SCORBUTIC ARTHROPATHY IN THE GUINEA PIG

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THE PATHOLOGIC defects which develop in the bones in acute and chronic scurvy have been the subject of numerous studies, based on both human and experimental material.[1] It was first suggested [2] and then proved experimentally [3] that a deficiency of ascorbic acid (vitamin C) induces pathologic changes not only in the osseous structures but also in all the tissues of mesenchymal origin throughout the body. Visceral lesions of scorbutic animals have also been described by numerous investigators. It seems probable, however, that many if not most of the visceral changes are due to the inanition and dehydration which always accompany the advanced stages of the scorbutic state rather than to ascorbic acid deficiency per se.

Despite the fact that from the time of the first descriptions of this disease the joints have been known to be involved in scurvy, relatively few well documented anatomic studies of the changes taking place in the articulations are available. Rinehart and co-workers [4] described the

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1. (*a*) Aschoff, L., and Koch, W.: Skorbut: Eine patologischanatomische Studie, Jena, Gustav Fischer, 1919. (*b*) Hess, A. F.: Scurvy, Past and Present, Philadelphia, J. B. Lippincott Company, 1920. (*c*) Höjer, J. A.: Acta pædiat. (supp.) **3**:8-278, 1924. (*d*) Wolbach, S. B., and Howe, P. R.: Arch. Path. **1**:1, 1926. (*e*) Dalldorf, G.: J.A.M.A. **111**:1376, 1938. (*f*) Meyer, A. N., and McCormick, L. M.: Studies on Scurvy, Stanford University, Calif., Stanford University Press, 1928. (*g*) Banks, S. W.: J. Bone & Joint Surg. **25**:553, 1943.

2. Aschoff and Koch.[1a] Höjer.[1c]

3. Wolbach, S. B.: Am. J. Path. (supp.) **9**:689, 1933.

4. (*a*) Rinehart, J. F.: Ann. Int. Med. **9**:671, 1935. (*b*) Rinehart, J. F.; Connor, C. L., and Mettier, S. R.: J. Exper. Med. **59**:97, 1934.

articular lesions of scorbutic guinea pigs, emphasizing that the lesions were similar to those usually found in human material in cases of rheumatoid arthritis. In the opinion of these investigators, the similarity was more striking in those animals in which infection was artificially imposed on a state of partial or chronic scurvy. Successive studies have stressed particular aspects of the changes taking place in and about the joints of guinea pigs in acute and chronic scurvy.[5]

The present investigation was undertaken with the purpose of studying further the nature of scorbutic arthropathy in the guinea pig, emphasis being placed on the course of this condition as shown by the histologic pictures of acute and particularly of chronic lesions, on the role of hemorrhage and of mechanical stress, and finally on the effect of ascorbic acid administered to animals with well advanced lesions. Another objective of this study was to evaluate the importance of ascorbic acid deficiency as a possible factor in the genesis of arthritis of the rheumatoid type. In addition, a more complete working knowledge of the changes of scorbutic arthropathy was a necessary preliminary step toward future studies in which other stresses would be imposed on conditions of ascorbic acid deficiency.

METHODS

Three groups of young guinea pigs, totaling 64, were used for the first part of this study. Of these, 54 weighed between 175 and 250 Gm. and 10 between 450 and 550 Gm. at the start of the experiment. They were offered three different scorbutogenic diets according to the formulas suggested by Rinehart, Connor and Mettier[4b] and by Crampton[6] with minor modifications, and a commercial chow,[7a] to which 1.5 per cent yeast was added.[7b] The amounts of ascorbic acid present in the three diets as determined by chemical analysis[8] were minimal, corresponding to daily intakes of 0.05 to 0.08 mg., compared with the normal requirement for young growing guinea pigs of approximately 2.0 mg. per day The results obtained with

5. (*a*) Schultz, M. P.: Arch. Path. **21**:472, 1936. (*b*) Ham, A. W., and Elliott, H. C.: Am. J. Path. **14**:323, 1938. (*c*) Mouriquand, G., and Dauvergne, M.: Presse méd. **46**:1081, 1938; (*d*) J. méd. franç. **27**:411, 1938. (*e*) Mouriquand, G.; Dauvergne, M.; Tête, H., and Edel, V.: Compt. rend. Acad. d. sc. **210**:515, 1940. (*f*) Mouriquand, G.: Vitamines et carences alimentaires, Paris, Albin Michel, éditeur, 1942.

6. (*a*) Crampton, E. W.; Collier, B. C.; Woolsey, L. D., and Farmer, F. A.: Science **100**:599, 1944. (*b*) Crampton, E. W.: J. Nutrition **33**:491, 1947.

7. (*a*) Purina® rabbit chow supplement, supplied by the Ralston Purina Company, St. Louis, Mo. Different batches of purina® rabbit chow used in other experiments were found to contain amounts of ascorbic acid as high as 10 to 15 mg. per hundred grams. This diet as such, therefore, should be used for experimental study of ascorbic acid deficiency only after analysis. The chow used in this experiment contained only 0.3 to 0.4 mg. per hundred grams. (*b*) Reid, M. E.: Biol. Symposia **12**:373, 1947.

8. Roe, J. H., and Kuether, C. A.: J. Biol. Chem. **147**:399, 1943.

the three diets used were essentially alike in regard to the development of scurvy and the pathologic changes induced. For the purposes of this study, therefore, no subdivision of the animals in groups according to diet was considered necessary. The animals were kept in single cages, with food and water fed ad libitum. In addition to the diet, all the animals received dry hay (swamp grass) for roughage, and vitamins A, D and E (tocopherol) in adequate amounts once weekly.[6b] Twenty-six guinea pigs (acute or total scurvy) received no supplement of ascorbic acid, 20 (chronic, low grade scurvy) 0.2 mg. per day and 18 (controls) 2 to 4 mg. per day of ascorbic acid or fresh lettuce. The ascorbic acid was administered orally every other day. In the control group no significant variations were noted either in the growth curves or in the clinical behavior of the animals fed the different amounts of ascorbic acid or excess lettuce. Weights were recorded twice weekly routinely but daily in the critical phase of the disease. Observations were made on the condition of the animals and particularly on the articulations at least twice weekly. To study the progress of the lesions, the animals were killed at different intervals of time ranging from one to nineteen weeks (chronic scurvy) from the beginning of the experiment. A complete autopsy was performed on every animal. Tissues were fixed in Zenker's solution with acetic acid replaced by solution of formaldehyde U. S. P. Bones were decalcified by the formic acid–citrate method, embedded in celloidin (concentrated pyroxylin) and cut at 10 microns. Multiple sections of the knee joints and one section of a few ribs were examined in the case of each animal. In addition, wrist, elbow, shoulder, ankle, hip and vertebral joints were examined in animals selected from each group. Sections were routinely stained with hematoxylin (Ehrlich's)-eosin. In selected cases, Mallory's stain for connective tissue (Heidenhain's azocarmine modification), Van Gieson's stain, Foot's reticulum stain, Hotchkiss' periodic acid routine and Mallory's phosphotungstic acid–hematoxylin stain were used. The degrees of severity of the lesions observed in and around the knee joint and considered characteristic of the scorbutic process were subjectively graded as slight, moderate, moderately severe and severe (from 1 plus to 4 plus) and tabulated in order better to evaluate the evolution of the pathologic changes.

