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INTERCELLULAR SUBSTANCES IN EXPERIMENTAL SCORBUTUS *

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The purpose of this report is to give the results of some simple experiments designed for the purpose of characterizing pathologically (on a histopathologic basis) the scorbutic condition. We have followed the histologic sequences in bone, connective tissue and teeth during the development of the absolute scorbutic condition and the immediate reparative processes following administration of antiscorbutics.

The pathology of human and experimental scorbutus has been extensively studied, and the main facts clearly established in a literature too extensive to be reviewed here. Hess,[1] in his admirable book, gives a good review of the pathology up to 1920. Special mention, however, must be made of the monograph of Aschoff and Koch [2] in 1919, though adequately quoted by Hess, and the monograph of Höjer.[3] The work of Aschoff and Koch is based on human postmortem material, that of Höjer principally on the experimental disease in guinea-pigs. Aschoff and Koch review previous contributions, and their paper deals largely with the changes in bone and cartilage found at costochondral junctions, and at the junctions of diaphyses and epiphyses. From previous work reviewed by them and from their own observations, the important features in the bone pathology may be enumerated as follows: cessation of new bone formation and rarefaction of existing bone of cortex and spongiosa; irregularities, absorption and disappearance of cartilage columns, yielding of the bone under strain and a zone of fragmentation

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1. Hess, A. F.: Scurvy Past and Present, Philadelphia, J. B. Lippincott Company, 1920.

2. Aschoff, L., and Koch, W.: Scorbut, Eine Pathologish-Anatomische Studie, Jena, Gustav Fischer, 1919.

3. Höjer, J. A.: Studies in Scurvy, Acta paediat., supp. **3**:8, 1924.

of bone trabeculae adjacent to the line of junction with cartilage. This is the "trümmerfeld" zone and actual separation by fracture may occur here. Hemorrhages occur. Osteoblasts assume elongated shapes and apparently disappear from regions of bone formation.

The marrow spaces of the shaft adjacent to the cartilage or trümmerfeld zone if that is present becomes filled with a loose textured connective tissue structure described as edematous or gelatinous. This is the "gerüstmark." In repair islands of bone formation make their appearance in subperiosteal regions and in the gerüstmark. Aschoff and Koch explain the condition as one of failure of osteoblasts to form osteoid tissue because of lack of essential materials. By inference they postulate a failure of cement substance in blood vessels, thus accounting for the hemorrhages.

Höjer's description of the bone changes does not materially differ from the foregoing, but he objects to Aschoff and Koch's hypothesis, and ascribes the deficient bone formation to . . . "a degeneration, or rather a receding of the bone forming cells, these being changed so as to form a qualitatively more and more degenerate as well as quantitatively more and more reduced bone, till their activity eventually leaves altogether." He therefore attributes the pathologic condition to defective function of cells, and excludes the factor of materials available by the osteoblasts. We believe that Höjer's interpretation was based on observations on animals in incomplete or partial scorbutus. Höjer's studies of changes in the incisor teeth were extensive and are beautifully illustrated. The incisors of rodents have "open roots" and continue to grow through the life time of the animals. Höjer's summary of the changes in teeth includes changes in morphology and arrangement of the odontoblasts, amorphous calcification of the predentin, new formation of bone instead of dentin by the odontoblasts, atrophy and resorption of pulp tissue, newly formed bone and the old dentin. In the healing of scorbutus he describes the "reorganization of the pulpa bone into irregular dentin, osteodentin with bone canals and dentinal canals."

We do not question the accuracy of Höjer's description. His findings are different in important respects from ours, as we found in the state of complete scorbutus no formation of "osteodentin" or pulp bone. We believe that his diets were not completely deficient, because we obtained tooth conditions answering to his descriptions only in guinea-pigs fed alternately on deficient and normal foods.

Höjer also demonstrates satisfactorily that in tuberculosis in scorbutic guinea-pigs there is much less collagen deposited by fibroblasts in the periphery of the tuberculous lesions. He also maintains without satisfactory presentation of evidence that there is in general an atrophy of collagen in connective tissue of various organs and in blood vessels, thus accounting for hemorrhages.

Our brief treatment of these two monographs is wholly inadequate for the credit they deserve for accuracy of description, illustrations and significance of conclusions. We have endeavored only to indicate how each has defined the problem of the pathogenesis of the scorbutic condition. Our own work was formulated and begun before Höjer's monograph appeared. While we have studied every tissue and organ from our animals, the present account concerns only the following observations made on guinea-pigs in the state of absolute scorbutus and during early repair following the administration of antiscorbutics: (1) sequences in the incisor teeth, (2) sequences in bones of growing guinea-pigs, (3) sequences in the repair of soft tissues and (4) sequences in the repair of bone injury.

Our results corroborate completely and extend the deductions of Aschoff and Koch, so that we characterize the scorbutic state as one due to the inability of cells of supporting tissues to produce intracellular substances and to maintain existing intracellular substances. In our descriptions of experiments we have purposely omitted histologic minutiae, and the account of structures not relevant to our thesis.

THE DIET

The diet employed by us consisted of soy beans, 50 parts; rolled oats, 29 parts; dried milk powder (Klim), 10 parts; brewers' yeast, 4 parts; butter, 5 parts; calcium carbonate, 1 part, and sodium chlorid, 1 part.

The soy beans were heated in the autoclave at 15 pounds for forty-five minutes as suggested by Mendel. The yeast (trade name Majestic) was obtained from the Liberty Yeast Company of Cambridge, Mass. The inorganic salts compensate the mineral deficiency of the cereal and legume. The ingredients were ground and the food thoroughly mixed, moistened with distilled water, rolled into thin sheets and dried in the incubator. Filter paper was given for roughage. This diet is deficient only in antiscorbutic substance. Eight cubic centimeters of orange juice daily was sufficient to afford complete protection against scorbutic symptoms. The animals ate on the average from 10 to 17 Gm. of the cracker daily. On this diet we have reared guinea-pigs after weaning to 3 or 4 years of age. Some of the animals attained a weight of more than 1,500 Gm. Reproduction also occurred normally on this diet.

The animals were kept in individual cages in a well lighted room with a nearly constant temperature. The cages and feeding dishes were kept scrupulously clean and the food given fresh twice daily.

The initial weight of most of the guinea-pigs used in our work was between 200 and 250 Gm.; a few weighed between 250 and 300 Gm.

SEQUENCES IN THE INCISOR TEETH IN SCORBUTUS AND IN REPA

These studies were made on sections through the skull at three lev each including the upper incisor teeth. The lower incisors were stu in sections at the level of the first molars. The material was fixe Zenker's fixative, decalcified in 5 per cent nitric acid, embedded in loidin and stained with hematoxylin and eosin. Sections for compari were occasionally stained with Van Gieson's stain and Mallory's c nective tissue stain.

