FROM THE SURGICAL DEPT. OF THE CENTRAL HOSPITAL OF BORAS. HEAD: W. MÖLLER, M.D.

ON THE INFLUENCE OF THE BONE SALTS ON THE RADIOGRAPH OF SO-CALLED ACUTE ATROPHY OF BONE

(Roentgen-experimental and histological examinations as well as a new alizarin staining technique).

BY

GUSTAF-ADOLF LANDOFF

The radiographic characteristics of what is generally called "atrophy of bone"[1] caught the interest of *Sudeck,* who made the picture more widely known.

He was the first to describe this picture exhaustively and he tried to interpret the underlying physiopathological processes of the disease. *Sudeck*'s classical verbal portrait of acute atrophy of bone still holds good. In the acute stage of the disease the radiograph is characterized by a cloudy and patchy appearance of the bone and by small nest-like rarefactions. When the process reaches a more or less chronic stage, the picture is characterized by numerically reduced, rarefied trabeculae and a cortex of diminished thickness.

As far as the underlying physiopathological processes are concerned, old conceptions have of late years been abandoned and replaced by a newer idea, according to which the process is considered to be the expression of a rapid remodelling of bone, which process in the acute stages does not differ in principle from that of normal bone (*Rieder* 1933, *Sudeck* 1938, etc.). This

[1] As the changes referred to in this paper are generally known under the name of "acute atrophy of bone", this designation has been retained, although on account of the nature of the processes, it is not an adequate term.

means that there is general accordance in opinion on the probably correct pathogenetic and physiopathologic causes of the changes. No general or acceptable explanation has however yet been produced as to how the anatomopathological changes give the radiograph its typical appearance, especially its cloudiness and patchiness. This problem was first dealt with by *Kienböck* (1901), who believed the above characteristics to be due to a rapid lacunar resorption of bone and especially of lime salts deposited in the bone meshes.

Friedl and *Schinz* assail this explanation on the grounds that the concentration of bone salts in the soft parts is insufficient to produce such a picture, and give instead thereof, a hypothesis according to which they ascribe to the bone a certain Bucky-effect, whereby the trabeculae serve as a sort of a secondary screen. They go on to contend that as the bone trabeculae are either completely destroyed or at least reduced in thickness by acute atrophy, the secondary radiation will be modified and give rise to a cloudy radiograph.

A third explanation is that suggested by *Lachmann* and *Whelan*. These authors have found that the radiograph will be cloudy not only after decalcification but also after maceration, and they have tried experimentally to disprove earlier theories. They successfully decalcified bone models in acids and followed the development thereof radiographically; the result was a picture very much like that of atrophy of bone. Various bones were examined and by paraffinning the cortex or the spongiosa of shaft bones and simultaneously decalcifying the non-paraffinned part of the bone, they tried to form a conception of the importance of bone structure i.e., the structure of the cortex and the spongiosa, with regard to the cloudiness and patchiness of the radiograph. These experiments showed inter alia that an average decrease of 15-20 per cent of the bone salts was necessary for any difference at all to be discernible in the radiograph. The above authors try to explain this phenomenon in a rather simple manner by regarding the radiograph as a picture of the entire structural material of the bone. They argue that if changes in the bone are due to atrophy, the trabeculae will be decalcified

in varying degree i.e., slight decalcification up to total destruction, and as the radiograph is a reflection of the sum of the trabeculae, a better defined picture than that of normal bone cannot be expected in cases of atrophy. They assert that the patchiness is to be ascribed to different degrees of decalcification in various parts of the bone. *Jaffe* and *Pommeranz* suggest that the patchiness is due to a gradual formation of new bone and consider the rarefaction dots to be the manifestation of cortical canals widened by perivascular resorption.

Sudeck dealt with this problem in 1938. He maintains that the radiograph shows destructive changes during the remodelling period of the bone until the osteoid is recalcified, whereas the repair or formation products, as long as they consist of osteoid tissue, at most tend to render the picture cloudy or make the bone contour "woolly". The patchiness observable in the radiograph during the remodelling period is indicative of a very rapid destruction indeed; if however, the process is somewhat slower, the cloudiness of the picture will be more diffuse and even, and thus indicate a more uniform remodelling process. Thus, according to this theory, the cloudiness is to be attributed to the osteoid substance in the bone being remodelled.

Other writers touching this problem in some way or another are *Herfart* and *Beck,* (1925), who more or less advocate *Kienböck*'s opinion. In 1917 *Maliwa* expressed the view that the radiographic picture of acute atrophy of bone is evidence of a transitional excitation stage prior to actual atrophy, but he gives no detailed account of the reasons for his belief. *Baastrup* believes the patchiness to be attributed to the rarefaction of bone, the zones being the reflection of the ramified ends of blood canals. According to *King,* local oedema and a swelling of the soft parts as well as the concentration of salts deposited in same, are essentialities for the cloudiness of the radiograph.

The above investigations are, to the knowledge of the present writer, the only attempts made to solve the problem under discussion.

In 1940 the writer published a preliminary essay in which he tried to explain the causes of the arisal of the cloudiness in

the radiograph of sub-acute atrophy of bone. He underlined the significance of the nutritional difference between the Haversian systems and the interstitial lamellae. The Haversian systems are better nourished than the interstitial lamellae *(Langer)* for which reason the former will be more exposed to possible circulation disturbances with consequential physicochemical changes than will the latter, and as these changes are believed to be the cause of acute atrophy of bone, it is obvious that a differential decalcification of bone is possible, the better nourished Haversian systems being influenced—remodelled or decalcified—both quicker and to a higher degree than the relatively ill-nourished, interstitial lamellae. This means that the latter—in virtue of their higher calcic density—will contrast with those zones that have a more generous blood supply, especially the zones of the Haversian systems. As a consequence of this, the bone might be expected to exhibit adjoining zones of differential calcic density. These zones, however, are of such a small order of magnitude[1] that they cannot be projected separately on the relatively course-grained X-ray film.

The sum of all these zones of varying calcic density together with the inability of the Roentgen film to reflect same separately, might be a plausible explanation for the radiographic cloudiness. When the remodelling and decalcification processes have advanced sufficiently for the calcium content of the bone again to be homogeneous, the cloudiness will disappear and leave behind a picture, characterized by relatively thin and sharply defined, comparatively sparse trabeculae and a cortex of diminished thickness (chronic atrophy).

As mentioned in previous papers the present writer based his hypothesis on earlier well known facts.

1. The fractional bone necrosis described by *Müller* and confirmed inter alia by *Jaffe* and *Pommeranz,* who had observed in an anemic and ischemic but otherwise vital bone, a local necrosis of the interstitial lamellae.[1]

[1]According to *Jaffe* (1929) the Haversian canals in normal bone have an average diameter of 50 microns.