RESULTS

Clinical Observations.—The first evidence of joint involvement became manifest about the end of the second or at the beginning of the third week in the totally scorbutic guinea pigs and one to two weeks later in the partially scorbutic animals. It usually appeared first at the knees and consisted of enlargement of the ends of the bones forming the joint, subcutaneous hemorrhages and progressively increasing tenderness in the same areas. The involved joints became swollen, and the animals had a progressively more difficult and painful gait. Healthy guinea pigs usually maintain a position of semiflexion of the knees (in angles of approximately 90 degrees) and slightly less flexion of the elbows (100 to 110 degrees). In the scorbutic animals the flexion, especially of the knees, became more acute (to 60 to 45 degrees) with progress and chronicity of the disease. Passive extension became progressively more difficult until there was complete immobilization of the joint in a position of sharp flexion. Similar changes took place in wrists and ankles, accompanied by a varying degree of fixation or ankylosis. The involvement of

hip and shoulder joints appeared to be considerably less severe, while that of the small carpal and tarsal joints did not lend itself to an accurate clinical evaluation. The vertebral column became gradually more rigid with either disappearance or exaggeration (accentuated kyphosis or hunching) of the normal curvatures. As a result, the scorbutic animal assumed a hyperflexed, hunched, rigid appearance, with voluntary motion reduced to an absolute minimum. There was evidence in some animals at least that the mandibular-zygomatic joints were also involved by the scorbutic process. This involvement probably was responsible, together with the loosened and apparently painful condition of the teeth, for the increasing difficulty in mastication.

In addition to the delayed appearance of pathologic changes and the prolonged course of the disease, the clinical picture of the chronically scorbutic guinea pigs presented some other features worth mentioning. The degree of ankylosis in general eventually became more severe than that noted in terminal acute scurvy (three to four weeks), but after having reached a maximum at about the twelfth week it remained stationary. In a few animals a mild "paradoxical" improvement was noted in the condition of the joints after the thirteenth and fourteenth week. Hemorrhagic manifestations about the joints, which reached a maximum between the sixth and the ninth week, diminished in intensity, as a rule, and in many cases ceased almost entirely after the twelfth week. Subcutaneous nodules measuring up to 2 to 4 mm. in diameter, at first semifirm in consistency and markedly hyperemic, later firmer and less congested, were observed in several animals. These nodules were located on the ulnar side of the wrist and near the small carpal and tarsal joints, particularly where enlarged bone structures and the skin were in close apposition. In some of the animals with advanced chronic scurvy, bony or cartilaginous spurs projecting under the skin could be detected, especially lateral to the knee joint.

Pathologic Changes in and About the Knee Joint.—In a normal guinea pig microscopic examination of a longitudinal section through the middle of a knee joint discloses a moderately cellular, smooth articular cartilage and the presence of cruciate ligaments. At the site of insertion of the cruciate ligaments the articular cartilage either is absent or presents transitional features leading to dense fibrous connective tissue. Cuboidal synovial lining is limited to the lateral recesses of the joint, while the more centrally located subsynovial fat pads are lined by flattened cells. Occasionally synovial villi are present in the lateral recesses and "piling up" of synovial lining cells is also seen.

The different pathologic changes which occur in and about the joints during the course of the disease will be considered separately.

Intra-articular hemorrhages were almost always seen in sections taken from animals that died or were killed during the hemorrhagic

phase of the disease, i. e., in the fourth week in acute scurvy and between the sixth and the ninth week in chronic scurvy. The intensity of the hemorrhages, however, varied considerably in different animals killed at the same time, and also appeared to be more severe in the totally than in the partially scorbutic animals (fig. 1). Part of the hemorrhage was quickly reabsorbed, and the corpuscular elements were rapidly destroyed. Fibrin was precipitated in the joint space, where at times it persisted for

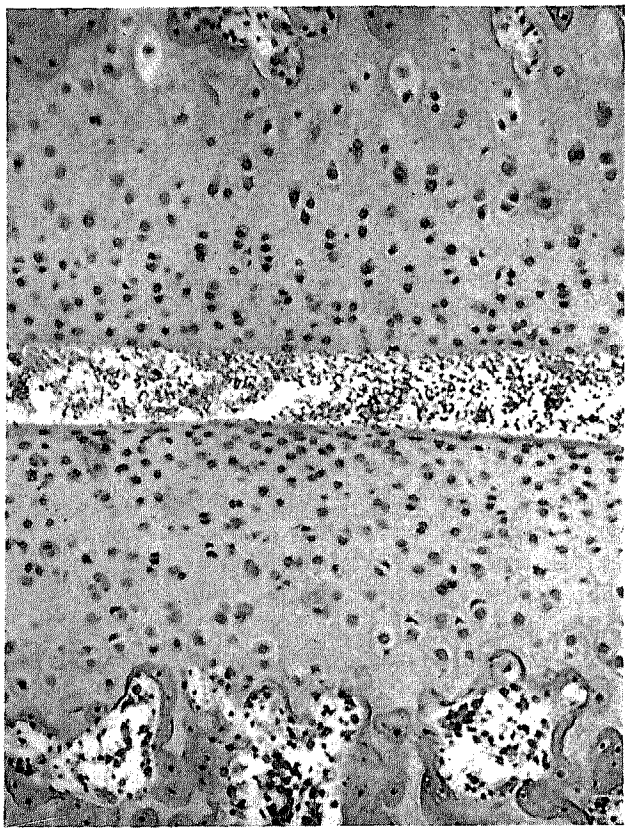


Fig. 1.—Recent hemorrhage in the knee joint of a guinea pig totally deficient in ascorbic acid; × 150 (approx.). (Armed Forces Institute of Pathology negative 218409-17.)