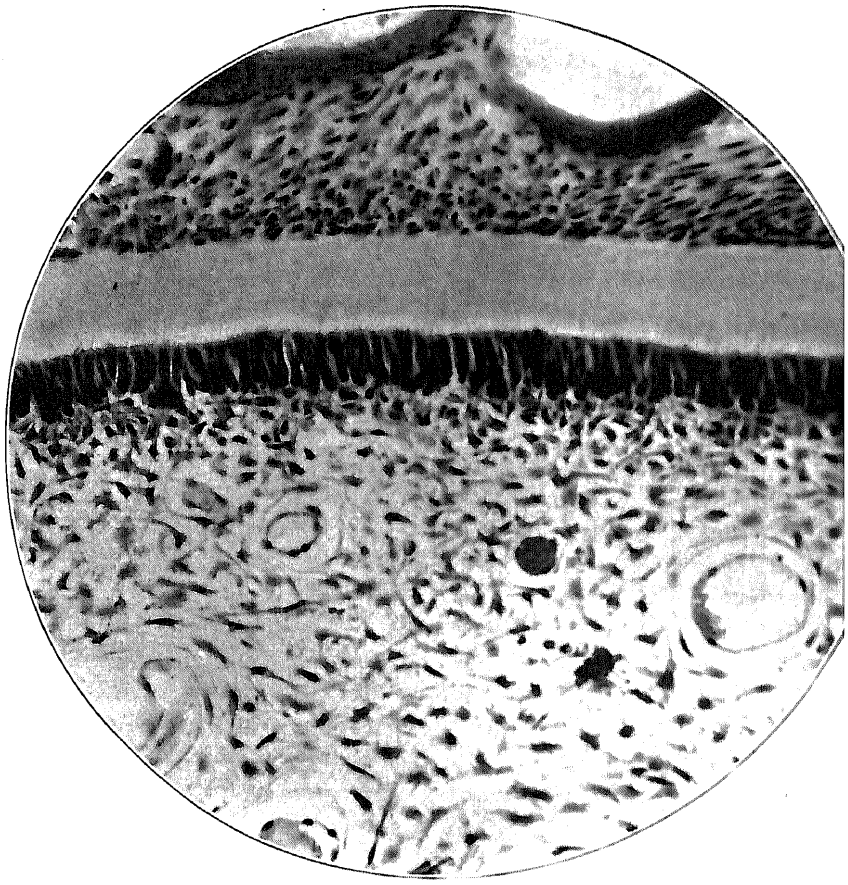

Fig. 1.—Detail of cross section of upper incisor of normal guinea-pig shown layer of odontoblasts, dentin and pulp. ×367.

In from seven to twelve days important changes were found in th odontoblast layer. As Höjer has pointed out, these changes occur fir at the apical end of the tooth. The normal odontoblast layer shown figure 1 consists of cells in orderly array and closely applied a homogeneously staining dentin. The earliest evidences of scorbut were separation of this layer from the dentin by a narrow margi

occasional irregular calcium deposits in the odontogenic zone (predentin) and irregularities in the odontoblast layer. The individual cells became smaller, and they stained more densely. The blood vessels in the adjacent pulp and capillaries in contact with and penetrating the odontoblast layer were more apparent through engorgement. In the odontogenic zone (predentin) there were occasional deposits of basic staining granular material interpreted as due to calcium salts, while between the

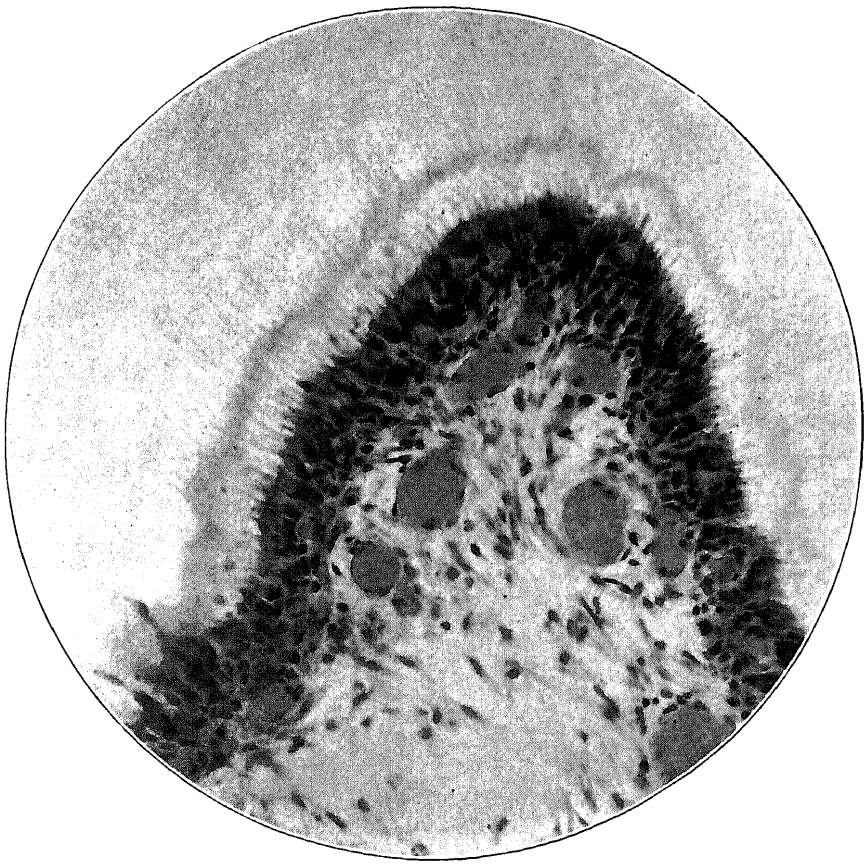

Fig. 2.—Incisor tooth showing early changes in dentin formation and beginning separation of layer of odontoblasts. Seven days on scorbutic diet. × 367.

processes of the odontoblasts evidences of continued dentin formation could be inferred because of the presence of globules of hyaline material which we interpreted as the matrix of spherites (fig. 2).

After a longer period, from twelve to fourteen days, we found the complete separation of odontoblasts from dentin. There was rupture of the processes of odontoblasts, and the spaces separating odontoblasts

and dentin contained no stainable material. Such spaces appeared first as vacuoles, and the conclusion that the accumulation of liquid material caused the separation was unavoidable (fig. 3).

The odontoblasts exhibited changes, the chief of which were diminution in size and increased density of staining. There were also changes in the pulp, edema in places and the deposit of finely granular material between the connective tissue cells possibly representing early deposit of calcium salts.