[1] In 1939 *Jaffe* published an investigation of a case of acute atrophy

2. Some phenomena seem indirectly to speak in favour of the cloudiness and patchiness of the radiograph being due to a certain extent to the bone structure. In sites of plexiform bone there is never any cloudiness or patchiness, e.g. children under school age, and attachment areas of bulky muscles and the insertions of tendons.

As a means of ascertaining the possible existence of the above supposed differential calcic density within various areas of the atrophic bone, the writer previously proposed spectroscopic measurement and light absorption determinations on ground disks taken from atrophic bone. The present crisis has however made it impossible to realize this proposal.[1] The writer has therefore now tried to find another solution to the problem of the radiograph of acute atrophy of bone and in this paper he will try to describe his experiments and the results he obtained.

Remarks on previous investigations and theories as well as propositions.

The above mentioned theories of the radiograph of acute atrophy of bone may be divided substantially into three groups:

I. That in which it is assumed that the cloudiness is due to the effect of lime salts—possibly dissolved—and possibly together with the changes in the trabeculae themselves (*Kienböck*).

II. That in which only or mainly those changes localized to the trabeculae play the decisive part, possibly in association with changes in the soft parts outside the bone, oedema, for example.

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of bone in which he observed a widening and a reduction of the Haversian systems and a few sprinkled signs of a partial necrosis in the interstitial lamellae.

[1] According to a method of analysis (Quantitative Micro- and Histochemical elementary Analysis by Roentgen absorption Spectography. Acta. Radiol. Suppl. LXIII, 1946) published recently by *A. Engström*, it is possible to measure a calcic content of only 0,1—100 μg in a square of 10×10 μ in ground preparations. The writer intends to try to carry out such experiments himself and will publish his results later.

III. *Sudecks* theory, according to which, the osteoid is the essential factor responsible for the cloudiness of the radiograph.

As pointed out above, *Friedl* and *Schinz* assailed Kienböck's theory on the grounds that the concentration of the dissolved salts deposited in the bone meshes is insufficient to exert any influence on the radiograph. In radiographs of skeletal affections, especially near fractures, *Cohn, Heydemann, Leriche* and *Jung* have however discerned shadows of calcic density in the soft parts, and they attributed these shadows to lime salts dissolved in the tissues (Kalkwanderung).

Lachmann and *Wehlan* attacked the theory of the Bucky-effect, and one might very well ask why this effect should be present only in the acute stage, and not in the chronic, in which the trabeculae are both fewer and sparser.

One objection that might be raised against *Sudeck*'s theory is that although the osteoid often seems to be scanty, the radiograph is nevertheless cloudy, as is sometimes the case with osteitis in spite of a total absence of osteoid substance.

The fact that the skeletonized bone retains its cloudy appearance in the radiograph, contradicts *King*'s hypothesis in which he says that oedema of the soft parts is an essential factor. The capacity of water to absorb Roentgen rays is in fact only $^1/_{40}$ of that of calcium (*Friedl* and *Schinz*). To this must be added of course, the effect of the dissolved salts.

The propositions of my investigations are:

1. Is there an increase in the quantity of lime salts dissolved in the bone meshes of atrophic bone comparable with that in normal bone and if so, what influence does it exert on the radiograph?

2. Are there any histological reasons for assuming the existence of differential decalcification and if so, how does the decalcification seem to be localized in relation to the various lamella systems of the bone?

MY OWN EXPERIMENTS

Material: It was very difficult indeed to collect suitable bone material from homo. In a few cases it was nevertheless possible to obtain preparations from cases with arthritis, tumours and extremital emboli in the lower extremities, the specimens always being chosen from a bone distant from the seat of the disease, generally one of the small bones of the foot, in some cases even fragments from the lower part of the tibia, in which it was possible radiographically to observe signs of atrophy of various stages. The material was complemented with rabbit bone, whose texture is very similar to that of human bone (*Demeter* and *Mátyás*). According to *Rieder,* the remodelling processes of rabbit bone are analogous to those of human bone, and in the histological pictures he published, the changes are identical. An allergic gonit ad modum Klinge was produced in these animals (see Acta Chir. Scandinav. Suppl. 71). In these cases specimens were taken from the tibia and the calcaneum. For the radiographic examinations of bone models, also a piece of a normal shaft bone from an ox was used in one case.

I. RADIOGRAPHIC EXAMINATIONS[1]

In order to find out to what degree one might assume the cloudiness of the radiograph to be due to the dissolved lime salts deposited in the bone meshes, a number of experiments were carried out on models of macerated bone.

(a) *Methodics:* With the exception of one case (ox bone) all the bone models were first radiographed. This operation was carried out under optimal conditions, whereafter the bone was macerated in accordance with an anti-formin method described by *Sahsin* and *Riemer*.[2]

[1] For this purpose the X-ray apparatus used, consisted of a tube P_{20} with rotatable focus. The radiographs were taken under analogous conditions (42 kw. and 115 cms. focal distance). The times for the various preparations varied from 0.1-0.4 sec. The method of development, which was the same for all, lasted exactly five minutes.

[2] The fresh bone was first weighed and dried until its weight became

The preparations treated in this manner were sometimes damaged when being handled, small chips or fragments being broken off the edges. This applies especially to the most atrophic bones, whose calcic stroma was extraordinarily slender and brittle. The dried, macerated bone was weighed and in some cases the liquids used for the maceration evaporated to a vol. of about 20 ml, whereafter the filtrate was measured and the amount of lime salts present in the solution was determined by *Aron's* method, as modified by *Hammarsten*. After being macerated and dried the bone preparations were radiographed under the same conditions as therebefore. The bone was then charged with a 10-25 per cent solution of gelatin in destilled water, in some cases with an addition of calcium chloride corresponding to a quantity of 0.5-2 per cent of the gelatin solution; the charging or imbibition process consisted in heating the bone 3-4 hours in a water bath in a solution of calcium chloride until the expulsion of air bubbles had ceased.[1] Gelatin was then added until the solution had the desired concentration whereafter the whole was maintained for a further 3-4 hours at a temp. of 80° C. and then allowed to set. In those cases in which the bone was treated solely with gelatin, it was first boiled in distilled water until all the air bubbles had been given off, whereafter the calculated quantity of warm gelatin solution was added and the solution then also allowed to stand 3-4 hours in a water bath whose temp. was maintained at 80° C.

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constant, after which it was rinsed under running water and then treated 4-6 hours in a 10 per cent solution of antiformin in a water bath whose temperature was maintained at 80° C. The bone was then rinsed 2-4 hours under slowly running hot water and again treated for 24 hours in a 5 per cent solution of hydrogen peroxide. Subsequent hereto the bone was carefully dried at a temperature of 20° C and then defatted in Xylol for 12 hours at a temp. of 80° C, after which it was dried and bleached with a 5 per cent solution of hydrogen peroxide.

[1] Instead of calcium phosphate this salt was chosen on account of its easy solubility. This choice will however be of negligible importance, because examinations have shown that the absorption increases at the rate of four times the atomic number, and therefore calcium is the greater shadowing factor (*Friedl* and *Schinz*).