several weeks, slowly undergoing organization (figs. 2 and 3). The fibrin present was often abundant, forming dense brilliant eosinophilic masses in the lateral recesses or a thin layer covering the articular surfaces. Its morphologic appearance and the results obtained with special stains (such as phosphotungstic acid–hematoxylin) made identification of this material as fibrin reasonably certain. Organization of the hemorrhage was already under way in the acutely scorbutic animals at the time

of death, and was completed about the eighth or the ninth week in the guinea pigs with chronic scurvy. The fibroblasts which were responsible for this process originated either from the subsynovial connective tissue or, more probably, from modified synovial lining cells. Evidence in favor of the latter interpretation was the fact that organization of the fibrin masses often assumed a pattern which simulated synovial proliferation with formation of atypical villi. Typical or true synovial hyperplasia with formation of villi was observed in a relatively small number of cases in a more advanced stage of the disease (fig. 4). This process

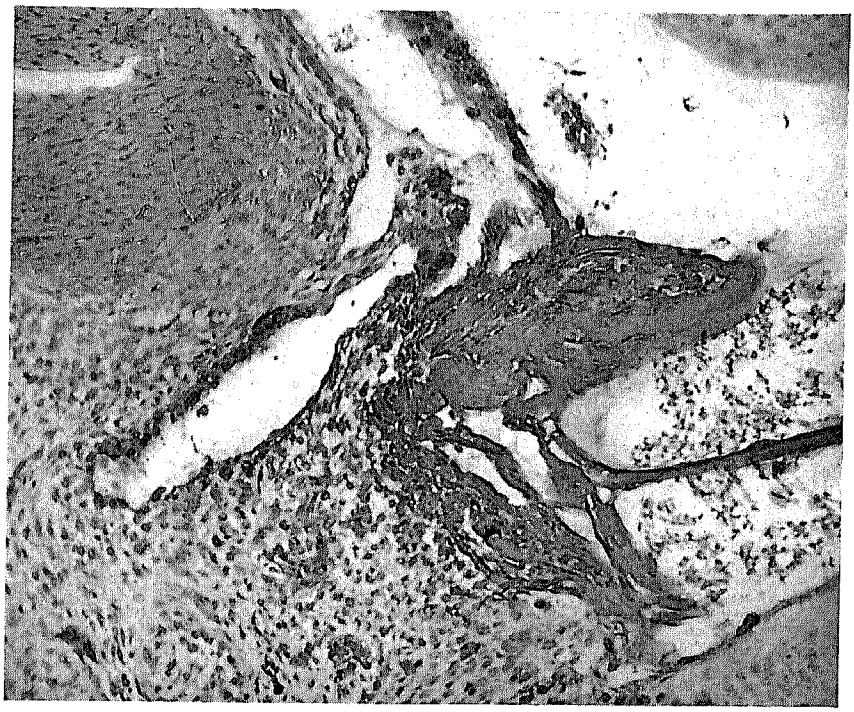


Fig. 2.—Organization of a recent hemorrhage in the knee joint of a guinea pig totally deficient in ascorbic acid. Note the degenerating blood corpuscular elements, the masses and strands of fibrin and the proliferation of fibroblasts; × 120 (approx.). (Armed Forces Institute of Pathology negative 218409-13.)

appeared to represent a reaction of the synovial cells to hemorrhagic material acting as a foreign body in the joint space. Even in the more chronic cases the connective tissue resulting from organization of the hemorrhage did not appear to be mature. Fibroblasts often maintained an immature appearance, failing to evolve to fibrocytes, and the intercellular substance could be shown to contain fairly abundant argentaffin fibrils (reticulum) but little or no mature collagen as revealed by Van Gieson's stain.

Simultaneously with the occurrence and organization of intra-articular hemorrhages, considerable fibroblastic proliferation took place in the subsynovial tissue, especially in the fat pads and the periarticular tissue. Although fairly extensive hemorrhages were often noted in these areas, it was difficult to evaluate their role in determining the final pathologic picture. Accumulations of fibrin were never observed in these regions, and also striking was the almost total absence of blood pigment–laden macrophages in all stages of the disease. In the most severe cases,

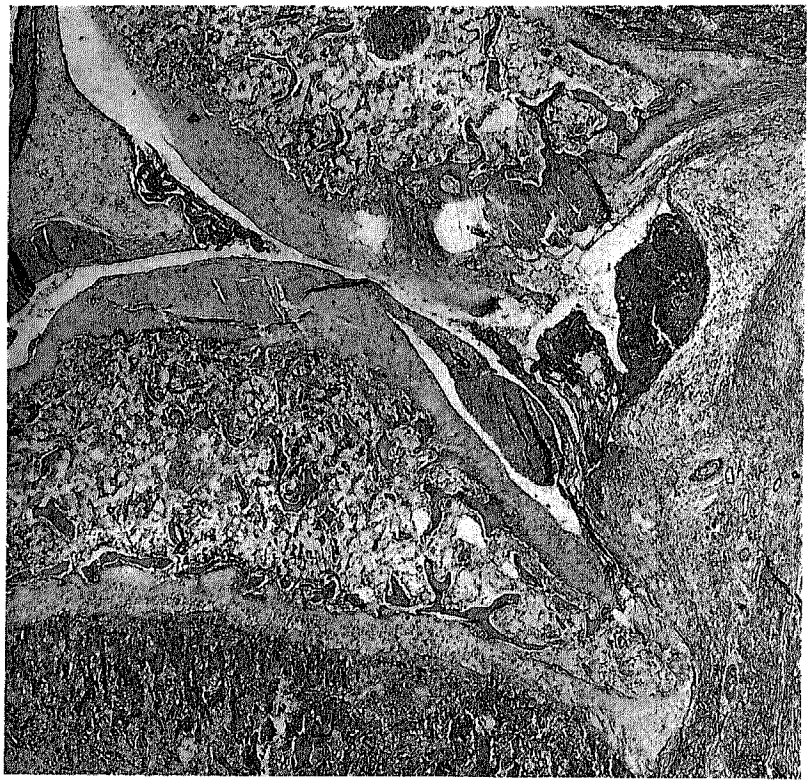


Fig. 3.—Knee joint showing scorbutic arthropathy in the acute stage; ×27. The anterior ("extensor") synovial pad (at left) shows considerably less change than the posterior ("flexor") one (at right). Note the abundant hemorrhagic, serofibrinous exudate in the joint space and the typical scorbutic changes in the bones. (Armed Forces Institute of Pathology negative 218407-17.)

fibroblasts entirely replaced the adipose tissue of the synovial pads which, as a result, became considerably larger and often entirely filled the recesses of the joint space. Fibroblastic proliferation, as a rule, was considerably more marked in the anterior than in the posterior synovial pad (fig. 3). This difference may have been caused by different degrees of hemorrhage per se in the two pads but more likely was the result of

