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THE REPAIR OF EXPERIMENTAL FRACTURES DURING LONG-TERM ANTICOAGULANT TREATMENT

An Experimental Study on Rats

By

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Little has been written concerning the effect exerted by anticoagulant treatment on the healing of fractures. *Stinchfield, Sandaran & Samilson* published in 1955 their observations on the effect of heparin and dicumarol as regards the healing of spongy bone transplants. In their experiment, the consolidation of an autologous spongy bone transplant on the crest of the ilium was studied in rabbits and dogs. Both heparin and dicumarol caused delayed formation of new bone, with marked appearance of fibrous tissue. The delaying effect of anticoagulant treatment on the formation of callus was most clearly observable in the animals to whom both heparin and dicumarol were administered immediately after the operation. Later, *Minola, Nasta & Granato* (1958) found in their work concerning experimental radius fracture in rabbits that heparin and dicumarol produced a persistent fracture haematoma and a more marked and prolonged cartilaginous stage in the fracture repair. On the other hand, *Aulisa* (1960), in his studies of experimental tibial fracture in guinea-pigs, could not demonstrate any distinct differences between heparin-treated animals and controls.

Since the observations made by *Stinchfield et al.* may possess practical significance, attention has been paid in our Clinic to the potential effect of anticoagulant treatment on fracture repair. In addition to a clinical study (*Solonen* 1963), the present experimental study is intended to serve the purpose of clarifying the effect of long-term anti-

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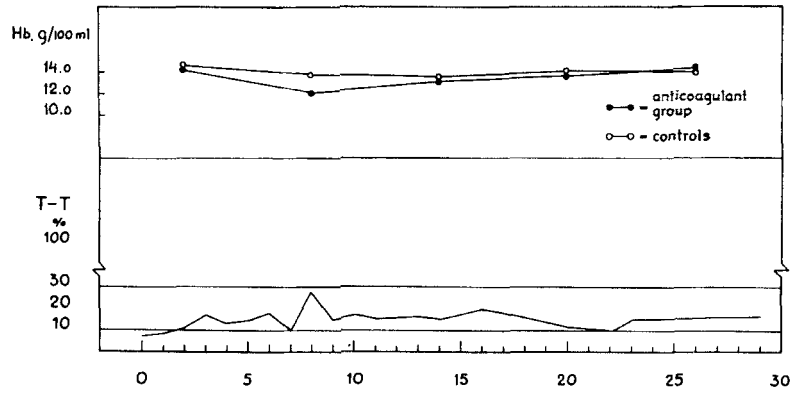

Fig. 1.

Haemoglobin values and prothrombin variations by Owren's TT method, in the test groups at different times after commencement of the anticoagulant treatment.

coagulant treatment on the healing of experimental diaphyseal fractures.

MATERIAL AND METHODS

In the experiments, 70 white, female rats of 5–6 months age and weighing between 138 and 180 g. were expended. The right tibia was fractured by hand in ether narcosis and no subsequent fixation was applied (cf. *Koskinen* 1959). Long-term anticoagulant treatment was given to 45 animals, the fracture being produced in 30 of them after commencement of this treatment, while in the case of the other 15 animals the anticoagulant treatment was started on the third day after the fracture. 25 animals constituted a control group. The animals were sacrificed and the fracture areas examined at intervals of six days, the oldest animals being sacrificed 30 days after the operation.

As *anticoagulant agent,* the preparation Sintrom® (Geigy) of dicumarol type was used. The effect of the anticoagulant treatment was checked by *Owren*'s (1959) TT method, which was found, in preliminary tests, to be applicable to rats. Initially, the TT variations were checked daily until a stable therapeutic level of 10–15 per cent had been reached. The haemoglobin values were checked at varying times after commencement of the anticoagulant treatment. No significant differences in respect of haemoglobin values could be established in comparison with the control group (Fig. 1).

After each animal had been sacrificed, *roentgenologic examination* of the fracture was carried out, and a print was made of the roentgenograph at 5 × magnification. For accurate determination of the callus size, the contour of the callus as seen in the print was traced by pencil and the area of the resulting figure was determined with the planimeter. For *histologic examination,* the specimen consisting of the fractured area was fixed in neutral formalin and decalcified by the EDTA method. Cuts made in the sagittal plane were stained by the haematoxylin-Weigert-van Gieson or the eosin-haematoxylin method. The histochemical method employed in this work was

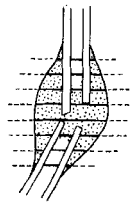

Fig. 2.
Illustrating the procedure in the line samling analysis of the callus components. The occurrence of the different tissue components was recorded along transects drawn at right angles to the proximal bone fragment, at intervals of one diameter of the microscope's field of vision. Depending on the size of the callus, the number of transects varied between 4 and 10.