The calcium content of the gelatin was calculated by *Aron's* method and was found to be 1.12 per cent Calcium. The meshes of the bone models were thus charged with a substance whose nature is much like that of the organic tissues in bone and whose composition also includes a certain amount of soluble salts. After the gelatin had set, all excess gelatin was removed from the bone model with a wooden spatula, whereafter the models were radiographed under the same conditions as at first, except for the fact that the time of exposure had to be varied in a few cases. Subsequent hereto the model was boiled 4-6 hours, the water being changed continually to separate the gelatinous substance properly from the model, which was then dried again and weighed and thereafter re-charged with gelatin and calcium chloride of higher concentrations. In this manner it was possible to study the contrast changes occurring in the bone when its meshes were charged with lime salts of various concentrations. Altogether 5 bone models were used for these experiments.

1. Shaft bone from ox.
2. Normal os naviculare ped.
3. Fragment of atrophic talus with cloudy radiograph (tbc-arthritis).
4. Osteitically altered Os cuniforme (tbc).
5. Os metatarsale (Normal bone).

The four last mentioned bones were from homo.

(b) *Experiments.*

The first experiment was made on bone excised from the region between the diaphysis and the metaphysis of one of the long shaft bones of an ox. The bone was sawn longitudinally into two parts, only one thereof being used for the experiment. Already after a charge of 0.75 per cent calcium[1] in a 25 per cent gelatin solution the trabeculae became less distinct and the entire radiograph more diffuse than therebefore. The radiographic changes disappeared again after the gelatin and calcium

[1] This includes both here and hereinafter also the salt content (1.12 %) proper to the gelatin itself.

had been removed by boiling, but reappeared with still greater prominence when the bone was charged with a 25 per cent solution of gelatin containing 1.3 and 2.3 per cent calcium.

2. The radiographic outline of the trabeculae of the normal os naviculare ped. after imbibition of gelatin containing 0.5 per cent calcium was considerably less distinct and the whole picture more diffuse than before imbibition; when treated with 1 per cent calcium in gelatin, the outline was still more diffuse and the general picture still more cloudy.

3. Specimen from talus taken from a case of tbc ped.[1] After the bone had been skeletonized, the radiograph revealed a cloudy somewhat spotty atrophy with sprinkled signs of glassiness a picture very much like that obtained from the bone in situ. (See Fig. 1 a). After maceration the calcic stroma was rather well defined. (Fig. 1 b). The calcium content—calculated by *Hammarsten*'s method—of the filtrate of the bone macerated according to *Sashin*'s and *Riemer*'s method, gave a quantity corresponding to 0.28 per cent of the organic substance plus H_2O of the bone. Blank tests of similarly macerated normal bone gave 0.02 per cent. According to this calculation there was 0.26 per cent calcium in the bone marrow. A charge of a 15 per cent gelatin solution containing altogether 0.28 per cent calcium again blurred the radiograph of the bone considerably. The trabeculae were illdefined, some of them being difficult to distringuish (Fig. 1 c). When the gelatin was boiled out again, the stroma appeared distinct anew, although somewhat deformed.

4. The radiograph taken of the os cuneiforme (tbc osteitis) was considerably cloudy (Fig. 2 a). After maceration the trabeculae appeared somewhat more distinctly in the radiograph, but the osteitically changed bone was still blurry. (Fig. 2 b). The cal-

[1] Specimen taken from the vicinity of the seat of the arthritis. The radiograph exhibits some denser margins. These are the result of a compression of the bone by the manipulations. A small piece of the bone was microscopically examined; only signs of atrophic, but not osteitic, changes were visible. No macroscopic signs of osteitis, although it was impossible with certainty to exclude the possibility of such signs in the centre of the preparation.

cium content was determined in the same manner as in Experiment 3 and it was found that the quantity of calcium incorporated in the organic substance of the bone was 0.40 per cent of the weight of the soft parts of the bone plus H_2O. After charging with 0.5 per cent calcium in a 25 per cent solution of gelatin, a picture is produced in which the trabeculae seem to be almost obliterated. (Fig. 2 c). The calcium and the gelatine were re-

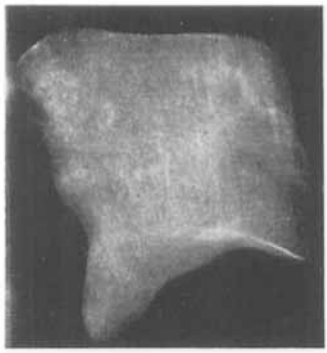

Fig. 1 a.

Fig. 1 b.

Fig. 1 c.

Fragment of talus taken from a case with tbc. ped. Histologically, the preparation exhibited a considerable destruction of the trabeculae by lacunar resorption and in the bone meshes it also displayed signs of bleding and oedema. Only relatively small quantity of osteoid in the preparation.

Fig. 1 a shows the fresh preparation. It has been somewhat deformed and its trabeculae compressed during preparatory treatment. See top left-hand corner of picture. A piece of the preparation was removed for histological examination, for which reason the preparation in Figs. b and c is somewhat smaller. Fig. b shows the macerated bone and Fig. c the bone when charged with a 15 per cent solution of gelatin (0.28 p. c. Ca).

moved and the radiograph became clearer again (Fig. 2 d). A new charge consisting of 0.11 per cent calcium in a 10 per cent gelatin solution again rendered the radiograph cloudy (Fig. 2 e).

Preparation 5. Os metatarsale I of normal bone. After sawing it longitudinally into two pieces, the bone was macerated, the two halves were joined together again and X-rayed. The spongiosa was very well defined in the radiograph. After the bone

had been charged with gelatin containing 0.5 per cent calcium, the spongiosa appeared possibly somewhat less clearly than before.

When carrying out radiographic experiments of this nature there are many sources of error. Above all, an adequate, constant

Fig. 2 a.

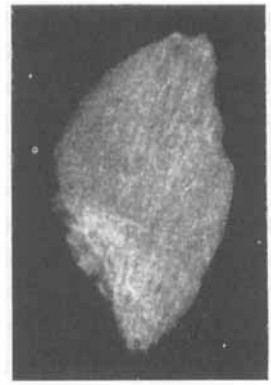

Fig. 2 b.

Fig. 2 c.

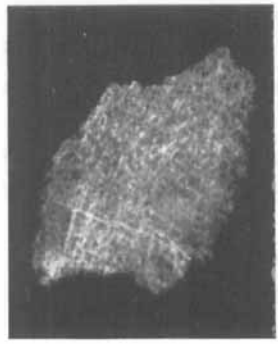

Fig. 2 d.

Fig. 2 e.

Fig. 2 (a-e) tbc osteitis.