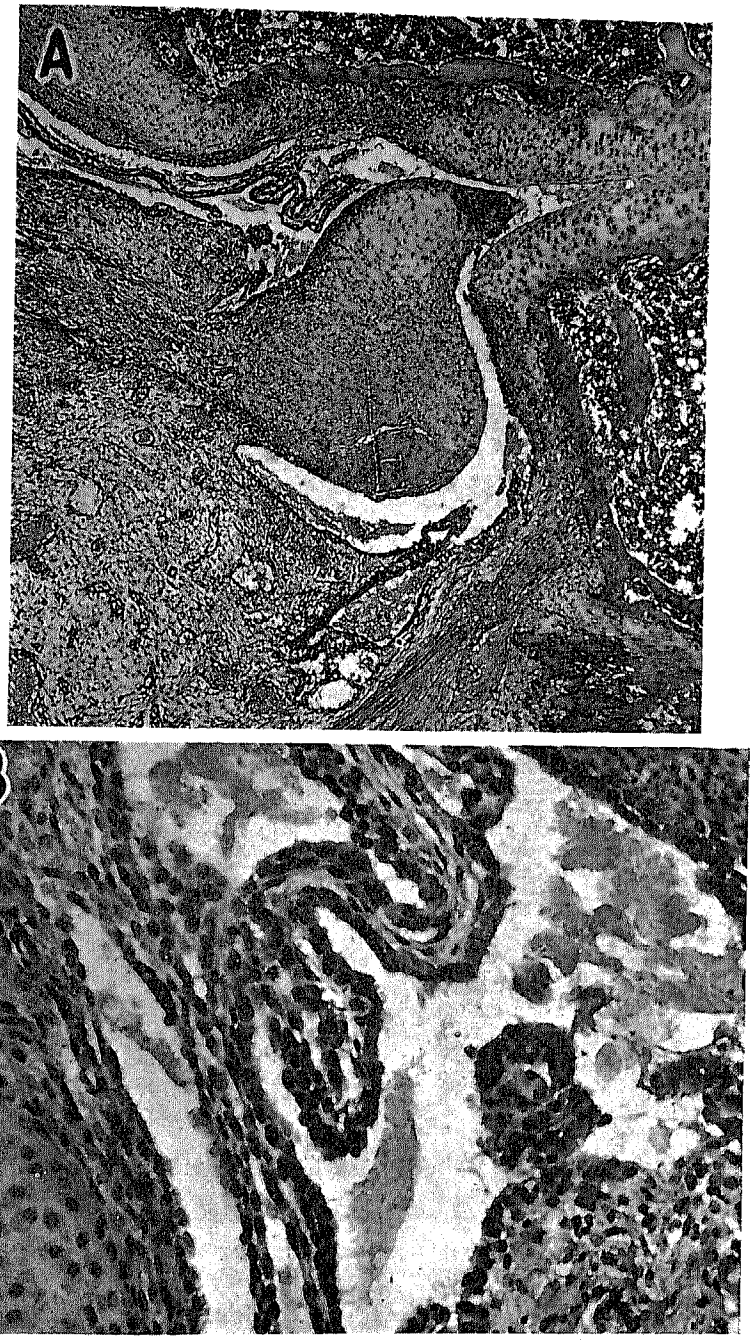


Fig. 4.—*A*, flexor recess of a knee joint in a subacute stage of scurvy, showing true synovial proliferation and large vascular spaces in the young fibrous connective tissue underneath the synovia; ×58. *B*, detail of *A* illustrating "true" synovial proliferation; ×250. Note the regular lining of synovial cells on newly formed villi.

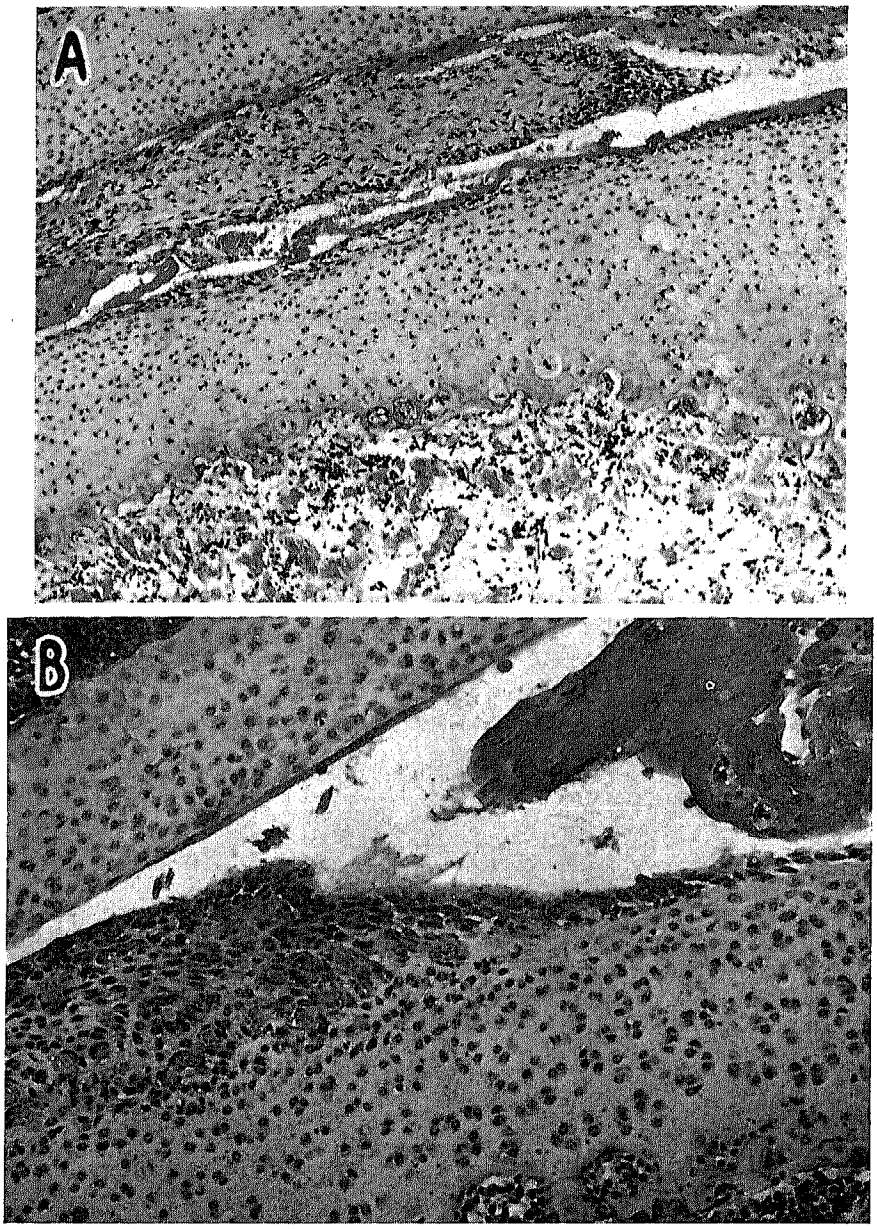


Fig. 5.—*A*, patellar-femoral space in late acute scurvy; ×91. Note the presence of fibrin and erythrocytes together with extensive pannus formation and slight regressive changes of the articular cartilage. (Armed Forces Institute of Pathology negative 218407-2.) *B*, patellar-femoral space in chronic scurvy; × 162. A large mass of fibrin is undergoing organization. The articular cartilage presents superficial vascularization, while the cartilage cells assume the appearance of fibroblasts. (Armed Forces Institute of Pathology negative 218407-5.)