Steedman's (1950) alcian blue staining in *Lison*'s (1954) modification. The methyl-metacrylate specimens, which had been subjected to autoradiographic and micro-radiographic examination, were ground down to 25–40 μ thickness and they, too, were stained with alcian blue upon removal of the metacrylate. The *line sampling method* devised by *Uotila* (1940) and by *Uotila & Kannas* (1952) is a histo-quantitative method previously employed by *Koskinen* (1959) in determination of the callus components. This method enables, with the aid of a microprojector, the different tissue components in the histological specimen under investigation to be quantitatively differentiated. The procedure employed in this investigation is illustrated by Fig. 2. On a number of transects drawn across each cut and spaced at distances equal to the diameter of the microscope's field of vision, the linear contribution of each of the following types of tissue components was recorded: new bone, cartilage in the process of becoming bone, hyaline cartilage, fibrous tissue, and bone cavities. The total transect length found for each component was then expressed in per cent of the sum of the transect lengths. A check of the method made by repeating the procedure a few times with one and the same specimen but with the lattice formed by the transects displaced by various fractional parts of the transect spacing, produced results on the strength of which the percentages found by one single determination as described can be considered reproducible within 3–5 per cent units.

Autoradiographic examination was undertaken in the case of 22 animals. They were given radioactive phosphorus P^{32} in the form of phosphate at a dosage of 1 microcurie per gram of body weight, by intraperitoneal injection 4 hours prior to sacrificing. Specimens of the fracture area embedded in methyl-metacrylate were ground down to about 80 μ and autoradiographs were made on Kodak Fast Autoradiographic Stripping Plates AR 10 with Kodak D 19b developer and 6 weeks exposure.

Microradiographic examination was performed on the same specimens as had been subjected to autoradiography. A Philips X-ray Apparatus type 11704 and Kodak Spectroscopic Plates 649 GH were used in this study.

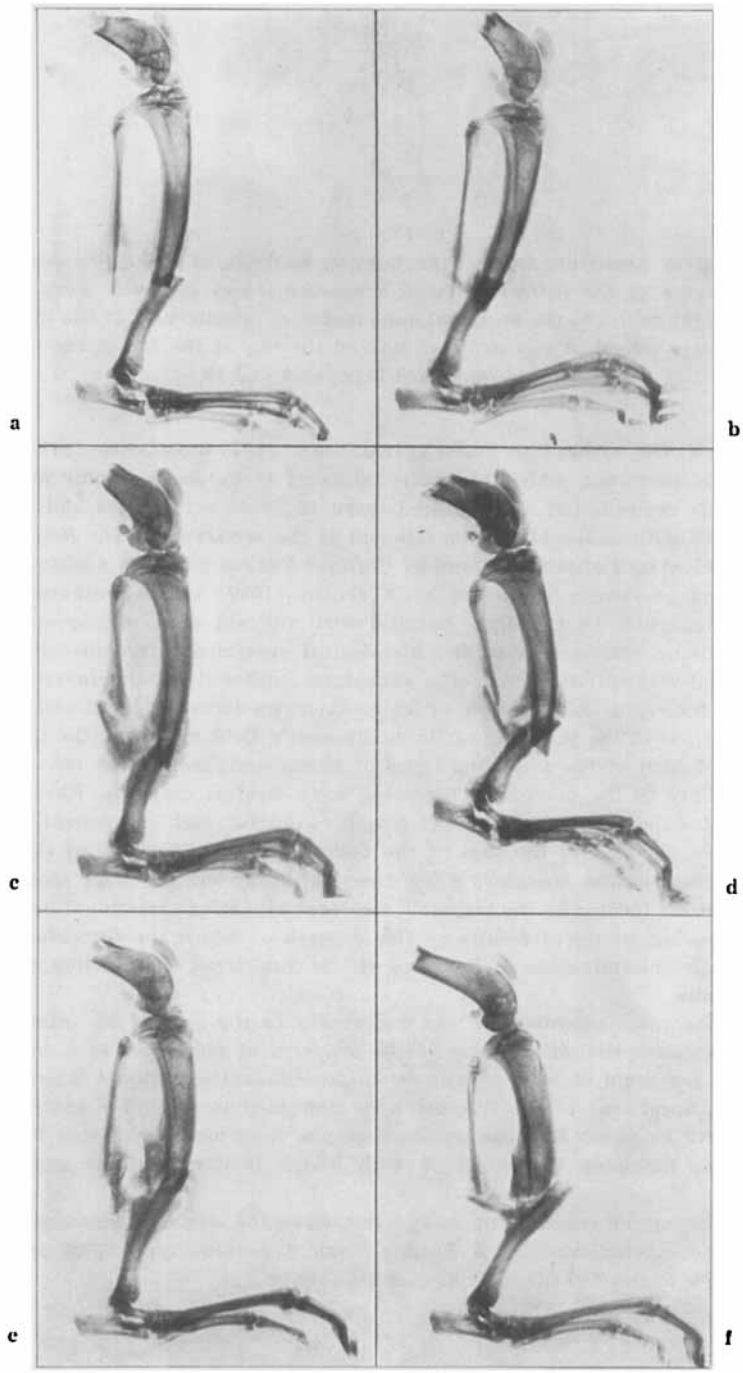

Fig. 3.