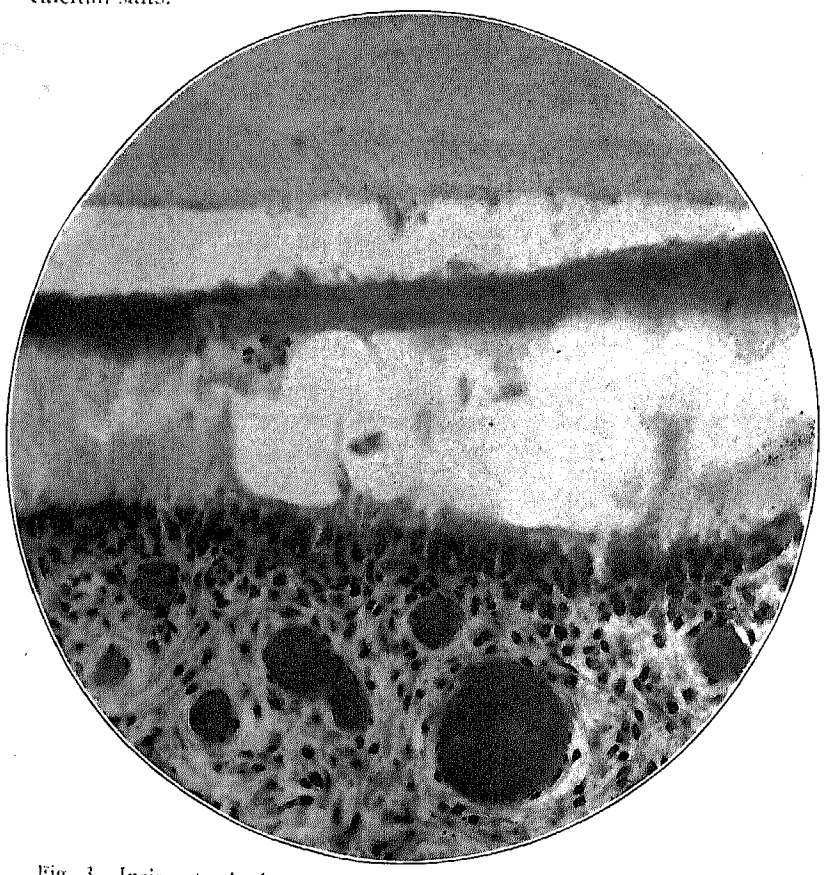

Fig. 3.—Incisor tooth showing separation of layer of odontoblasts and formation of vacuoles between dentin and odontoblast layer. The cleavage between dentin and zone of lime salt deposit is an artefact. The odontoblasts are diminished in size. Twelve days on scorbutic diet. ×367.

Finally, we had in absolute scorbutus the picture of a shrunken pulp completely freed from the dentin and apparently floating in a liquid material (fig. 4).

Contrary to Höjer, we found no formation of bone in the teeth in complete scurvy. The new formation of intracellular material matrix of bone and of dentin had ceased.

The administration of orange juice resulted in the prompt appearance of new dentin. In twenty-four hours, the administration of only 2 cc. of orange juice to a guinea-pig kept for twelve days on the scorbutic diet resulted in the formation of a zone of dentin on the separated odontoblasts. Eight cubic centimeters of orange juice daily for three days resulted in the complete filling of the space between dentin and odontoblasts in scorbutus of long standing (figs. 5 and 6). The newly formed dentin may be thicker than the original dentin and follows

Fig. 4.—Incisor tooth. Fourteen days on scorbutic diet. There is almost complete separation of the pulp from the dentin with rupture of the processes of odontoblasts.

the irregular contours of the odontoblast layer resulting from the scorbutic state.

Examination at twenty-four and forty-eight hour intervals after the addition of orange juice to the diet showed that the filling of the space by dentin proceeded from the surface of the osteoblasts outward. The rapidity of its formation, however, and its appearance before any discernible restoration of the odontoblasts to normal size and staining reac-

tions indicate that the process is one of setting or jelling of a liquid material. On this basis we may assume that the liquid separating odontoblasts and dentin in absolute scorbutus is a defective product of odontoblasts secreted in excess of the normal rate. This explanation must be accepted to account for the large volume of dentin as compared with the original dentin produced in so short a period. The missing factor or agent which the antiscorbutic enables the odontoblasts to supply is evidently one effecting the jelling or setting.

Fig. 5.—Incisor tooth. Fourteen days on scorbutic diet followed by three days with addition of orange juice. The guinea-pig was killed seventy-two hours after the first administration of orange juice. Note the newly formed dentin.

SEQUENCES IN BONES OF GROWING GUINEA-PIGS

The ribs of guinea-pigs gave us the best material for the study of scorbutic sequences because they became completely decalcified in twenty-four hours in Zenker's fixative, and because paraffin embedding and thin sectioning was possible. Accordingly, our most careful studies were made on ribs. A modified Giemsa stain was employed for routine, as we found it gave results superior to hematoxylin-eosin in the study of

cells, calcium salt deposits and matrices of cartilage and bone. Van Gieson's stain, Mallory's phosphotungstic acid hematoxylin and connective tissue stain were also used on selected material.

In addition to the ribs, we made studies from forty-five guinea-pigs on the bones of the skull, the scapula, the humerus including the shoulder joint, the femur including the hip joint and the femur and tibia including the knee joint. All this material was decalcified in 5 per cent nitric acid embedded in celloidin and stained with hematoxylin-eosin.

Fig. 6.—Incisor tooth. Nineteen days on scorbutic diet followed by seven days with addition of orange juice. The guinea-pig was killed seven days after the first administration of orange juice. The new dentin is irregularly traversed by processes of odontoblasts not continued into the old dentin. × 300.

The observations we record as of importance, although made on study of the ribs, were confirmed by the study of the other material. The sequences in costochondral junctions in growing guinea-pigs are qualitatively the same as those in the epiphysial line region of other bones.

We make no attempt to improve on the objective descriptions of the bone histology in scorbutus. We limit ourselves to observations on the

periosteum and growth of bone in relation to cartilage, in complete scorbutus and early reparative response to antiscorbutic treatment.