different degrees of mechanical stress (compression) either alone or combined with increased hemorrhage. Because of the normally flexed position of the knee joint in the guinea pig, it seems probable that much of the body weight rests on the posterior (flexor) rather than on the anterior (extensor) aspect of this articulation.

In some animals the proliferating connective tissue (pannus) covered the articular cartilage surfaces. Often, however, in spite of the close apposition of intra-articular connective tissue and articular cartilage, a

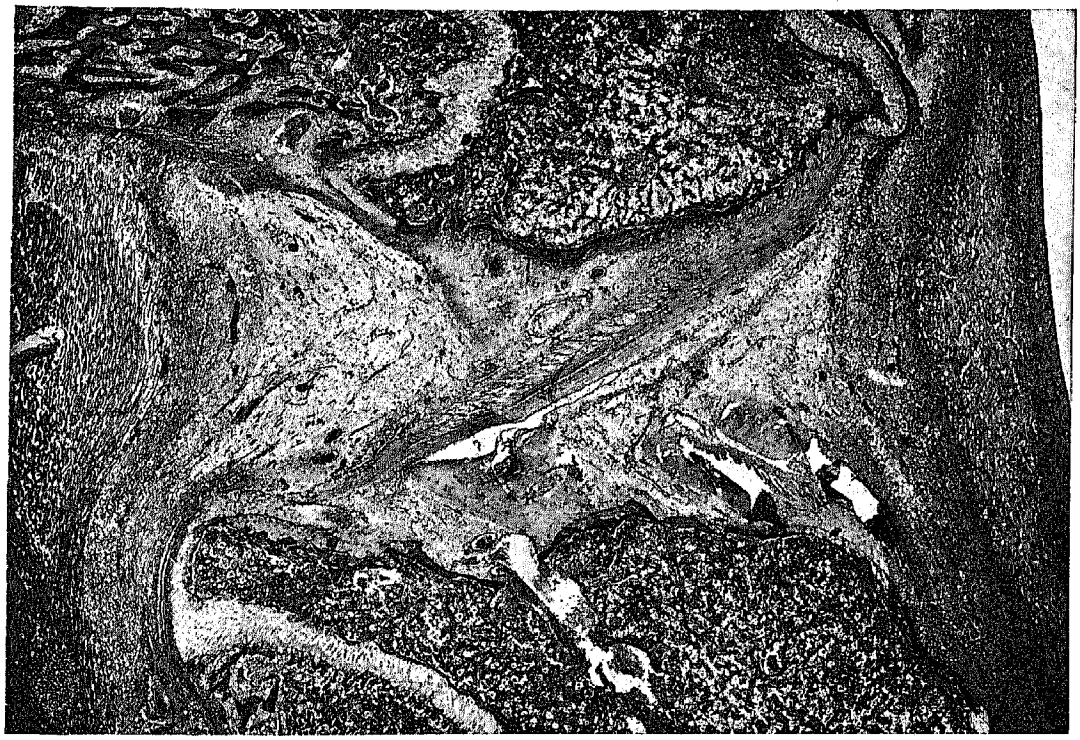


Fig. 6.—Knee joint in chronic scurvy; ×20. There is fibrous ankylosis with partial destruction of the articular cartilage. The entire joint cavity is filled by young proliferating connective tissue. Fibrosis of the periarticular tissue is severe on both the anterior and the posterior aspect of the knee and is continuous with the thick subperiosteal layer of fibrous tissue.

distinct demarcating line between the two structures could still be made out (fig. 5 *A*).

The articular cartilage was studied with particular attention in an attempt to detect changes either in the matrix or in the cells. In the areas where newly formed pannus was in close apposition, the cartilage cells occasionally showed retrogressive changes, assuming the appearance of fibroblasts. In some specimens, newly formed capillaries appeared in the

superficial layers of the cartilage (fig. 5 *B*). As a rule, the matrix of articular cartilage was more abundant and stained more darkly than did the matrix of epiphysial cartilage. This staining difference was observed both in the control and in the scorbutic animals but often was more obvious in the latter, indicating a different chemical or physical state of the matrix in the two types of cartilage, which was accentuated by a condition of ascorbic acid deficiency. However, no definite evidence of immaturity was observed by the use of either routine or special stains (Hotchkiss, Mallory's or Van Gieson's). In addition, the matrix of the articular cartilage, both in the control and in the scorbutic animals but especially in the latter, was stained more intensely in the deeper than in the superficial layers, irrespective of the stain used. This finding is difficult to interpret and may indicate either aging or abnormality of the deeper layers of cartilage. No typical fractures were observed in the articular cartilage, but occasionally this structure showed depressed areas, especially in the animals with chronic scurvy, in which the epiphysial bone contained few or no trabeculae. Evidence of excessive and irregular cartilage formation was found not uncommonly at each end of the epiphysial line, but it was rare and less obvious in the articular cartilage. In most instances the articular cartilage showed only minor changes in spite of the considerable amount of fibrous tissue within the knee joint. In 1 animal complete fibrous ankylosis was associated with partial destruction of the articular cartilage (fig. 6).