RESULTS

During the tests, two animals in the anticoagulant treatment groups were lost owing to haemorrhage.—In the test groups as well as in the control group the fractured extremity was as a rule sufficiently stable after 18 days to bear weight normally. In the anticoagulant treatment groups a larger size of the callus by palpation was found, as compared to the control group, although no distinct difference could be manually observed in the solidity of the fractured bone. Two instances of pseudarthrosis were noted in the control group, but none in the anticoagulant treatment group.

Roentgenologic Changes.

12 days after the fracture it was already observable in the roentgenographs that the callus area was clearly larger in extent in the anticoagulant treatment group than in the control group. The differences became even more marked in the further course of the tests (Fig. 3).

The fractures were similar in the different animals. In the subjects of the anticoagulant treatment groups a sizeable callus of loose structure was seen. There were occasional radiolucent spots and even changes resembling cysts (Fig. 4).

Measurements of the longitudinal extension of the callus by the roentgenographs revealed that this dimension was greater in the anticoagulant treatment group by 9 per cent on the anterior and by 29 per cent on the posterior side than in the controls. The differences between the groups as regards callus size are readily evident in the graphs showing the callus areas found by planimetration (Fig. 5).

In the test group consisting of animals whose fracture had been produced during anticoagulant treatment, the measured callus was nearly twice as large as in the control series. In addition the animals in whose case the anticoagulant treatment had not been commenced until three days after the fracture, showed a clearly observable deviation from the control series in this respect.

Fig. 3.

Roentgenographs of the fractured tibia of animals, 12, 18 and 24 days after the fracture, revealing a greater amount of callus in the animals under anticoagulant treatment (a, c, e) than in the controls (b, d, f).

Fig. 4.
Cyst-like radiolucent spots in the callus of an animal subjected to anticoagulant treatment. Roentgenograph taken 30 days after the fracture.

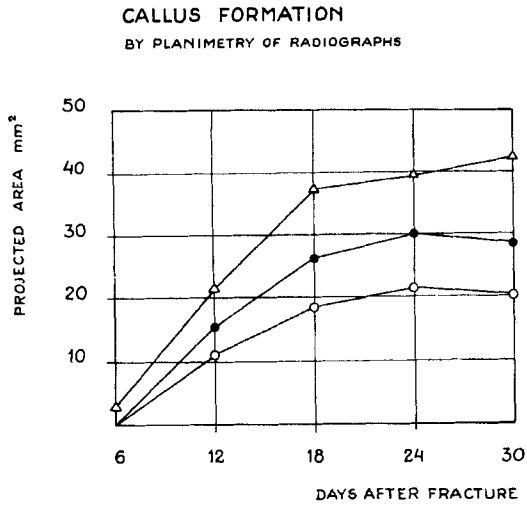

Fig. 5.
Area of the callus shadow in the roentgenographs, determined by planimetry.
△——△ Anticoagulant treatment in progress when the fracture was induced.
●——● Anticoagulant treatment commenced on the third day after the fracture.
○——○ Controls.

Histologic Changes.

In the subsequent histological description, the normal repair process of the bone encountered in the control group are presented, while with regard to the anticoagulant treatment groups only circumstances indicating deviation from this normal behaviour are brought to the fore.

In the control series, the ends of the fragments were surrounded after 6 days by a haematoma, which was in its turn encircled by fibrous tissue. The osteogenic layer of the periosteum was thick and proliferative close to the point where it was detached from the bone surface. In this collar of the periosteum new bone was already seen in scanty amount at this stage. Adjacent to it, some hyaline cartilage was seen and more peripherally fibrous tissue. In the ends of the fragments, some bone cavities were anuclear and the bone itself displayed poorer stainability. In the animals under anticoagulant treatment, the formation of new bone in the collar started as in the controls, but the associated hyaline cartilage was more abundant.

After 12 days signs of haematoma were still visible between the fragments in the control series. Formation of new bone was in progress closer to the centre, and the hyaline cartilage was scanty. The connective tissue zone observable on the margin of the callus was wide, and the periosteum was thicker than before. In the anticoagulant treatment groups ossification took place in the same area as in the controls. The callus was considerably richer in hyaline cartilage, and larger on the whole. The fragments were mutually connected in some places by a cartilaginous bridge in addition to the fibrous zone. It could also be seen that detachment of the periosteum had occurred over a wider area than in the controls.