The Periosteum.—The rarefaction (osteoporosis) of cortical bone is an established observation in experimental as well as in human scorbutus. After from seven to nine days of the scorbutic diet, an increase in spindle-shaped basic staining cells was found in the

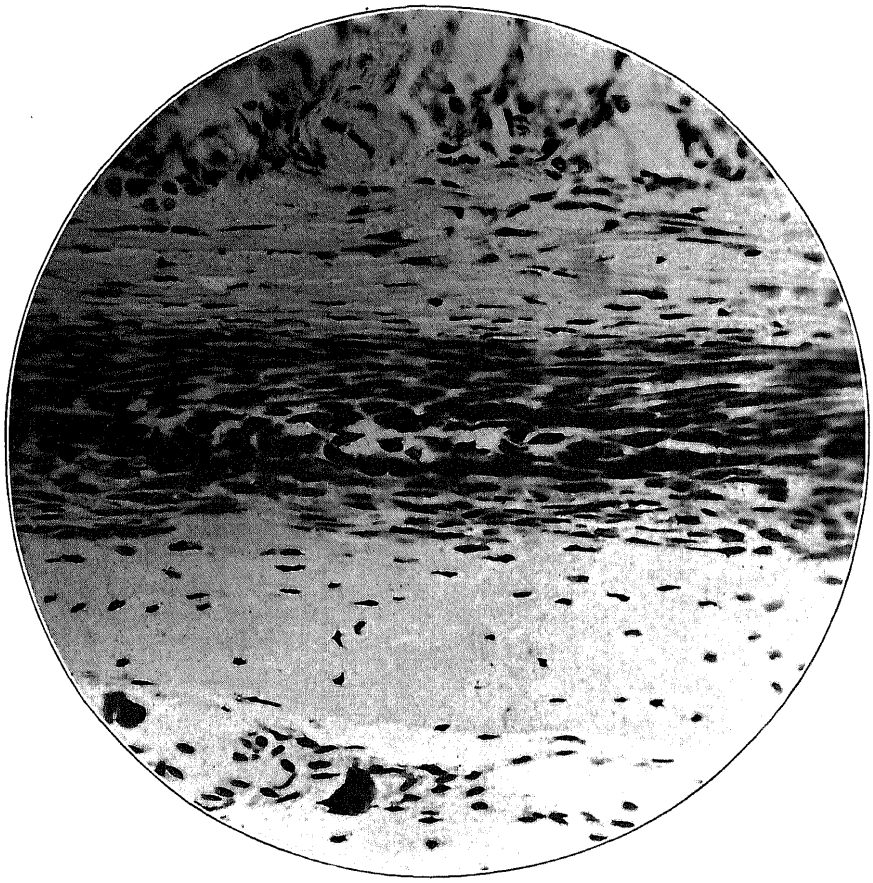

Fig. 7.—Periosteum. Rib of guinea-pig twelve days on scorbutic diet followed by a single dose of 2 cc. of orange juice and killed twenty-four hours later. There has been an increase of spindle shaped osteoblasts, some of which have formed bone matrix following the administration of orange juice. × 266.

osteogenic zone of the periosteum. Multiplication of these cells continued by mitotic division up to the twelfth to the nineteenth day. These accumulations of cells were not uniformly distributed on the individual bone. They have been observed in ribs, jaws, scapula, pelvis, long bones and skull. One determining factor we believe to be

mechanical stress, because the accumulations were greatest where there were muscular attachments. These cells were without intercellular substance. The addition of orange juice to the diet in twenty-four hours produced a change in the morphology of these cells and the deposition of a homogeneous matrix between them (fig. 7). Continuation of the orange juice for from three to six days was sufficient to yield

Fig. 8.—Rib showing cortex and periosteum with recent bone formation. From a guinea-pig kept for ten days on the scorbutic diet followed by six days with the addition of orange juice; killed on sixth day after the first administration of orange juice. × 367.

a fairly extensive layer of osteoid tissue (fig. 8). In as short a period as six days trabeculated new bone was present.

Repeated observations of the foregoing sequences in all the situations mentioned compel the conclusions that for a period after the state of complete scorbutus is established the osteoblasts on the surfaces of

growing bone continue to multiply in the form of spindle-shaped cells, unable to produce intercellular material, and that the administration of antiscorbutics is followed by a prompt formation of bone matrix and the resumption of osteoblast morphology. We shall not endeavor to describe the end-result of continued scorbutus in the periosteum, because of the complicated picture resulting from hemorrhages, and because it is not pertinent to the present thesis.

Fig. 9.—Costochondral junction of a guinea-pig after twelve days on the scorbutic diet. The drawing shows an increase in number and a change in the morphology of the osteoblasts adjacent to a bone column in continuity with the cartilage. Resorption of the bone is also apparent. × 675.

Costochondral Junctions.—After from seven to nine days on the scorbutic diet, there was an increase in the number of osteoblasts applied to the ossifying cartilage columns (fig. 9). As in the case of the periosteum, the osteoblasts became spindle-shaped, were intensely basic in staining reaction and were frequently in mitotic division. The proof

that these spindle-shaped cells were osteoblasts was furnished by the fact that they produced bone matrix after the giving of orange juice to a companion scorbutic guinea-pig.

In a later stage, from twelve to sixteen days, on the scorbutic diet, these cells of osteoblastic origin were found at a considerable distance

Fig. 10.—Costochondral junction of a guinea-pig after nineteen days on the scorbutic diet. The deeply stained structures are remnants of cartilage columns. The marrow has now assumed the appearance of the gerüstmark of German authors. It is impossible to distinguish between osteoblasts and fibroblasts. × 540.

from the cartilage columns. Mitotic figures continued to be numerous. The succession of histologic appearances between the seventh and twelfth to sixteenth day of diet indicated that the osteoblasts migrated from

their original position and assumed the shapes of fibroblasts; meanwhile mitotic division continued (fig. 10). During this period there was resorption of the bone deposited on calcified cartilage columns. The source of the cells composing the gerüstmark (framework marrow) is thus seen to be from osteoblasts. The appearance of the gerüstmark is that of a loosely textured connective tissue frequently described as edema-