Fig. 2 a shows the fresh bone, 2 b after maceration, 2 c when charged with 0.5 per cent Ca in gelatin (the calcic spots on the picture are the reflection of a thread charged with gelatin + Ca; the thread was drawn through the preparation to facilitate the handling of the latter. Fig. 2 d shows the preparation after the removal of gelatin, and Fig. 2 e, after the charging of the preparation with 0.11 per cent Ca in a 10 per cent solution of gelatin.

current and exposure time are of paramount importance. In the cases dealt with in the investigation made by the writer these factors have been observed, i.e., the current has been both adequate and constant and only in some cases has the exposure time varied somewhat. The maceration of the bone and the charging of same with gelatin are manipulations which might easily damage the bone and thus involve structural changes that might make it difficult to compare the various pictures of one and the same preparation. It was also found difficult completely to remove all the gelatin from the bone, especially from atrophic and osteitic bone (Exp. 3 and 4), because the preparation was so very brittle after the removal of the gelatin. To avoid this, in some cases the writer added to the gelatin already at the beginning of the operation, a quantity of calcium corresponding to that ascertained by the calcium determination of the soft parts. Another source of error is that the bone might possibly not be charged properly throughout with gelatin with the consequence that some of the cell systems will still contain air. To prevent this possibility, however, the preparations were first boiled in a sufficiently strong watery solution of calcium chloride until the expulsion of air bubbles from the bone ceased. After this step, gelatin with a corresponding quantity of calcium was added. By adding methylene blue to the salt solution and by sawing through the bone, it was then possible to check whether the imbibition of gelatine was satisfactory throughout the whole bone.

The amount of calcium determined to be present in the filtrate cannot be regarded as being more than approximative. Blank tests on normal bone were of course made with a view to diminish the number and degree of severity of the many possible errors.

Summary of Results and Discussion.

The results of the above experiments show:

1. If we charge the meshes of normal bone with calcium and gelatin and the concentration thereof be less than 0.5 per cent

calcium in a 25 per cent solution of gelatin, the radiographic outline of the bone will be somewhat ill-defined.

2. An atrophic bone giving a cloudy radiograph, will have a better defined trabecular system after maceration than therebefore, and most of the general cloudiness of the radiograph will also have disappeared.

3. Experiments made to determine the calcium content of the marrow of bone showed that the calcium content of the bone marrow in two cases of atrophic and osteitic bone had a concentration of 0.26 and 0.40 per cent respectively, calculated on the basis of the organic substance plus H_2O of the bone.

4. The macerated atrophic bone after being charged with quantities of calcium corresponding roughly to those found by chemical analysis again shows a considerably cloudy bone texture differing somewhat however from that displayed before maceration. After the removal of the lime salts and gelatin, the radiograph of the bone is again better defined.

5. Corresponding experiments were made on a spongious osteitic bone and the results obtained were similar, although a certain cloudiness in the bone remained also after the maceration.

These experiments indicate that salts possibly present in the bone marrow can undoubtedly play a rôle in the arisal of the characteristic cloudiness in the radiograph of atrophic bone. By means of imbibition tests it was possible to obtain pictures similar to certain extent to those of atrophy. The experimental imbibition also gave a certain measure of the concentration of the lime salts in the soft parts of the bone that is necessary for rendering the above-mentioned picture indistinct.

One disadvantage is, however, that calcium chloride was used instead of calcium phosphate. Nevertheless as pointed out above, it is certainly the calcium that plays the greater part in the absorption of the X-rays. But the importance of the phosphate components dare not be underrated and therefore it can be assumed that the quantity of calcium necessary to make a radiograph of living bone cloudy, is much less than that necessary in the experiments, namely, about 0.11-0.5 per cent. Ex-

periments made on models of normal bone show also that the bone structure, i.e., proportion between the spongiosa and the compacta, exerts a certain influence, because purely spongy bone can show changes with a lower concentration of dissolved lime salts which changes are not visible in a more compact, calcic bone. This is also a possible explanation for the reason why changes in the spongy regions appear first and always are most prominent just there.

In the osteitically changed bone preparation it was not possible to obtain a well-defined radiograph after maceration. We may therefore assume that, at least in some cases the changes in the trabeculae are partly responsible for the arisal of the cloudy picture in such changed preparations.

II. HISTOLOGICAL INVESTIGATIONS

But few investigations of so-called *acute* atrophy or acute remodelling processes of bone are known to the literature, and the first to shed certain light on the underlying anatomopathological processes were *Jaffe* (1930), *Rieder* (1933-36), and *Sudeck* (1938), who investigated os homo and rabbit bone. The main fact established by all these writers is that there is a cellular destruction of bone in which the granulation tissues emanating from the connective tissues and the vessels, seem to be the essential factors. Resorption occurs also lacunarly and the osteoclasts in the lacuna are small and spindle-like. Giant osteoclasts have been observed less frequently. In addition hereto so-called perivascular absorption was also observable. Besides this, however, these writers also believes in the existence of a chemical destruction in the form of decalcification. As well as the destruction, which is of differential prominence in various parts of the bone (it is for this reason we speak of a histologocally patchy or cloudy picture [*Rieder*]) there are simultaneous repair processes of various degrees. As pointed out above, it is a question of a remodelling of bone caused by cellular reaction and, possibly, also of a simultaneous chemical decalcification. It has hitherto been impossible with certainty to establish the presence of signs

of an osteolysis, for the simple reason that it is not possible to decide whether the existence of the osteoid in the preparation is a result of destructive or repair processes. Many writers such as *Leriche* and *Policard, Häbler, Jaffe* (1930) and *Gurd* assume, however, that in cases of acute atrophy of bone accompanied by cellular destruction, there is osteolysis, which however, cannot be distinguished as a separate phase. Furthermore, it might be mentioned that no histological investigations of the behaviour of the bone salts in atrophic bone have hitherto been made.

In his investigations of an artificially produced acute atrophy of bone on a rabbit[1] the writer noticed a certain heterogeneity in the bone substance.

In a number of preparations stained with Haematoxylin (Ehrlich) and Chromotrop 2 R he found that there were some zones that would not stain as much as the rest of the bone. These zones, generally localized to the interstitial lamellae were poorly demarcated and the bone seemed to be so to say "patchy". In these zones it was often possible to observe a large number of light corpuscules, possibly suggestive of a more rapid destruction of bone—a de-meshing process (*Häggquist*). Similar phenomena —although less pronounced—were nevertheless visible in the apparently normal bone, for which reason no conclusions can be drawn.

Achard (1935), found similar changes in decalcified normal bone, and he believed them to be the result of a differential composition and of varying electric charges of the colloids in the ground substance, due to the differential age of the trabecular systems in the bone. By means of alizinar staining *Cameron* (1930) could also show a difference in the staining capacity of old and young bone, which inter alia expressed itself in a differential stainability between the Haversian systems and the rest of the bone.