After 18 days the callus in the controls was seen to be composed of new bone and fibrous tissue. Hyaline cartilage occurred but little. The callus was scanty in quantity and the process of ossification was moving into the area between the fragments. In one of the cuts, bony consolidation was seen between the fragments. In the anticoagulant treatment groups the callus was still greater and more hyaline cartilage-dominated. Ossification of the callus was in progress from both fragments outwards, though on the other hand independent centres of ossification could be observed on the marginal areas of the sizeable callus (Fig. 6).

After 24–30 days, still a scanty and mainly fibrous callus was encountered in the control series. The hyaline cartilage was exceedingly scanty. The fractures showed bony consolidation except for two cases, in which the fragments were joined merely by a fibrous callus. At the

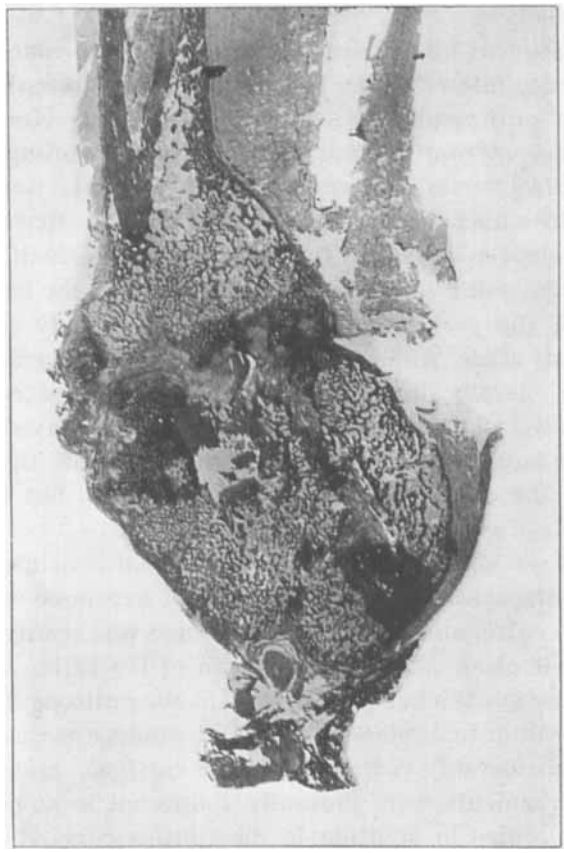

Fig. 6.
Histological micrograph of a specimen taken 18 days after the fracture, of an animal whose fracture had been induced during anticoagulant treatment.
Abundant cartilaginous tissue is seen in the large-sized callus.—Alcian blue stain, magnification 50 ×.

ends of the fragments anuclear areas of large extent were encountered, but new bone occurred in close association with this necrotic area. In the anticoagulant treatment groups even now hyaline cartilage could be seen in greater abundance than in the controls. The process of new bone formation had moved into the space between the fragments and in some cuts bony consolidation could be seen to have occurred in the marginal parts. Furthermore, independent centres of ossification with hyaline cartilage domination were observed.

Endostal formation of bone was scanty in all specimens, in the control as well as the anticoagulant treatment groups.

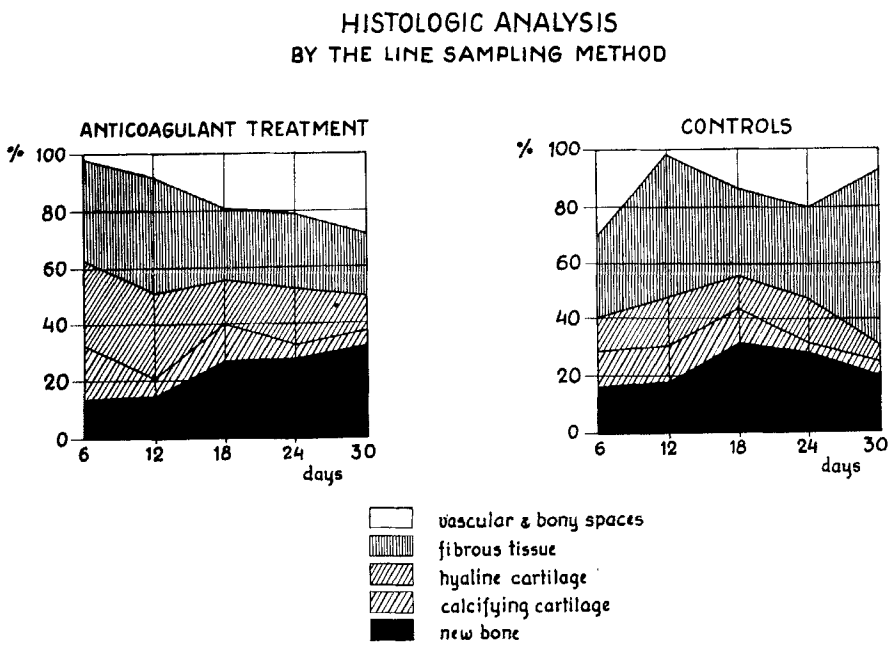

Fig. 7.