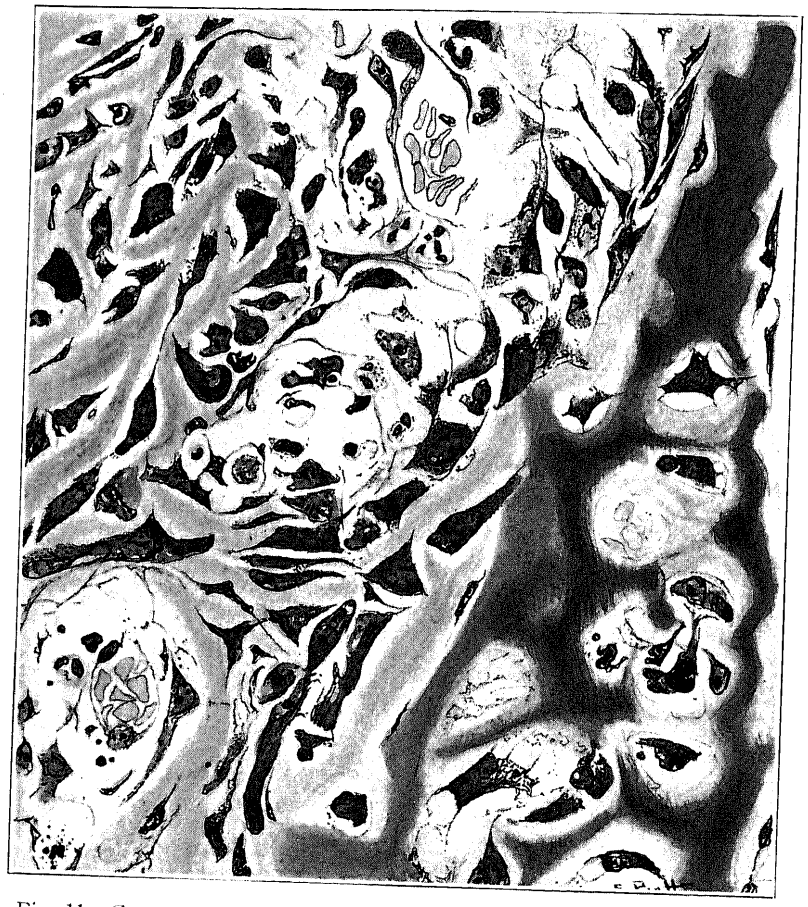

Fig. 11.—Costochondral junction of a guinea-pig after fourteen days on the scorbutic diet and three days with the addition of orange juice. Killed three days after the first administration of orange juice. There has been a deposit of bone matrix between the cells of the gerüstmark, thus proving the osteoblastic origin of the cells. × 675.

tous or myxomatous in appearance. This loose texture we believe to be caused by the presence of a liquid intercellular material. The cells have all the appearance of fibroblasts, including fibroglia fibrils. Red blood corpuscles, possibly by diapedesis, are frequently present between the

cells of this gerüstmark. The blood corpuscles do not undergo phagocytosis. They become enveloped in a material the nature of which is disputed. Aschoff and Koch have called it fibrin. Höjer, discussing it at length, called it bone. This material if fibrin would seem too abundant to have accompanied the number of red corpuscles present. As hemosiderin is a product of phagocytosis, the absence of pigment is of no importance in the discussion. Strands of fibrin are present in this material and stain densely blue with the phosphotungstic acid hema-

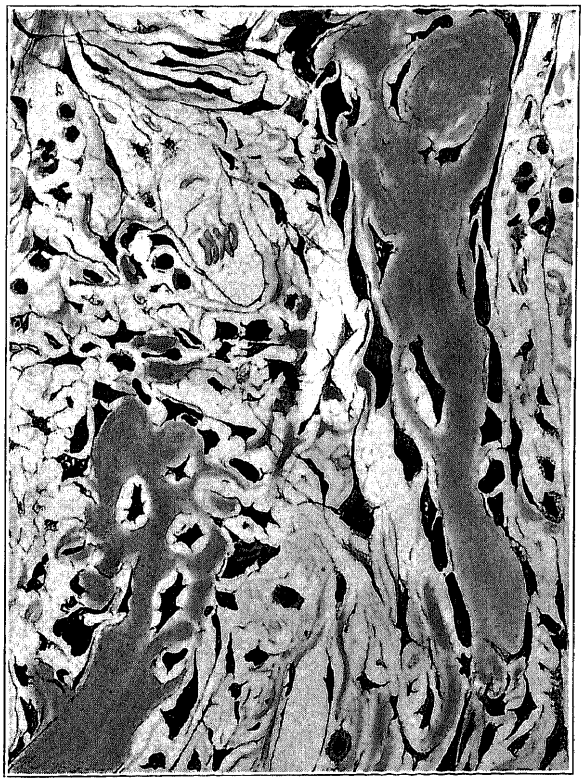

Fig. 12.—Costochondral junction of a guinea-pig after twenty-five days on the scorbutic diet followed by the addition of orange juice for six days. Killed six days after the first administration of orange juice. Bone trabeculae have formed in the gerüstmark; resorption of some of the newly formed bone matrix is in evidence. × 540.

toxylin stain. In addition to the fibrin, there is a homogeneous light brownish staining matrix comprising this material. The most abundant deposit of this fibrin and matrix was found adjacent to the cartilage after the nineteenth to the twenty-third day. Its occurrence coincided with the period of rapid dissolution of cartilage preceding and causing the infractions so common in scorbutus.

Our explanation is that this material has as its basis a product of the cells of the gerüstmark, probably liquid until added to by materials from blood plasma or cartilage matrix resorption. The amount of fibrin varies, but it is always considerable after infraction. We cannot agree with Höjer that it is of the nature of bone matrix, because following antiscorbutic treatment we found bone matrix deposited about this material.

The dissolution or resorption of cartilage columns, which we have constantly observed during the third week, was followed in the fourth

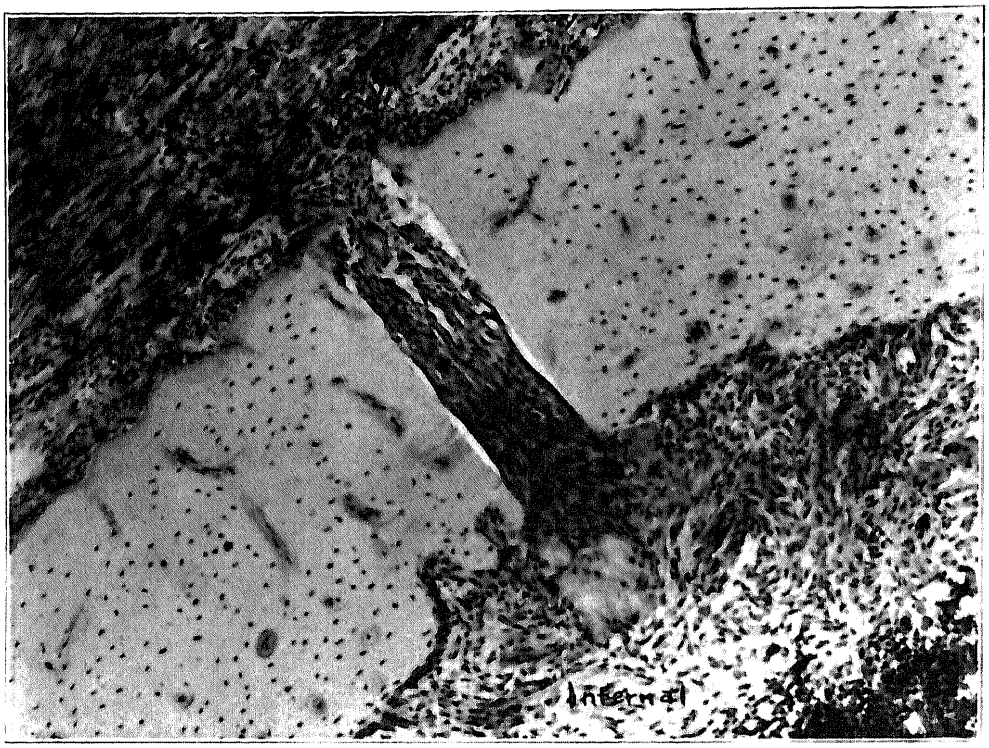

Fig. 13.—Incision of shaft of femur. Operation on tenth day on scorbutic diet. The guinea-pig was killed at the end of six days. In this instance there was only slight damage to the marrow, so that the bone defect has filled with fibroblast-like cells unaccompanied by capillaries and without intercellular substance. × 300.

week by a similar process in the cartilage distal to the costochondral junction. This breaking down of the cartilage matrix begins in the center of the rib, and results in a cavity without cells filled with basic staining granular material.