W. Müller, Jaffe (1930) and *Schmidt* observed similar phenomena in necrotic bone, but point out that such pictures are also encountered in normal bone. *Jaffe* found that in the early stages

[1] Acta Chir. Skandinav. suppl. LXXI 1942.

of acute atrophy there was a granulation and dissolution of the bone substance of the same nature as the present writer found in his investigation in 1942. It is very difficult to interpret such a picture, and it is impossible to draw any conclusions therefrom, because the changes might possibly be artefacts (*Landoff* 1942).

Examinations of decalcified bone have not led to any definite result when it comes to establishing a possible osteolysis or recording a differential distribution of the lime salts in atrophic bone. Much, however, points towards the decalcification being a purely chemical process under certain circumstances and *Cohn*'s and *Heydemann*'s radiographic examinations of fractures, in which they found stripes of calcic density in the soft parts, speak in favour of this assumption. *Leriche* and *Jung* examined the calcium content of the soft parts in the immediate neighbourhood of fractures and were able chemically to determine the calcium concentration, which was higher than normal, especially in cases of pseudarthrosis or complicated fractures.

The calcium content of the blood, however, is not affected. *Rieder* and *Never* experimented on dogs in vivo and were able to show a decalcification of bone by running venous blood through it.

This suggests the necessity of histologically examining the calcic stroma itself, if possible, in order to obtain some fundamental evidence that might serve as a starting point for further studies. As pointed out above, such investigations have apparently not been made. As mentioned in the introduction of this paper, the light absorption determinations and the spectrographic examinations I suggested, could not be carried out, for which reason I was obliged to resort to a purely histological investigation by staining the lime salts of ground disks of prepared bone taken from various sorts of atrophic bones. The staining methods mentioned most in the literature are: *Kossa*'s staining with Ag NO_3, the tannic acid method, the pyrogallol method, staining with lead acetate and alizarin staining (*Lison, Krause, Schmorl,* etc.). None of the above staining substances seem to be pure calcium stains, because they also stain other

metals and alkaline salts in the bone to a certain extent. They also have a certain affinity for the organic substance of the bone. The methods have been compared and criticized exhaustively by *Cameron, Lison,* etc., for example. The most reliable of the above calcium staining methods seems to be the alizarin staining process (*Cameron*). Sometimes, however, the alizarin stain will with both Fe and Al produce a colour that is hardly distinguishable from stained calcium. As already mentioned, *Cameron* believes that changes in the ground substance can give rise to a certain irregular staining, which expresses itself most in the differential staining capacity of old and new bone, the latter exhibiting a considerably greater affinity for alizarin than does old bone, which sometimes almost refuses to be stained.

After carrying out experiments in which $AgNO_3$ and haematoxylin were used as stains the writer went over to the use of alizarin.

1. Technique. The fresh bone preparations were first cured in alcohol of 95 per cent concentration in which they were afterwards kept, because bone preparations preserved in formalin solution can undergo decalcification by the formic acid formed in the solution. A section of the bone about 0.5-1.0 mm. in thickness was sawn out by means of a saw with two parallel blades and ground manually between two oil stones until it was reduced to a thickness of about 10-15 μ. It was then carefully rinsed with distilled water and subsequently stained with a solution of alizarin in alcohol. Use was thereby made of a saturated standard solution of alizarin disolved in 95 per cent alcohol as suggested by *Lundwall.* This solution was diluted with 70 p. c. alcohol in the proportion 1:9. The preparations were stained 3-6 hours, after which they were differentiated in 70 p. c. alcohol and dehydrated in absolute alcohol for 3-4 hours and thereafter mounted in the usual manner in Canada balsam. In some cases, in order to avoid as much as possible the influence of the organic tissues of the bone and of the salts deposited therein, the writer stained the disk after maceration. After being ground down to a desired thickness of 10-15 μ the disks were

treated three hours in a 10 per cent solution of antiformin at 80° C, after which they were carefully rinsed 3-4 hours under running warm water. The preparations were then allowed to remain 24 hours in a 5 per cent solution of hydrogen peroxide, then dried and treated 3 hours with xylol at a temperature of 80° C., whereafter they were stained by the above method and mounted in the usual manner. In some cases attempts were made at vitally staining young rabbits. An aseptic gonit ad modum Klinge was produced in the animals, and in two cases attempts were made at instigating an acidotic condition in the animals by introducing ammonium chloride through a rubber tube into the stomach. After 7 and 14 days the animals were given subcutaneous injections of 5 ml. of a 2 per cent watery solution of alizarin. The last injection was given to the animals about an hour before they were killed.

2. Material. For these staining experiments 10 preparations from homo were used, the preparations being chosen from bone in various stages of atrophy, and in about 20 cases the preparations consisted of rabbit bone.

As the purpose of this investigation was mainly to find out whether there is any difference in the nature of the various trabecular systems in bone, especially the Haversian systems and the interstitial lamellae, the writer used chiefly bone with a relatively rich proportion of compacta. It is also easier to obtain better ground disks of compacta than of spongoid bone.

The only detectable difference in many of the preparations was an increase in vascularization and a lacuna destruction by the granular tissues and small uninuclear osteoclasts. Nowhere could clusters of osteoclasts betraying a somewhat more rapid destruction—de-meshing—be observed. In a few cases, which will be dealt with more in detail herebelow, certain tinctorial properties could be observed.

Prep. 4. Osteosarcoma femoris E. J. 15 yrs, (Orthopaedic Hosp., Hälsingborg, 1940). In a preparation taken from the diaphysis of the tibia, there was a richly vascularized compacta with large dilated canals. Furthermore the greater part of the Haversian systems in the preparation stained deep red, con-

trasting in some places sharply against the paler interstitial lamellae. In the Haversian systems the tissues nearest the vessels were stained brightest. (See Fig. 3).

Prep. Nr. 8. Embolia art. fem. A. N. 36 yrs (Borås 1076/43). In the ground disk of the preparation taken from the fresh bone there is, apart from a rather rich vascularization, a distinct staining capacity. The Haversian systems are mostly more redd-

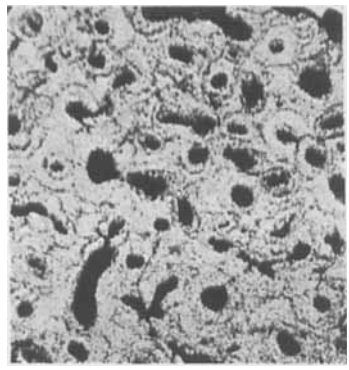

Fig. 3.
Fragment from the diaphysis of the tibia from a case with osteosarcoma femoris. The preparation after maceration and alizarin staining. The Haversian osteons are conspicuously demarcated against the ground substance.

ish-violet than are the interstitial lamellae, which in some spots are hardly stained at all. In a macerated ground disk taken from adjacent areas of the bone, the tinctorial phenomena are more pronounced, and everywhere in the preparation are the beautiful above-mentioned contrasts to be seen (Fig. 4 a). In order to find out whether the above changes might be due to variations in the ground substance, another preparation was taken from the same area and then first macerated and subsequently decalcified in the usual manner in a 10 per cent solution of nitric acid and stained in the same way as was the previous one. Unlike the two previous preparations taken from the same case, a pale yellow, absolutely uniform staining of both the Haversian

systems and the interstitial lamellae throughout the whole preparation was observable. (See Fig. 4 b).