Proportions of the different tissue components in the callus of animals whose fracture was induced during anticoagulant treatment and of the controls. See text for comments.

Line Sampling.

The results relating to differentiation of the callus tissue by its various components, obtained on the basis of the line sampling measurements, have been plotted in Fig. 7 showing the means of the percentages found for several replicate animals. The group of rats whose tibia was fractured while they were already undergoing anticoagulant treatment is compared in the figure to the control group. In the controls, fibrous tissue accounts for the greatest share in the callus (30 per cent) after 6 days, and its contribution remains considerable throughout the period of observation. The combined percentage of hyaline cartilage and calcifying cartilage is virtually unchanged (about 25 per cent) during the first 18 days and decreases in the final stage of bone repair. The contribution of new bone is seen to increase during the period up to 18 days, after which its gradual decrease is noted, parallelling the decrease of calcifying cartilage. At 6 days in the anticoagulant treatment group, hyaline cartilage and calcifying bone present the highest contribution with 51 per cent; their share remains dominant up to 18 days (28 per

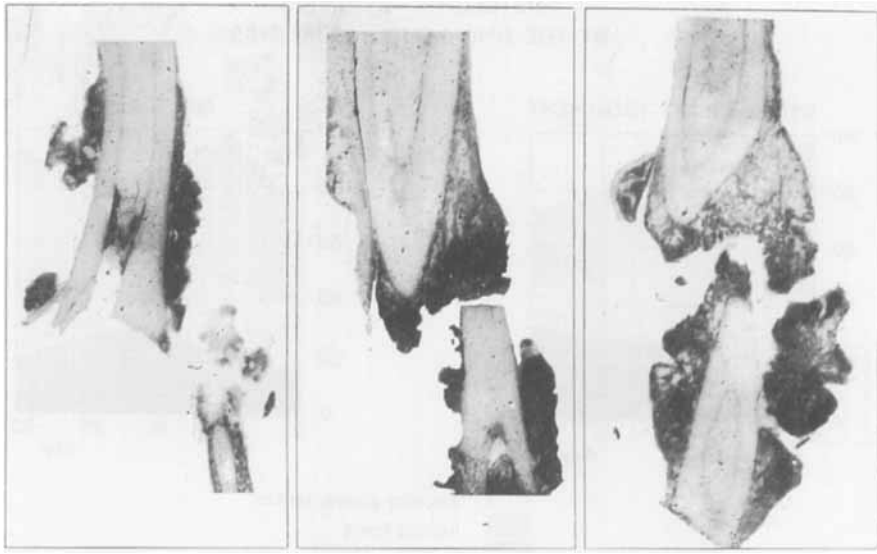

Fig. 8.
Autoradiographs made with the aid of radioactive phosphorus of the callus of animals whose fracture had been induced during anticoagulant treatment, 12, 18 and 24 days after the fracture. Accumulation of phosphorus in the initial stage in the area of the periosteal collar is clearly seen. Later, the highest deposit was observed in the centre of the callus.

cent), and the subsequent decrease of the cartilage components is seen in this group, too. The share of new bone in the entire callus shows a gradual increase throughout the period of observation. At 18 days closely equal proportions of the different components are seen in both groups.

Autoradiographic and Microradiographic Changes.

The deposition of minerals as demonstrated by the P^{32} phosphate, at 6 and 12 days, was subperiosteal in the collar. At 18 days, the highest uptake was seen to be mineral reaction bands on a level with the fragment ends of the fractured bone. In the anticoagulant treatment groups the uptake was found to be greater than in the control group. At 24 and 30 days, the highest uptake was seen still closer to the centre of the fracture area, though in less strength than at the earlier times (Fig. 8).