Considerable fibrosis was observed in the periarticular areas, especially in chronic scurvy. Here again the fibrous tissues appeared cellular and immature, with a limited amount of collagen but with numerous, often distended, thin-walled vascular spaces. Fibrosis was particularly prominent in the flexor aspect of the leg in the popliteal region and adjacent tissues. Where seen, it was clearly distributed according to lines of greater mechanical stress—for instance, along tendons and at muscle insertions. In the advanced lesions it was continuous with the proliferating connective tissue of the synovial pads and the joint cavity and especially with the actively proliferating subperiosteal layer of connective tissue along the diaphysis. This frankly thickened periosteum was the result, first, of hemorrhages which had undergone organization and, second, of a considerable proliferation of osteoblasts from the adjacent bone (fig. 7). These osteoblasts appeared immature, had lost their property of producing bone and gradually had fused with the surrounding mass of fibroblasts. This process could also be observed around the cortex of the epiphyses and occasionally was of considerable magnitude (fig. 8 *A*). Therefore it appeared that some of the "fibroblastic" proliferation around the joint was actually due to multiplication of osteoblasts originating from the epiphysial bone. In a few specimens,

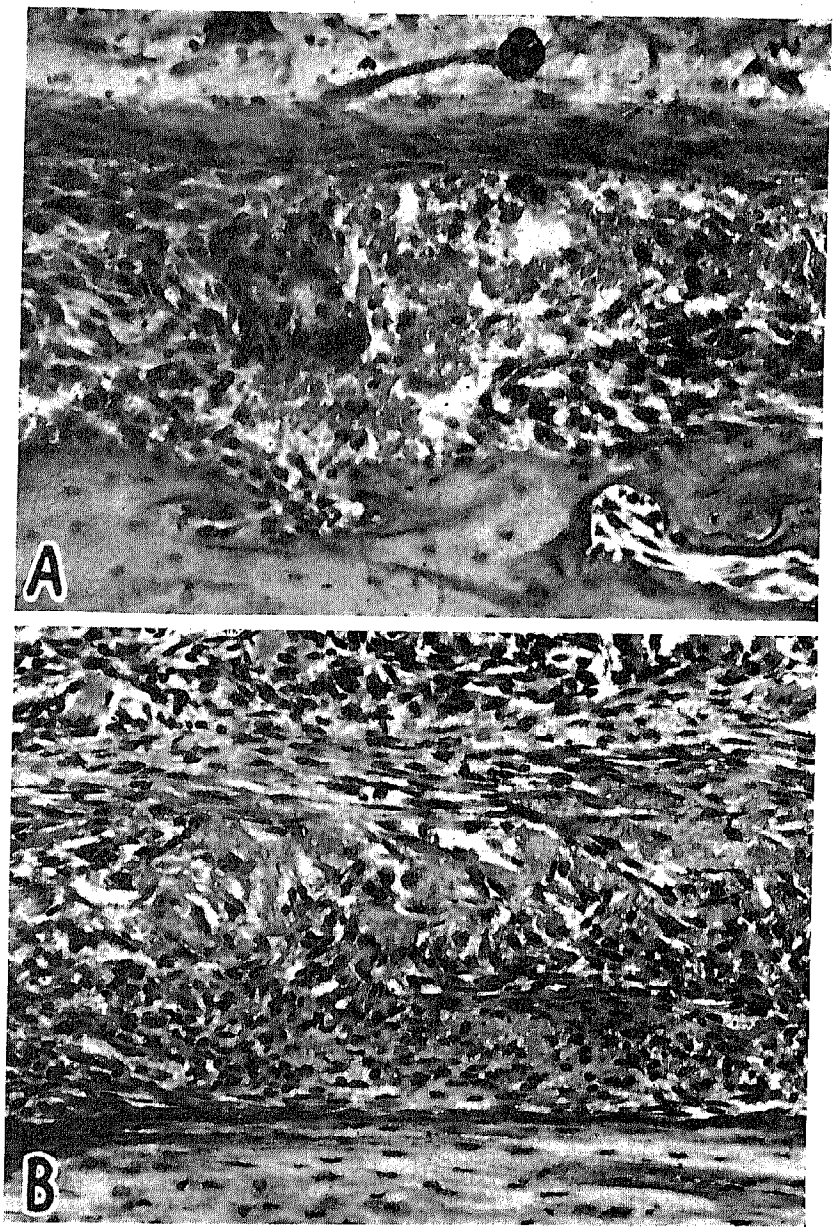


Fig. 7.—*A*, subperiosteal hemorrhages with separation of periosteum (above) and osteoblastic and fibroblastic cellular proliferation in acute scurvy; ×250. *B*, subperiosteal fibrosis with active fibroblastic proliferation; ×250. The periosteum is still recognizable above.

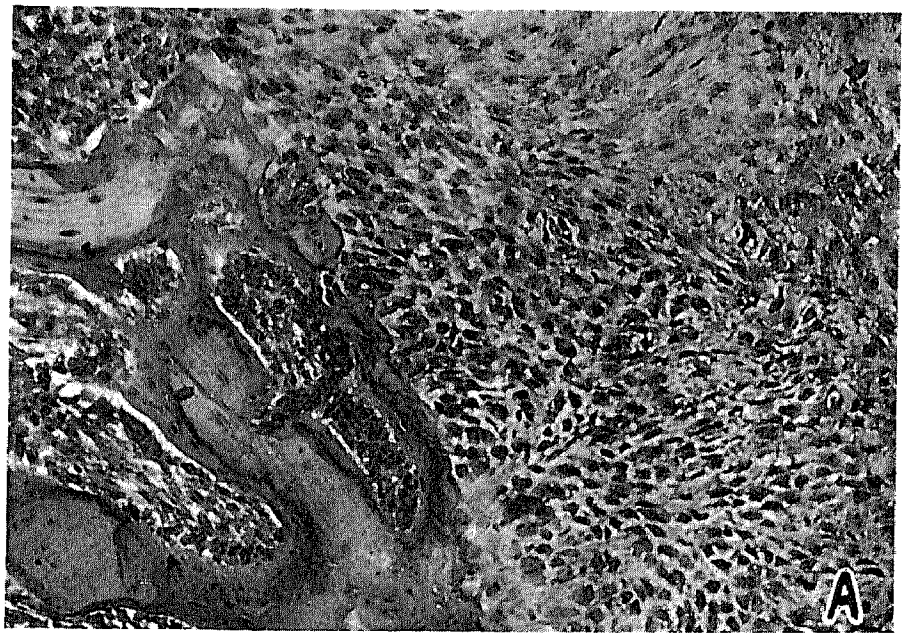
plaques of cartilage cells were also noted at a considerable distance from the joint space and even outside the joint capsule in the popliteal space (fig. 8*B*). It was impossible to say whether they represented a true heterotopic formation of cartilage or were cartilage cells displaced either from the articular surface or from the semilunar cartilages.

Throughout this impressive fibroblastic proliferation that took place both within and around the joint space, inflammatory cells were strikingly absent except for occasional lymphocytes and macrophages in the perivascular spaces.