Prep. Nr. 13. Tbc. ped. 70 yrs. (Borås 619/43). In alizarin-stained ground disk taken from the diaphysis of the tibia the

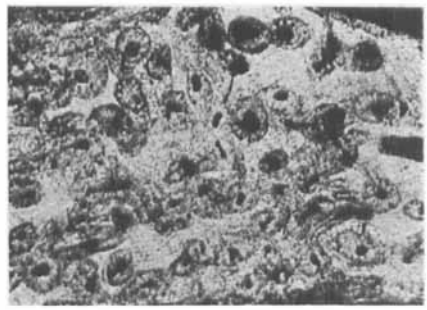

Fig. 4 a.

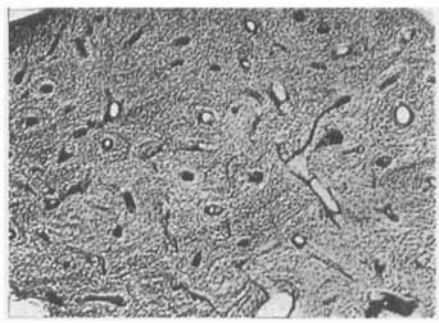

Fig. 4 b.

Fig. 4 a-b. Fragment of tibia from a case with embolia art. fem. Fig. 4 a shows a piece from the cortex in the tibia. The preparation is macerated and stained with alizarin. Its Haversian systems are stained deeper whilst the interstitial lamellae are paler. Fig. 4 b shows the bone after maceration and decalcification. No observable difference between the staining of the Haversian systems and the interstitial lamellae.

same changes in staining capacity are observable between the various lamella systems as those described above, although the changes are not quite so pronounced.

In a few cases in the tibia of a rabbit an aseptic gonit was

produced for 7-14 days, similar changes were recorded, whereas no changes could be observed in the control leg.

Sources of Error.

Apart from the differential staining capacity of bone due to varying age, as described inter alia by *Cameron* and to which I shall revert later in this paper, there are other factors that might influence the result of an investigation. For instance, the pH value at which the staining process is carried out. In all of the above cases the staining took place in a neutral solution. The judging of a preparation is also rendered more difficult by the fact that the disk cannot be reduced to a thickness of less than about 10-15 μ. This facilitates the arisal of certain artefacts, for example, by the overlapping or super-imposition and impression of lamellae normally lying in different planes, which lamellae, when super-imposed, can give zones of differential staining capacity. Comparisons have always been made of such zones of the preparation that most definitely do not exhibit any such superimposition. Furthermore it is also feasible that owing to the differential hardness of the Haversian systems and the interstitial lamellae, the surface of the disk may be unevenly ground and consequently not be of uniform thickness throughout. A microscopic examination of the disk, however seemed to contradict this assumption, the Haversian systems and the interstitial lamellae being apparently of the same thickness and adjoining zones being equally well defined in one and the same adjustment of the microscope. The grinding operation might also involve a few scratches and consequential rarefactions, which, however, are easily discernible and can therefore be allowed for when judging the disk.

The possibility that the changes should be caused by the fractioned necrosis of bone described by *Müller,* is not probable, at least not in Prep. 4. where the patient is relatively young. Also the increase in vascularization of all the above preparations contradict this.

Summary and Discussion.

As mentioned at the beginning of this paper, a certain difference in the staining capacity of the osteoid and the interstitial lamellae is known to the literature. *Achard* believed this difference to be due to varying colloid-chemical changes in the ground substances, especially in the pH value. *Cameron* showed by alizarin-staining that the differential staining capacity of the various lamella systems of bone varies with age. Also he ascribes this phenomenon mainly to changes of the ground substance. Other writers who have observed this phenomenon (e.g. *Müller*) in both normal and pathological bone (Fractioned bone necrosis) also share his opinion.

The staining experiments carried out by the present writer, however, prove:

(1) That also old bone, taken, for example, from a 70 year old person can be stained by the methodics applied just as well as bone taken from an adolescent.

(2) That results obtained by the staining methodics in question revealed nothing but possibly indicated very faint differences in the stainability of the various lamella systems in normal bone, this being true also in cases of rather slowly progressing atrophy.

(3) That in cases of rather acute atrophy it was possible to prove:

(a) A repeatedly observable, clear difference in stainability between the Haversian systems and the interstitial lamellae, the latter being considerably less stainable than the osteons.

(b) This difference is augmented after maceration, by which it is possible to remove a fair amount of organic substance and blood which seems to exert a certain influence on the picture. This applies especially to the blood, because Fe with alizarin gives an intensive, dark red stain.

(c) The substance remaining after maceration and decalcification with nitric acid stains absolutely homogeneously and the tinctorial phenomena in the bone disappear altogether.

The experiments made thus show that all bone, immaterial

whether young or old, can be stained by the methodics applied by the writer. The difference found in certain atrophic bone between the staining capacity of the Haversian systems and that of the interstitial lamellae disappeared after maceration and decalcification. It would therefore seem that these regional differences in stainability are dependent on the bone salts and not the ground substance. The latter can admittedly be stained pale yellow, but the stain is absolutely homogeneous. On the basis of these experiments it can thus be assumed that there is a difference in the calcic stroma, although it cannot yet be said whether this difference is quantitative or qualitative. A possible explanation for this difference in the calcic stroma is that owing to the underlying pathophysiological processes of atrophy of bone, especially the blood circulation, there will be a certain pH difference in the tissues, which in turn will exert an influence on the bone salts whose composition will thus be modified. In this conjunction it might be remembered that some writers found a differential mobility of the calcium in various parts of the bone. *Rutishauser, Farwager* & *Queloz,* for example, found by injections of lead on animals that relatively greater quantities of lead salts are deposited in growth zones and those areas exposed to the greatest strain and compression than was the case elsewhere in the bone. By subsequent acidotic tests they discovered that relatively more lead and calcium was released from these areas than from the rest of the skeletal parts. *Bauer, Albright* & *Aub* found by alizarin staining and parathormon injections that calcium could be mobilized easiest in the metaphysis.

III. THE SUBCHONDRAL CHANGES ESPECIALLY WITH REGARD TO THE CYST-LIKE RAREFACTIONS

The structure of bone, especially the proportion between the spongiosa and the compacta is of great importance in the radiograph i.e., characteristics of the picture of acute atrophy of bone (e.g. *Lachmann* and *Wehlan*) because atrophy in the spongiosa is detectable earlier and it is also in the spongiosa

that the changes are most marked. This has to a certain extent been confirmed by the radiographic experiments, carried out on models by the present writer. Nevertheless the architecture of the bone alone cannot be responsible for this fact because

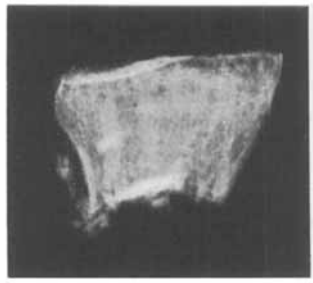

Fig. 5 a.