The administration of orange juice after extensive building of gerüstmark was followed promptly by the deposition of bone matrix

between the fibroblast-like cells. This was a diffuse process, as seen at the end of three days' treatment with orange juice (fig. 11). By the sixth day there was a definite structure or architecture of osteoid and osseous trabeculae, between which we found evidence of resorption of some of the bone matrix first laid down (fig. 12). Percisely analogous sequences occurred simultaneously in the periosteal new bone formation. We did not carry our studies over a sufficient period to follow the slower reparative responses of cartilage.

We may summarize the effects of the scorbutic state on growing bone by the statements that the production of the matrix of bone ceases and that maintenance of existing bone matrix is interfered with, as shown by the osteoporosis. The appearance of the gerüstmark and the sequences following antiscorbutic treatment are best interpreted in the light of the sequences observed in the teeth to the effect that the osteoblasts continue to produce a defective product, liquid in nature. The proliferative activities of the osteoblasts are not diminished, and probably are augmented over a considerable period of time.

SEQUENCES IN THE REPAIR OF SOFT TISSUES

Two experiments furnished material for the study of repair by organization.

One experiment was that planned for the study of the repair of bone injury. Twelve guinea-pigs all weighing between 160 and 190 gm. were kept on the deficient diet for ten days. On the eleventh day, under ether anesthesia, the left femur of each was exposed, and a thin slit parallel to the long axis was sawed through the cortical bone by means of a small rotary dental saw 0.09 mm. thick driven by a dental engine. The wound was closed by skin sutures only. After the operations, six of the guinea-pigs were kept on the deficient diet, and six were given 8 cc. of orange juice and a liberal supply of lettuce daily in addition to the scorbutic diet. We thus had the opportunity to follow repair in scorbutus and under the influence of antiscorbutic treatment at the same time intervals. One guinea-pig of each set of six was killed at the end of two, four, six and nine days when it became apparent from the gross observations that the period of important sequences had passed.

The other experiment began with guinea-pigs from 400 to 600 Gm. in weight after twelve days on the scorbutic diet. The operation was devised with the intention of following repair of cartilage and the process of filling a defect by organization. The operation consisted in cutting a wedge-shaped piece from the ensiform, exposed and pulled into view through an incision made over the lower end of the sternum. After removal of the piece the ensiform cartilage was allowed to slip

back into position and the skin and muscle were sutured. We followed the repair at three, six and ten day intervals in guinea-pigs kept on the deficient diet and in those treated with orange juice and lettuce as in the first experiment.

In the first experiment, slight gross differences only were noted in the healing of the skin incision. All of the wounds remained dry, and there was no sloughing. The crust over the skin incision remained longer in the animals on continued scorbutic diet. In the second experiment, no difference was noted in gross until the sixth day, after which the wounds of the guinea-pigs on continued scorbutic diet showed evidence of failure to heal somewhere along the line of incision. By the tenth day the wounds of those on antiscorbutic diet were completely healed, while two remaining on the scorbutic diet presented ulcers, one discharging pus, the other dry and crusted.

The material from the first experiment was fixed in Zenker's fixative and was embedded in celloidin after decalcification in 5 per cent nitric acid. That from the second experiment was similarly fixed, but was embedded in paraffin without having been treated in nitric acid, so that fine details could be followed and special stains applied. Mallory's connective tissue and phosphotungstic acid hematoxylin stains were of great value in following and contrasting the formation of collagen in the treated and untreated guinea-pigs.

The Epidermis.—We could detect no difference in either experiment between the treated and untreated guinea-pigs as regards the regenerative activities of the epidermis. In the guinea-pigs kept on the scorbutic diet, the epidermis responded as promptly as in the controls, and when apposition was poor, covered the cut surfaces of the corium by down growth and massing of cells. In every instance in the first experiment the epidermis completely covered the wound and bridged over gaps filled with blood and fibrin into which fibroblasts had penetrated, but in which there was no blood vessel formation. In fact, owing to failure of organization of the defect in corium and deeper tissues, the products of epithelial proliferation were greatest in the animals kept after operation on the scorbutic diet.

Cartilage.—In the second experiment there was complete absence of reparative activity on the part of the ensiform cartilage in the guinea-pigs of both sets—those kept on scorbutic diet and those receiving antiscorbutic treatment following the operations.

Mononuclear Phagocytes.—In both experiments we found no difference in the activities of mononuclear phagocytes (endothelial leukocytes) in the reaction to various types of material. Foreign body giant cells appeared as promptly in guinea-pigs kept on scorbutic diet as in those

receiving treatment, and were present in the first experiment in both sets forty-eight hours after the operation, in response to bone fragments, fat crystals and degenerated tissue elements.

Skeletal Muscle.—The durations of both experiments were too short to follow to the end reparative sequences in muscle. As far as the early stages of regeneration of injured muscle fibers, there were no differences. Multiplication of nuclei of the sarcolemma was as prompt in occurrence and as voluminous in untreated guinea-pigs as in the treated.

Repair by Granulation.—In both experiments we could find no difference between treated and untreated guinea-pigs in the promptness and volume of fibroblastic proliferation, though striking contrasts were present in collagen formation and in growth of capillaries. In the guinea-pigs receiving antiscorbutic treatment after operation, organization by fibroblast and vascular ingrowth proceeded in normal fashion.

In the second experiment as early as the third day following operation in the guinea-pigs receiving antiscorbutics, there was new capillary formation. Fibroblastic proliferation was active, and collagen had been formed by cells which had penetrated fibrinous exudate and blood clots. In the companion guinea-pig kept on the scorbutic diet, fibroblastic proliferation and migration were equally active, but there was no trace of collagen formation and no new capillary formation. Efforts toward capillary formation were evident in capillaries and vessels of precapillary size in fat tissue adjacent to the operative defect in that endothelial cell mitoses in situ were numerous and because endothelial cells accumulated in the lumina of these vessels and outside of them as clumps or buds without arrangement.

In both experiments in the guinea-pigs kept on the scorbutic diet healing proceeded by avascular organization. Fibroblasts penetrated the defects filled with blood clot or fibrin and continued to divide by mitoses. These fibroblasts in some instances produced no demonstrable collagen; in other guinea-pigs, notably those with the bone operation, a small amount of collagen was deposited, so that by the ninth day following operation a cellular cicatrix resulted in which only traces of collagen could be demonstrated. The fibroblasts in this repair in complete scorbutus have easily demonstrable fibroglia fibrils, a fact strongly indicative of a chemical composition unlike that of collagen.

