skeletal structures. They demonstrated that following irradiation in a dose of 2000 r the exposed animals stopped growing at some time within one month after the treatment and that irradiation, even if given in such large doses, did not produce an immediate effect.

Our results show that the effect of roentgen irradiation on the epiphyseal cartilage is of an entirely different character to that produced for instance by injection of papain, or that seen in osteolathyrism. Roentgen irradiation produces primary damage to the osteoblasts, whereas both following injection of papain and in osteolathyrism the primary changes affect the epiphyseal cartilage.

On the whole the present findings showed good agreement with those reported by Hinkel (1943), among others whereas the changes 6 and 24 hours after irradiation merely differed in intensity from those observed after 48 hours.

SUMMARY

The early changes produced by roentgen irradiation of the distal radial and ulnar epiphyses in young rabbits are described. Following radiation in doses of at least 300 r, the roentgenogram shows a zone of decreased density straight across the metaphysis adjoining the epiphyseal cartilage. This clear zone can in microradiograms be identified as an interruption or narrowing of the bony trabeculae. The histologic

findings indicate that the damage primarily affects the osteoblasts and capillaries in the portion of the metaphysis which adjoins the epiphyseal cartilage. As a result of this, bone deposit on the calcified intercartilaginous septa fails to take place everywhere. No changes can be demonstrated in the epiphyseal cartilage within the first 48 hours following irradiation, but severe changes are evident after 10 days. During this period a clearly perceptible growth of the bone occurs.

RESUME

Description des modifications précoces produites par l'irradiation roentgen sur l'épiphyse distale radiare et ulnaire chez les jeunes lapins. A la suite de radiations à doses d'au moins 300 r, le roentgenogramme montre une zone de densité diminuée juste au travers de la métaphyse adjacente au cartilage épiphysaire. Cette zone claire peut être identifiée sur le microradiogramme comme une interruption ou un rétrécissement des trabécules osseux. Les trouvailles histologiques indiquent que le dommage primaire atteint les ostéoblastes et les capillaires dans la portion de la métaphyse qui est contiguë au cartilage épiphysaire. Il en résulte que le dépôt osseux sur la paroi intercartilagineuse calcifiée ne se fait pas régulièrement partout. On ne peut constater aucune modification du cartilage épiphysaire pendant les 48 heures qui suivent l'irradiation, mais des modifications graves apparaissent de toute évidence après 10 jours. Durant cette période il se produit une croissance nettement perceptible de l'os.

ZUSAMMENFASSUNG

Die durch Röntgenbestrahlung hervorgerufenen frühzeitigen Veränderungen der distalen Radius- und Ulnaepiphysen junger Kaninchen werden beschrieben. Nach Bestrahlungsdosen von zumindest 300 R, zeigen Röntgenaufnahmen eine Zone herabgesetzter Dichtigkeit quer über die dem Epiphysenknorpel benachbarte Metaphyse. Diese klare Zone kann im Mikroröntgenbilde als eine Unterbrechung oder Verschmälerung der Knochentrabekel erkannt werden. Die histologischen Befunde deuten darauf hin, dass in erster Reihe die Osteoblasten und Kapillaren in dem Epiphysenknorpel benachbarten Teile der Metaphyse geschädigt werden. Das Resultat davon ist ein Versagen der Knochenablagerung in die verkalkten intercartilaginären Septa auf der ganzen Linie. Veränderungen am Epiphysenknorpel innerhalb der er-

sten 48 Stunden nach der Bestrahlung können nicht nachgewiesen werden, aber schwere Veränderungen sind nach 10 Tagen sichtbar. Während dieses Zeitraumes geht ein deutlich wahrnehmbares Wachstum des Knochens vor sich.

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ACKNOWLEDGEMENT

We are greatly indebted to Bengt Nohrman, M.D., Head of the Department of Radiotherapy, University of Uppsala, for his valuable help with the roentgen irradiation and in revising this manuscript.