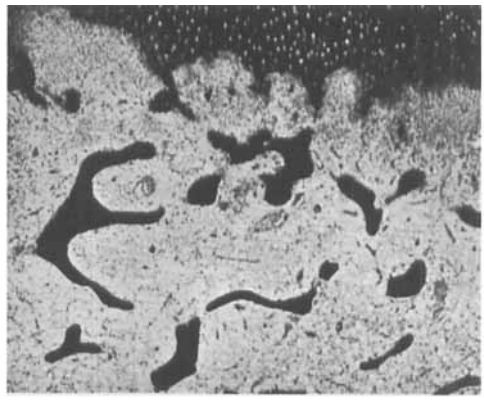

Fig. 5 b.

Fig. 5 a small sub-chondral rarefaction dots. Fig. 5 b is a histological section from the area of the rarefaction dots. The picture shows reduced trabeculae with signs of resorption by the connective tissues and especially sub-chondrally there are some signs of bleeding and infiltration of round cells. In the marrow space, which is widened considerably, there is a fairly pronounced oedema. No osteoid visible.

much speaks in favour of differential atrophy in one and the same zone of a bone. This is probably due to the inter-relationship between the circulation changes caused by the primary process

and the atrophy. This occurrence has since long been recorded by inter alia *Lehmann, Hilgenreiner, Herfarth* and *Heydemann* etc., who found that atrophy appears first in the metaphysal regions and especially in the subchondral layers. *Heydemann* describes the subchondral rarefactions accompanying arthritis and attributes there arisal to the intimate relationship existing between the subchondral vessels and the capsular vessels. These rarefactions typical of acute atrophy have been described by various writers such as *v. Manteuffel, Sundt, Leriche & Policard*, but generally speaking only relatively little attention has been focussed upon them, and they have not been made the subject matter of exhaustive investigations. The changes, which may now and then be the first signs of acute atrophy appear in the radiograph in the form of small, rather ill-defined or woolly, subchondral rarefactions localized mainly to the phalanges, metatarsal and metacarpal bones.

In a preparation (basal part of metatarsal I) taken from a man with senile gangrene in the outer phalange of a toe, the writer compared the radiographic and histologic pictures thereof. In the radiograph (Fig. 5) subchondral, small, cyst-like rarefactions of the above mentioned nature and signs of atrophy of bone were discernible. Histologically it was possible to observe extensive corresponding changes that were especially pronounced in the sub-chondral layers. There were no other bordered destruction areas to be observed. In the layer underneath the cartilage one could observe considerable hyperemia and here and there a moderate amount of oedema. The trabeculae throughout the whole bone bore signs of a patchy, rather pronounced, lacuna resorption by small, not very numerous osteoclasts. Besides this, especially sub-chondrally, where the hyperemia seems to be the greatest, collections of small, rather diffusely bordered bundles of granular tissues were apparent. (See Fig. 5 b).

The general atrophy in the subchondral layer together with the granulation tissues present there are presumably the fundamental causes of the changes in the radiograph. In the remainder of the bone there are also reduced trabecular systems and lacuna destruction, whilst in the preparation as a whole there are no

signs whatsoever of remodelling, which is probably due to a diminished regenerative capacity of the bone. The picture, when viewed from this angle, therefore corresponds best to *Sudeck*'s dystrophic stage.

We thus have a picture of acute remodelling corresponding to that described inter alia by *Sudeck* and *Rieder* and which is characterized by a strong exudative reaction in the bone, limited especially to the subchondral areas, which are most richly vascularized.

The histologic picture illustrates in a beautiful way the connection between changed vascularization and atrophy of bone and thus supports the vascular theory.

SUMMARY OF THE RESULTS OF THE INVESTIGATION

The investigation reported above does not prove my hypothesis of 1940 concerning the cause of the characteristics of the radiograph of acute atrophy of bone. Experiments on models of normal and atrophic bone have indicated that the typical cloudiness may be caused by the dissolved lime salts present in the bone meshes, the concentration of same apparently not exceeding that given by other writers (*Leriche* & *Jung*) in the soft parts around the fractures. The theory advocated by *Kienböck* is therefore probably correct. It cannot however be denied that also changes arising in the calcic stroma and newly formed osteoid, may possibly attribute to a certain extent to the character of the radiographic picture. No quantitative difference could be recorded histologically. It was however possible in a nice way to show that there are certain changes in the lime salts of bone suffering from atrophy. These differences seem to be regional and to correspond to the microposcopic units of the bone. Whether the changes are an expression of a regional decalcification or quantitative or qualitative changes in the calcic stroma of the bone cannot yet be determined.

The experiments also show that differences made visible by alizarin staining must depend on the bone salts themselves and that such changes are thus not to be ascribed to any changes

in the ground substance. Changes in the bone due to age, as described by *Cameron*, for example, expressing themselves in differential stainability of the bone tissues probably depend less on colloid—chemical changes in the ground substance than hitherto supposed. It is very probable that a changed chemical composition of the bone salts plays a rôle here. It is a well known fact that the CO content and the Mg content in the bone are modified with advancing age (*Aron*). It would also seem rather improbable that the changes proved by the present writer should be solely age-changes of the nature described by *Cameron* because the observed differential stainability of various adjacent areas which does to a certain extent agree to *Cameron*'s description could not be proved by the methodics applied by the present writer in cases of normal bone taken from elderly individuals or in bones with rather chronic atrophy; moreover no changes could be observed in control-examinations of rabbit bone although changes were observed in the experimental bone. The changes described, which cannot be shown in normal or chronic atrophic bone but very well in bone with acute atrophy, apparently speak in favour of a transitional change in the inorganic stroma of the bone, probably of chemical or electro-chemical nature.

SUMMARY

In an earlier paper the author brought forward a theory according to which the cloudiness and the patchiness of the radiographic design peculiar to acute atrophy of bone is due to a differential decalcification of the latter. According to this hypothesis the structure of the bone tissues and the differential degree of nutrition play an important part, the better nourished Haversian systems decalcifying more rapidly than the interstitial lamellae, so that adjacent patches of differential density will arise and thus to a certain extent explain the diffuseness of the radiograph.