At microradiographic examination, 6 days after the fracture, incipient subperiosteal mineralization was seen in the region of the collar. It spread from there to the central callus area. At the same time an in-

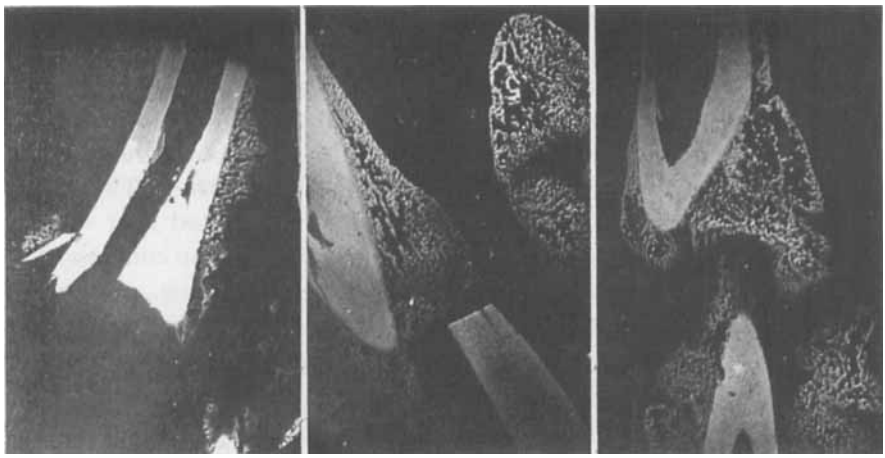

Fig. 9.

Microradiographs of the callus, made of specimens from the same animals as in Fig. 8.—The mineralization starts at a great distance from the ends of the fragments, proceeding at lesser strength towards the central area of the fracture. At the points where strong P^{32} uptake is seen (Fig. 8), the microradiograph reveals incipient mineralization.

crease in strength of the mineralization in the collar could be noted. However, the density of old bone was not attained during the entire period of observation. At a later stage mineralization of different degrees was seen in the specimens: dense bone trabeculae in the collar area, and reticular, less strongly mineralized tissue more centrally in the callus area. In the anticoagulant treatment group the initial stage of mineralization was consistent with that in the control group, but greatly different degrees of mineralization could be observed later at different points of the large callus in this group (Fig. 9).

DISCUSSION

Roentgenologic examination revealed distinct differences between the different test groups in size and structure of the callus. It could be demonstrated by planimetry that the area of the callus shadow was about twice as large in the case of the animals subjected to anticoagulant treatment as in the control series. Considering the entire volume of the callus, the estimate can be made that the callus in the anticoagulant treatment group was about seven times the size of that in the controls. The changes in callus size were most distinct with the animals

whose fracture had been produced while they were being administered anticoagulant, but it is an interesting observation that anticoagulant treatment instituted at a later time also results in a large callus formation. Measurements of the roentgenographs revealed that the new bone extended to a greater distance from the ends of the bone fragments in the anticoagulant treatment group than in the controls. This is obviously due to the fact that the periosteum is detached over a wider area in the animals of the test groups proper than in the controls. This phenomenon could also be seen at histologic examination.

In the roentgenographs radiolucent spots and cyst-like changes were observable in the animals subjected to anticoagulant treatment. Similar radiolucency has been observed with haemophiliacs, who often present considerable osseous changes (*Crock & Bony* 1960). In haemophilic persons these changes are considered to be caused by continuous periosteal and cortical haemorrhages (*Ghormley & Clegg* 1948). The radiolucent spots in the callus of the animals under anticoagulant treatment, too, are evidently due to persistent minor haemorrhages in the fracture area. These effusions of blood cause resorption of new bone trabeculae, which later results in the formation of cavities.

Both in the standard roentgenographs and in the microradiographic preparations the observation was made in all groups that the formation of new bone started at a great distance from the ends of the bone fragments, at the point where the periosteum could be seen, histologically, to have become detached from the bone. This is consistent with earlier reports (*Urist & McLean* 1941, *Ham & Harris* 1956). Subsequently, the mineralization of the callus moved gradually closer to the centre of the callus.

A notable observation in the present study was that of the abundant occurrence of cartilaginous tissue in the callus of the anticoagulant-treated animals. *Ham & Harris* (1956) have demonstrated in their studies concerning normal bone repair that the periosteum plays a decisive rôle in callus formation: immediately after the fracture, very strong proliferation of the two osteogenous layers of the periosteum takes place and it gradually builds a bridge from fragment to fragment. At points where the osteogenous cells happen to grow in well-vascularized surroundings, they change into osteoblasts while they become chondroblasts at points where the vascularization is less abundant. Cartilaginous tissue is thus a normal occurrence in the callus.

Direct ossification only occurs in the places where the vascularization is optimal. In other places the calcification of the cartilaginous tissue

is closely dependent on the penetration of new blood vessels into the callus (*Göthman* 1962). The abundant occurrence of cartilaginous tissue seen in the callus in the animals subjected to anticoagulant treatment and the slower maturation of the callus are obviously explainable on the basis of these observations. As a result of the anticoagulant treatment, an extensive fracture haematoma can be observed, and also fargoing detachment of the periosteum along the diaphyses. These circumstances seem to induce powerful osteogenic activity, which leads to the formation of a large-sized callus in the fracture area. Since the osteogenic cells, which even normally display lively growth, tend to outgrow the capillaries, it appears reasonable to assume that the vascularization in this large callus mass already remains deficient at an early stage. As a consequence of the poor vascularization, a considerable part of the osteogenous cells is then converted to cartilage. The observations are clearly in support of *Harris & Ham*'s (1956) contention that "the fracture haematoma appears to be more of an obstacle than anything else to the growth and fusion of the collars of osteogenic tissue that bring about union".