In both experiments for the study of repair, sections of the skull were also made through the incisor teeth from each guinea-pig. The study of these sections brought to light the interesting fact that in the guinea-pigs kept on the scorbutic diet, some new formation of dentin followed the operation. This interruption of the sequences in the progress of absolute scorbutus proves that antiscorbutic materials are

liberated in the destruction of tissue. Therefore the formation of collagen by fibroblasts in the repair of soft parts injured in the bone operation procedure may be accounted for, in part at least.

SEQUENCES IN THE REPAIR OF BONE INJURY

In the guinea-pigs with antiscorbutics added to their diet after the operation, described above, repair of the bone defect proceeded with rapidity. At the end of the fourth day the gap was filled by fibro-

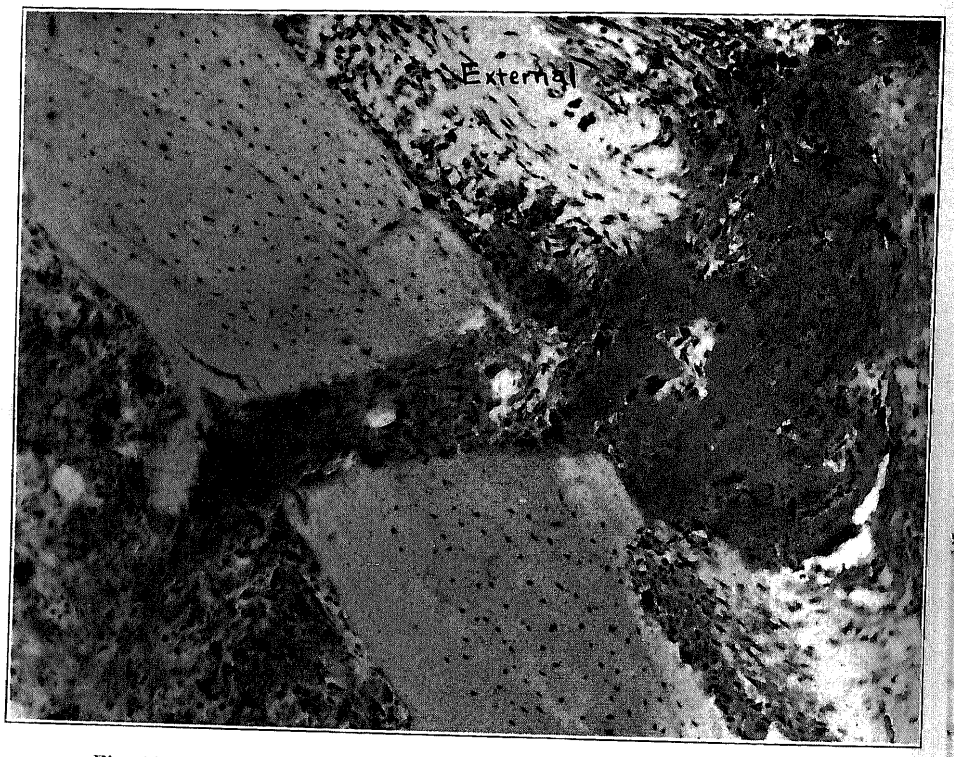

Fig. 14.—Same as figure 13 but ten days following the operation, scorbutic diet continued. There is almost no repair. The bone defect is invaded by a few spindle shaped cells. On the external surface is shown the fibrin-like substance discussed in the text. × 300.

blast-like cells accompanied by capillaries. The formation of an internal callus was in progress, and osteoblasts had begun to deposit bone matrix on the cut edges of the bone, extending outward from the internal callus (fig. 15). At the end of the sixth day, we found abundant external and internal callus formation and the defect nearly filled with new bone matrix incorporating a single row of osteoblasts on each side of the gap. New bone was separated from the cut edges of old bone by a thin line of

finely granular material, probably derived from fibrin but now staining bluish with Mallory's connective tissue stains, purplish with hematoxylin and eosin.

At the end of nine days, the new bone has practically filled the gap. Several rows of osteoblasts are incorporated on each side of the gap in the new bone. New and old bone are still sharply demarcated, evidence we believe in attributing the sole source of bone formation to osteoblasts from endosteum and periosteum (fig. 16).

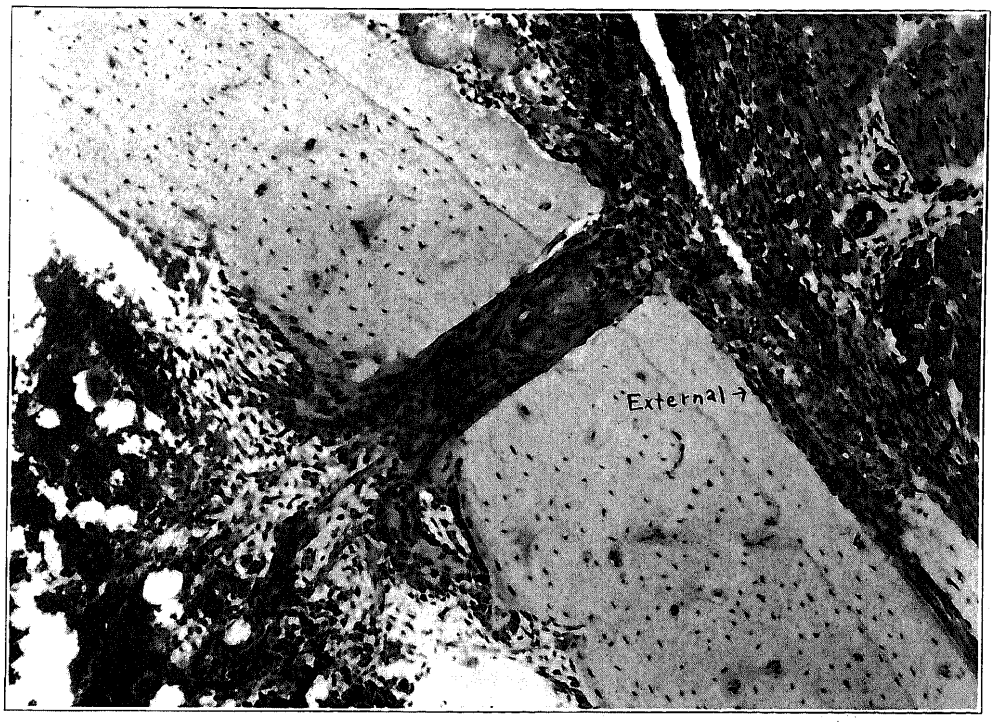

Fig. 15.—Incision of shaft of femur. Operation on tenth day on scorbutic diet, continued for four days with the addition of orange juice. The guinea-pig was killed four days after the first administration of orange juice. There is an early internal callus formation. The bone defect is filled with cells, presumably chiefly osteoblasts accompanied by capillaries. × 300.