As the radiographic cloudiness might feasibly be due to inter alia dissolved calcium salts deposited in the bone meshes (*Kienböck*), the author made a few experiments on bone models, the

tissues of the soft parts of which had been removed by treatment with antiformin. The approximate content of dissolved calcium salts in the soft parts was determined in two cases and found to be 0.26-0.4 per cent calcium. The bone model was then charged with gelatin containing different proportions of calcium whereby it was observed that when the bone models were allowed to imbibe gelatin containing less then 0.5 p. c. calcium, the radiographic design of the bone was patchy and cloudy. The results of the investigation speak in favour of *Kienböck*'s theory, but disclose no evidence in support of the author's hypothesis. However, the possibility cannot be excluded that the changes the Haversian systems and the interstitial lamellae undergo in acute atrophy of bone are nevertheless differential. The correctness of this assumption is supported by the histological examinations described by the author in the latter part of his report. By treating the ground preparations of atrophic and normal bone by a special alizarin staining method, it was possible to produce beautifully conspicuous differences in the stainability of the lamellae in preparations from acutely atrophic bone. These changes could not be produced in normal bone or in bone with chronic atrophy. After maceration of the bone by antiformin treatment the contrasts were still more perspicuous. When however, the ground preparation of an atrophic bone was decalcified and stained, a completely homogeneous yellow resulted. All the above staining phenomena thus seem to be related to the bone salts.

A histological examination of preparations from acute atrophy of bone in which nest-like subchondral rarefactions were discernible also evidences the correlation between atrophy of bone and the circulation.

ZUSAMMENFASSUNG

In einer früheren Arbeit hat Verfasser die Theorie aufgestellt, dass die Schleier und Schattenflecke im Röntgenbilde für die akute Knochenatrophie charakteristisch seien und auf einem Gradunterschied in der Dekalzifizierung des Knochens beruhten.

Nach dieser Hypothese spielen die Struktur des Knochengewebes und der verschiedene Ernährungsgrad eine wichtige Rolle, indem die besser ernährten *Havers*'schen Systeme schneller dekalzifizieren als die interstitiellen Lamellen, so dass angrenzende Flecke von verschiedener Kaldichte entstehen, wodurch die Weichheit des Bildes bis zu einem gewissen Grade zu erklären ist.

Da die Schleier des Röntgenogramms möglicherweise u. a. von aufgelösten Calcium-Salzen herrühren, die sich in der schwammigen Knochensubstanz abgesetzt haben (*Kienböck*), stellte der Verfasser einige Experimente an Knochen an, deren Weichteilsgewebe durch Behandlung mit Antiformin entfernt worden waren. Der annähernde Gehalt an gelösten Calcium-Salzen in den Weichteilen war in zwei Fällen auf 0.26-0.4 % Calcium bestimmt worden. Das Knochenpräparat wurde dann mit Gelatine gefüllt, die zu verschiedenen Teilen Calcium enthielt, wobei man die Beobachtung machte, dass das Röntgenbild des Knochens fleckig und verschleiert wurde, wenn die in die Knochenpräparate eingezogene Gelatine einen Gehalt von weniger als 0.5 % Calcium hatte. Die Ergebnisse der Untersuchung sprechen für die *Kienböck*'sche Theorie, bringen aber keinen Beweis zugunsten der Hypothese des Verfassers. Trotzdem kann die Möglichkeit nicht ausgeschlossen werden, dass die Veränderungen, welche die *Havers*'schen Systeme und die interstitiellen Lamellen bei akuter Knochenatrophie erleiden, sich nichtsdestoweniger unterscheiden. Die Richtigkeit dieser Annahme wird durch die histologischen Untersuchungen gestützt, die im zweiten Teil dieses Berichtes vom Verfasser beschrieben werden. Durch Behandlung der Ausgangspräparate von atrophischem und normalem Knochen mit einer besonderen Alizarin-Färbemethode war es möglich, in den Präparaten von akut atrophischem Knochen deutliche Unterschiede in der Färbbarkeit der Lamellen zu erzielen. Diese verschiedenen Tönungen liessen sich in normalem Knochen oder in Knochen mit chronischer Atrophie nicht sichtbar machen. Nach Mazeration des Knochens durch Antiformin-Behandlung waren die Kontraste noch deutlicher. Wenn man aber das Ausgangspräparat eines atrophischen Knochen dekalzifizierte und färbte, zeigte sich ein völlig homo-

genes Gelb. Alle obengenannten Färbungserscheinungen scheinen also mit den Knochensalzen in Verbindung zu stehen.

Eine histologische Untersuchung von Präparate bei akuter Knochenatrophie, bei denen cysten-ähnliche subchondrale Rarefaktionen sichtbar waren, beweist ebenfalls den Zusammenhang zwischen Knochenatrophie und dem Kreislauf.

L'influence des sels minéraux sur l'image radiographique dans « l'atrophie osseuse aigue », de *G. A. Landoff.*

RÉSUMÉ

Dans une communication antérieure l'auteur a déja émis l'hypothese selon laquelle les « flores » et les taches qui caractérisent les radiographies des cas d'atrophic osseuse aiguë, s'expliquent par une décalcification variable des différences parties de l'os. La structure osseuse et la nutrition différente des tissus jouent un grand rôle, les canaux de Haver qui sont les mieux nourris perdent leur chaux plus vite que les lamelles interstitielles, de sorte que des taches de densité différente se touchent et jusqu'à un certain point expliquent le « flou » de la radiographie.

Il est possible que « flou » est dû surtout à la dissolution des sels de chaux — de préférence à d'autres sels — qui se trouvent dans les tissus spongieux de l'os (*Kienböck*) ; l'auteur a fait des essais sur quelques os après traitement par l'antiformine, afin d'éliminer les tissus mous. La quantité approximative de sels de chaux dans les tissus mous a été déterminée deux fois : on a trouvé de 0.26 à 0.4 p. Ct. — Après le traitement par l'antiformine, l'os était ensuite trempé dans une solution de gélatine, contenant des sels de chaux dans des proportions variables ; chaque fois que le pourcentage de chaux était inférieur à 0.5, la radiographie était « floue » et montrait des taches. Le résultat parle en faveur de la théorie de Kienböck, mais ne prouve par l'hypothèse de l'auteur ; néanmoins la possibilité n'est pas exclue, que les transformations des canaux de Haver et des lamelles interstitielles dans l'atrophie osseuse aiguë ne soient les mêmes.

Les examens histologiques décrits par l'auteur à la fin de son rapport tendent à prouver que son point de vue est exact.

Par une technique spéciale de coloration des préparations d'os normal et d'os atrophié par de l'alizarine il a été possible de montrer des anomalies nettes dans la coloration des lamelles, quand il s'agissait de cas d'atrophie osseuse aiguë. Ces anomalies ne se produisaient pas, quand il s'agissait d'un os normal ou bien d'un os atteint d'atrophie chronique, elles devenaient plus nettes après traitement préalable par de l'antiformine.

Quand au contraire une préparation d'un os atrophié était décalcifiée avant coloration, on ne voyait qu'un jaune uniforme. Tous les phénomènes de coloration ci-dessus mentionnés semblent dûs aux sels minéraux contenus dans l'os.

L'examen histologique (microscopique) de préparations d'atrophie osseuse aiguë, avec raréfactions sous chondrales en nids, met en évidence les relations entre l'atrophie osseuse et la circulation.

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