The high percentage of cartilaginous tissue and of cartilage in the process of calcification (51 per cent) in the callus during the initial stage of bone repair was clearly demonstrated in the anticoagulant treatment group by the line sampling measurements. The differences between this group and the controls diminished in the course of the period of observation, and very nearly the same composition of the callus was seen at 18 days, that is, at the time when most fractures appeared clinically stable. Subsequently, in the control series the share of new bone, of calcifying cartilage and of cartilage decreased as an obvious consequence of the remodelling of the callus. In the anticoagulant treatment group, however, the repair process continued further also after a period of 18 days and this, too, can be considered a sign of immaturity.—As regards the method of line sampling analysis, which was first applied by *Koskinen* (1959) as a means of studying the callus formation, it may be noted that the present modification with a number of transects examined in each cut, presented a clear and perspicuous picture of the occurrence of the tissue components in the entire callus area.

The autoradiographic and microradiographic examinations yielded results in support of the observations made in the roentgenologic, histologic and histoquantitative studies. It could be clearly demonstrated both in the anticoagulation treatment group and in the control group

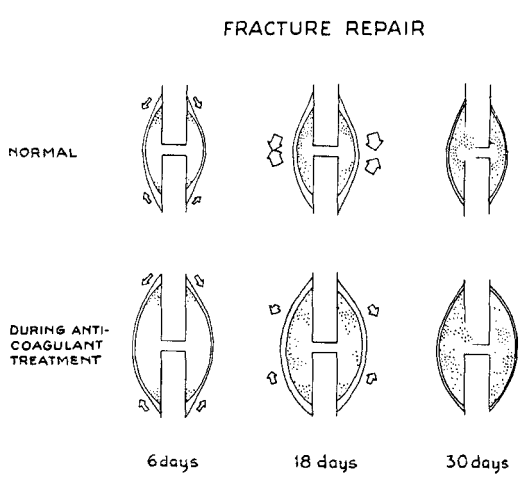

Fig. 10.

Schematic diagram illustrating the normal repair of a fracture and that during anticoagulant treatment.—Normally, the osteogenic activity of the periosteum creates an external callus. Ossification proceeds, in principle, accordingly as the callus is vascularized. The large fracture haematoma produced as a result of anticoagulant treatment leads to a large-sized callus, which needs a longer time to become vascularized. As a consequence, the bony consolidation, which takes place normally in other respects, is delayed.

that the process of ossification had its start in the periosteal collar, moving continuously closer to the centre of the fracture area in the course of time.

On the strength of the findings and inferences presented in the foregoing, a schematic characterization of the stages of progress occurring in the anticoagulant treatment group and in the control group is possible (Fig. 10).

Stinchfield et al. were the first to note the detrimental effect of anticoagulant treatment on the repair of experimental fractures. This effect was attributed either to deficient fibrin formation in the fracture haematoma or to histotoxic action. Neither conjecture finds support in the present observations. The differences in the callus found in the present study to have been produced by anticoagulant treatment are due, in the writers' opinion, to the extensive fracture haematoma, to the great size of the callus and to its poor vascularization. Consistent with the present findings is the abundance of cartilaginous tissue in the callus as a consequence of heparin treatment observed by *Minola et al.*

Stinchfield et al. advise caution in the administration of anticoagu-

lant agents to fracture patients. On the strength of their experimental observations, *Minola et al.* did not consider anticoagulant treatment detrimental to the bone repair process. Although the changes established in the present study do not justify inferences in respect of clinical work, the observation that prolonged anticoagulant treatment produces distinct changes in the callus at the site of the fracture in experimental animals should be kept in mind in clinical practice.

SUMMARY

The purpose of the study was to clarify the effect of prolonged anticoagulant treatment on the healing of fractures of the tibia. Sintrom® (Geigy) was administered as an anticoagulant agent by intramuscular injection to 45 rats with fractured tibia. The fracture was induced in one group while the animals were being given anticoagulant treatment, whereas such treatment was not started in the other group until the third day after the fracture. In a control group of 25 animals similar fractures were induced but no treatment applied. The period of observation was 6–30 days.