In marked contrast, in the guinea-pigs kept on the scorbutic diet no new bone formation is found at the conclusion of the experiment at the end of the ninth day after operation. In the guinea-pig killed seven days after the operation the gap in the bone was filled with fibroblast-like cells without trace of collagen and unaccompanied by capillaries (fig. 13). This avascular organization was possible in the animal because the cut was sawed with minimum injury to the marrow. In the other untreated

guinea-pigs there was no repair of the bone gap. At the end of nine days, we found the defect in the bone filled with blood corpuscles and fibrin penetrated by a few fibroblast-like cells. Cells having the appearance of osteoblasts were absent, and there was no suggestion of bone matrix formation (fig. 14).

The soft tissues external to the bone react as has been described in the account of healing of soft tissue. After six days, we found a homogeneous fibrin-like material in immediate contact with the bone similar to that which we discussed in our account of the sequences in

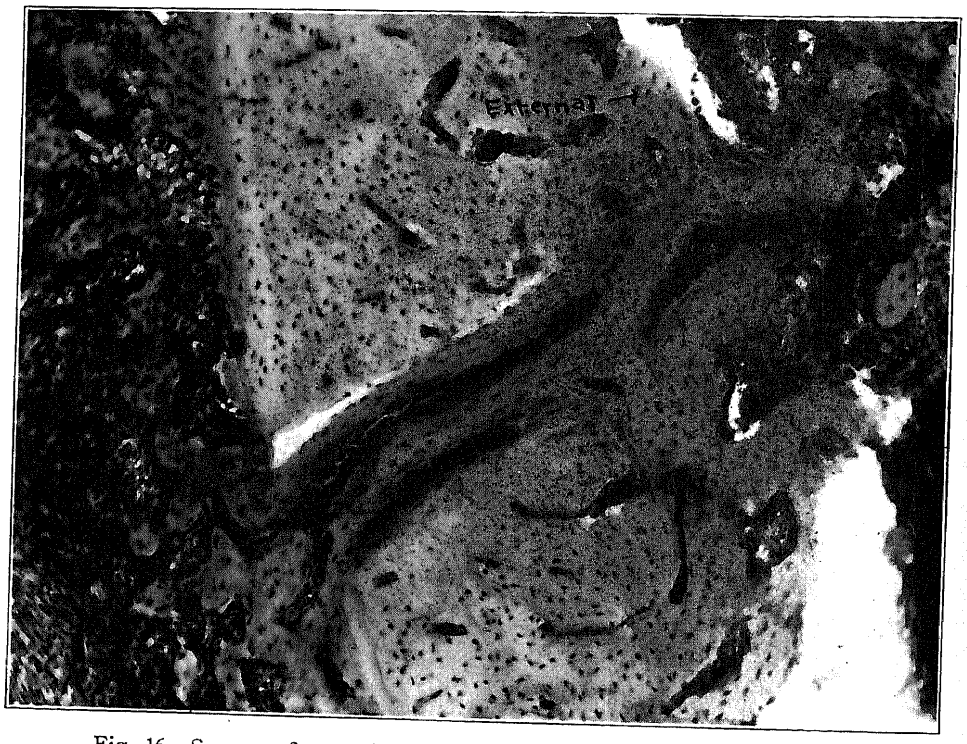

Fig. 16.—Same as figure 15 but ten days following the operation; orange juice addition to scorbutic diet. The bone defect is nearly filled with newly formed bone; there are well developed internal and external callus formations. × 300.

growing bones and which is found at epiphysial lines and costochondral junctions. It was called fibrin by Aschoff and Koch and homogeneous bone by Höjer. This material, although containing fibrin threads, consists of a homogeneous matrix already noted as staining brownish with phosphotungstic acid hematoxylin. With Mallory's connective tissue stain it takes the blue coloration, and I repeat our opinion that this material is an intercellular product jelled or set by material derived from injured tissues or blood plasma.

In the marrow the injury caused by the saw cut is followed by avascular repair. The cells derived both from fibroblasts and from endostial osteoblasts form no intracellular material except where they are in contact with fibrin or dead tissue, and here again we found the local production of the peculiar matrix under discussion.

This experiment showed complete absence of repair of bone in scorbutus other than by avascular organization without formation of collagen or bone matrix, and gives additional evidence that the fundamental condition in scorbutus is the failure of cells to form intracellular material.

SUMMARY AND CONCLUSIONS

We have described the morphologic concomitants of the condition of complete scorbutus and the immediate responses in repair. Our work establishes the hypothesis of Aschoff and Koch and confirms some of the observations of Höjer, although our methods of procedure and resultant material give us few points of contact.

We characterize the condition of scorbutus as inability of the supporting tissues to produce and maintain intercellular substances. Direct proof of this conclusion has been obtained in study of teeth in regard to dentin, in the study of growth and repair of bone in regard to bone matrix and in the study of repair of soft tissue in regard to the collagen of connective tissues. Our proof in regard to cartilage is incomplete.

The failure of capillary formation can be explained reasonably in the light of knowledge of other intercellular substances as due to failure of endothelial cells to form cement substance, an inference that Aschoff and Koch arrived at. We have at least shown that proliferative activity of the vascular endothelium is not at fault.

The proliferative power of epidermis, endothelium, fibroblasts and osteoblasts is not diminished in scorbutus. We are reasonably certain that it is augmented in the case of osteoblasts, which, however, undergo striking morphologic change.

Study of the sequences following antiscorbutic treatment has enabled us to control our observations at every stage in regard to the nature of cells contributing to the histologic ensemble of scorbutus. Osteoblasts, in spite of the great change in morphology, with complete similitude to fibroblasts, preserve their chemical potentialities and produce bone matrix.

Study of the sequences in teeth in progressive scorbutus, namely, the separation of the odontoblast layer from the dentin, led us to the theory that these cells continued to produce a liquid material. The same theory accounts for the edematous appearance of the fibrous-tissue-like structure in bones called gerüstmark. The theory as a whole is supported by the promptness and volume of matrix formation following antiscorbutic treatment.

We therefore advance the theory that the failure of cells to produce intercellular substance in scorbutus is due to the absence of an agent common to all supporting tissues which is responsible for the setting or jelling of a liquid product. Antiscorbutic substance is liberated in the destruction of tissues. The osteoporosis suggests further the hypothesis that this reaction is in some degree a reversible one.

We hope that the observations recorded in this report may suggest an approach to the study of the physiology of intercellular materials.