Histologically, the subcutaneous nodules mentioned previously consisted of fibroblasts either irregularly distributed or arranged in whorls (fig. 9). Although there was a little immature collagen between the cells, there were no areas of necrosis. Fibrinoid degeneration was not observed. Nodules, however, were not examined microscopically in the early stages of development, when grossly they appeared markedly hyperemic and soft in consistency. Their typical position was outside a joint where the enlarged ends of the bones were closely apposed to the overlying skin. Hemorrhages, frequently seen in these regions, and the movement of the bone against the subcutaneous tissue were apparently the factors responsible for the formation of these structures.

Pathologic Changes in Joints Other Than the Knee.—The lesions observed in other joints were essentially similar to those described for the knees. The process was severe at wrists, ankles and elbows but considerably less, as a rule, at hips and shoulders. In the spine the costovertebral articulations were also involved, often severely, while the intervertebral articulations showed only slight changes in the few specimens that were studied. In addition, active fibrocellular proliferation was noted about the spinous processes and the longitudinal ligament, explaining the rigidity of the vertebral column observed clinically in many of these animals. The zygomatic-mandibular joint was examined in 2 guinea pigs with chronic scurvy. Typical scorbutic changes were observed both in the mandible and in the zygomatic bones. In addition a moderate degree of periarticular fibrosis with occasional hemorrhages was noted. The joint proper did not appear to be involved. These findings were believed to be important in accounting for the difficult and painful mastication.

In spite of the considerable differences in the severity of the scorbutic changes in different joints and also between two symmetric joints, it is clear that this process affects practically every articulation in the body. The difference in severity of the lesions appeared to depend mainly on the degree of mechanical stress and hemorrhage in different areas. At the knee the extent of the articular and periarticular involvement was somewhat proportional to the osseous changes in the adjacent bones.



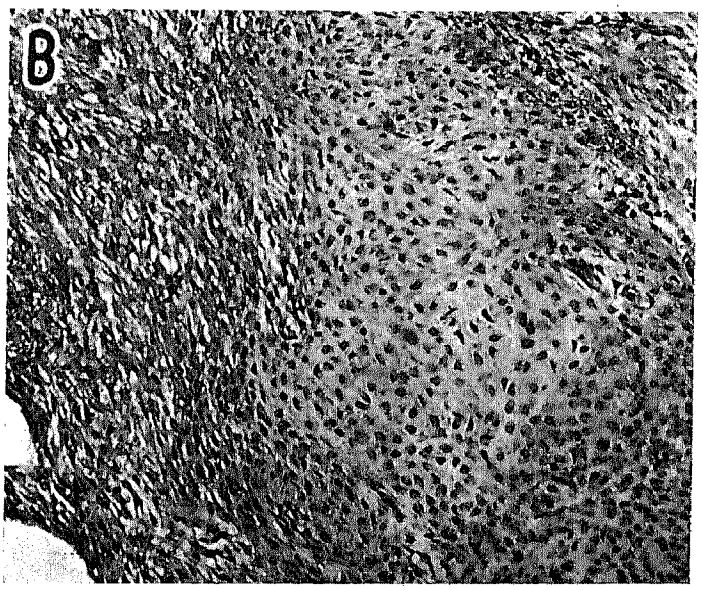


Fig. 8.—*A*, chronic scurvy; × 350 (approx.). Osteoblasts are proliferating from the epiphysis of the femur into the periarticular fibrous connective tissue. (Armed Forces Institute of Pathology negative 218409-7.) *B*, chronic scurvy; × 200 (approx.). There is heterotopic formation of cartilage in periarticular fibrous connective tissue. (Armed Forces Institute of Pathology negative 218409-11.)

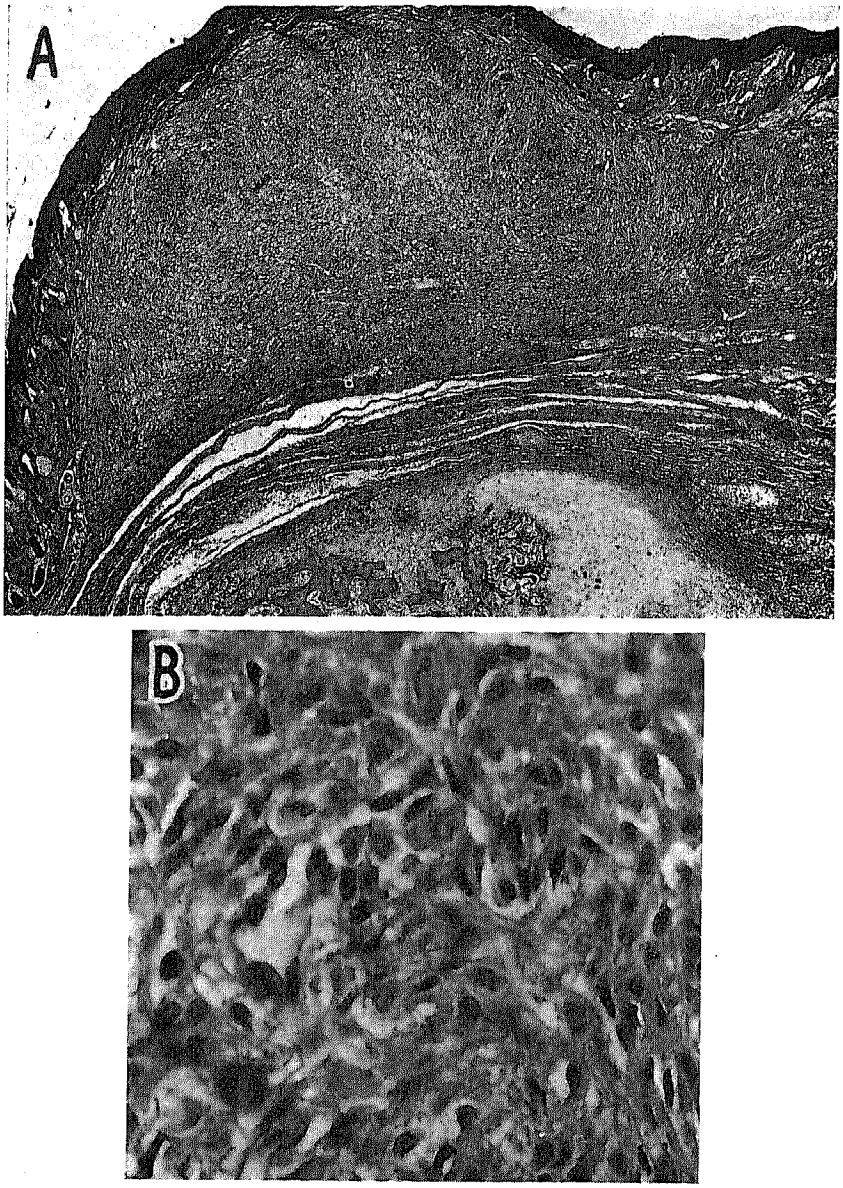


Fig. 9.—*A*, chronic scurvy; ×27. The subcutaneous fibroblastic nodule at the radial aspect of the wrist is in close apposition with the bone. *B*, detail of *A* demonstrating the arrangement of the fibroblasts; ×500.