The *roentgenographic examination* revealed a callus of larger size and looser structure in the anticoagulant treatment groups than in the control group. Even cyst-like changes were encountered. *Planimetric study* showed that the callus in the anticoagulant treatment group was twice the size of that in the control group. *Histologic examination* demonstrated that ossification occurred in a normal manner in all groups, proceeding from the collar mainly subperiosteally, but in the anticoagulant treatment group the callus was found to be larger and more strongly cartilage-dominated. The abundant occurrence of cartilaginous tissue seems to be due to poor vascularization. Reshaping of the callus had already commenced in the control group 18 days after the fracture but had not yet started in the anticoagulant treatment group by the 30th day. This inference is also supported by the histoquantitative results yielded by the *line sampling method.* The *autoradiographic and microradiographic studies* gave evidence to the effect that the process of mineralization progressed from the periosteal collar towards the centre of the fracture in essentially identical manner in all test groups.

RESUME

Le but de l'étude était de clarifier l'effet d'un traitement anticoagulant prolongé sur la guérison des fractures du tibia. Sintrom® (Geigy)

a été administré comme agent anticoagulant par injection intramusculaire à 45 rats ayant un tibia fracturé. La fracture a été provoquée dans un groupe alors que les animaux étaient soumis à un traitement anticoagulant, tandis que le traitement ne fut entrepris dans l'autre groupe que le 3ème jour après la fracture. Dans un groupe de contrôle de 25 animaux, une fracture similaire fut provoquée, mais sans appliquer de traitement. La période d'observation a été de 6 à 30 jours.

L'examen radiographique révéla un cal de plus grande dimension et de structure plus lâche dans le groupe traité à l'anticoagulant que dans le groupe de contrôle. On a même observé des modifications ressemblant à un kyste. Une *étude planimétrique* montra que dans le groupe auquel a été adminstré le traitement anticoagulant, le cal a deux fois la grandeur de celui du groupe de contrôle. Un *examen histologique* a démontré que l'ossification se fait de manière normale dans tous les groupes, progressant du col, principalement subpériostalement. Toutefois, dans le groupe traité par anticoagulant, le cal était plus grand et plus fortement cartilagineux. La présence d'un abondant tissu cartilagineux semble être due à une pauvre vascularisation. La formation du cal commence dans le groupe de contrôle déjà 18 jours après la fracture, mais n'avait pas encore commencé dans le groupe traité au coagulant le 30ème jour, Cette constatation est aussi appuyée par les résultats histoquantitatifs obtenus par la *méthode de la ligne de suture*. Les *études autoradiographiques et microradiographiques* ont rendu évident que le processus de minéralisation progresse du périoste du col vers le centre de la fracture d'une manière essentiellement identique dans tous les groupes d'essai.

ZUSAMMENFASSUNG

Der Zweck dieser Untersuchung war es die Wirkung einer langdauernden antikoagulierenden Behandlung auf die Heilung von Tibiabrüchen aufzuklären. Sintrom® (Geigy) wurde als Antikoagulant mittels intramuskulärer Injektion an 45 Ratten mit gebrochener Tibia verabreicht. In einer Gruppe wurde der Bruch erzeugt während die Tiere antikoagulierende Behandlung erhielten, während in einer anderen Gruppe diese Behandlung nicht vor dem dritten Tage nach dem Bruche eingeleitet wurde. In einer Kontrollgruppe von 25 Tieren wurde ein gleicher Bruch erzeugt, aber keinerlei Behandlung gegeben. Die Beobachtungszeit war 6–30 Tage.

Die *röntgenographische Untersuchung* zeigte einen ausgedehnteren Kallus mit loserer Struktur in der mit Antikoagulantia behandelten

Gruppe als er in der Kontrollgruppe zu finden war. Sogar zystenartige Veränderungen wurden angetroffen.

Planimetrische Studien erwiesen, dass der Kallus in der Antikoagulantgruppe doppelt so gross als der in der Kontrollgruppe war.

Die histologische Untersuchung demonstrierte dass die Verknöcherung in allen Gruppen auf normale Weise vor sich ging indem sie vom Kragen hauptsächlich subperiostal fortschritt, aber in der Antikoagulantgruppe war der Kallus ausgedehnter und mehr von Knorpelbildung beherrscht. Das reichliche Vorkommen von Knorpelgewebe scheint mit der schlechten Blutgefässversorgung im Zusammenhang zu stehen. Neuformung des Kallus begann in der Kontrollgruppe bereits 18 Tage nach dem Bruche, während sie in der Antikoagulantgruppe noch nicht nach 30 Tagen begonnen hatte. Diese Beobachtung wird auch durch die Resultate, die sich aus der *Line Sampling Methode* ergeben, unterstützt. Die *autoröntgenographischen und mikroröntgenographischen Studien* bezeugten, dass der Prozess der Mineralisierung vom periostalen Kragen gegen das Zentrum des Bruches bei allen Untersuchungsgruppen wesentlich in gleicher Weise vor sich ging.

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