However, this was not generally true for other joints. For example, extensive changes may be found in the small carpal or tarsal joints without lesions of a corresponding severity being present in the small bones. It appeared, therefore, that in these regions the articular lesions were largely independent of the bony alterations. However, since the severity of the osseous changes of scurvy is known to be dependent in part on the activity of the epiphysial plates[9] and, as a rule, a more severe arthropathy is found in those joints which are formed by the more actively growing bones (i. e., the knee), the concept that there is a partial parallelism between articular and osseous lesions must be accepted.

Course of Scorbutic Arthropathy.—The evolution of some of the more important pathologic changes occurring in the joints over the experimental period considered is summarized in figure 10.

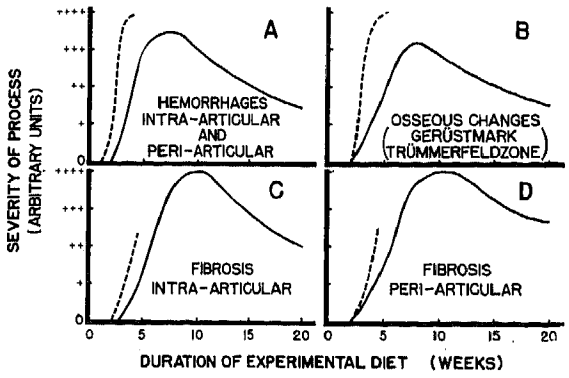


Fig. 10.—A schematic presentation of the changes occurring in the joints of scorbutic guinea pigs. Acute scurvy is represented by a broken line. The guinea pigs showing acute scurvy had been restricted to a diet practically free of ascorbic acid. Chronic scurvy, represented by an unbroken line, was produced by restricting the animals to an intake of ascorbic acid of 0.2 mg. per day.

Intra-articular and periarticular hemorrhages, which are considered together (fig. 10*A*), developed earlier and were more severe in the acutely scorbutic than in the chronically scorbutic animals. In the latter group these hemorrhagic manifestations reached a maximum between the sixth and the ninth week to decline progressively to only slight or moderate intensity between the fourteenth and the nineteenth week. It is interesting to note that most of the guinea pigs of the group with chronic involvement either died or appeared critically ill between the eighth and the tenth to eleventh week. Those which survived this period appeared to adapt themselves to some extent to the experimental conditions and showed slight gain in appetite and weight, improved appearance and activity, and possibly slightly increased mobility of joints,

9. Glasunow, M.: Virchows Arch. f. path. Anat. **299**:120, 1937.

although this may have been due merely to a disappearance of pain and tenderness. The reason for this decline in intensity of the hemorrhagic manifestations was not clear. However, the number and the size of the capillaries and vascular spaces in the areas of fibroblastic proliferation were considerably reduced after the eleventh week. In addition, with the progress of the disease, the areas of fibrocellular proliferation appeared more dense and, although only small amounts of collagen were formed, the walls of the capillaries were probably better supported by the adjacent tissue. Changes, such as fibrosis and at times hyalinization of the smaller arteries, or endothelial hyperplasia and thickening of the capillary wall, were observed in many cases but apparently were too irregular and inconsistent to have played an important role in diminishing the intensity of the hemorrhagic manifestations. Other factors which may have been of importance in this phenomenon were the reduction of mechanical stress on the joint area due to the extremely reduced mobility of the limbs in chronic scurvy and the reduced blood volume, and probably sluggish circulation, which appeared to have been present in these animals.

The severity of the characteristic osseous changes of scurvy such as the *Gerüstmark* (rarefied trabeculae and loose fibrous marrow) and the *Trümmerfeldzone* (zone of dense abnormal calcified cartilage) followed a pattern similar to that of hemorrhages during the course of the disease (fig. 1*B*). As a rule, at least at the knee, there was a considerable parallelism between the severity of the articular and that of the osseous changes. In the chronically scorbutic animals after the twelfth week both the *Gerüstmark* and the *Trümmerfeldzone* underwent resorption, to disappear almost entirely in many cases after the sixteenth week. It was interesting to note that resorption of the abnormal trabeculae (*Trümmerfeldzone*) and of the fibrous tissue (*Gerüstmark*), together with the fibrin and amorphous bone matrix present in this region, had taken place in the absence of any detectable activity either of osteoclasts or of macrophages. In this respect the resorptive process was similar to, although more complete than, that of the intra-articular fibrosis present in the same animals. While forming of new bone at the epiphysial plate had ceased almost entirely, new, apparently defective bone was formed in the cortex of the diaphysis, which appeared then considerably thickened. Control guinea pigs of the same age group, which had been under experimental observation for corresponding periods of time, showed persistent bone formation at the epiphysial plate and a thinner cortex. It appeared, therefore, that chronic severe ascorbic acid deficiency had induced an accelerated aging in the process of bone formation as evidenced by the fact that in the guinea pig epiphysial bone formation ceases and periosteal bone formation may continue, even in excess of normal, when the ascorbic acid intake is approximately one tenth of what is considered the normal requirement. These findings can be

explained in part by the fact that the untoward effects of ascorbic acid deficiency become less apparent as the growth rate declines, or are retarded as a result of other physiologic inadequacies resulting from the deficiency.

Intra-articular and periarticular fibrosis (fig. 10 *C* and *D*) was also considerably more severe in the chronically scorbutic than in the acutely scorbutic animals. It was first noticed about a week after the onset of the hemorrhagic manifestations and reached its maximum between the eighth and the twelfth week. It then declined more in the intra-articular than in the periarticular region. Within the joint space the fibrin was reabsorbed. Synovial proliferation was progressively less notable, and there was reappearance of adipose tissue in the synovial pads. In the periarticular region, however, during this period the fibrosis was reduced only slightly, so that functional improvement, as a rule, was limited and the fixed position of the knee joint was not appreciably changed. This condition can be called pseudoankylosis in contrast with true fibrous ankylosis, which occurs at the height of the disease.

Role of Mechanical Stress.—The importance of mechanical force in determining and aggravating the scorbutic lesions of the bones and adjacent tissues is well known. To investigate this point further, with special regard to the joint changes, the left leg of each of 10 young adult guinea pigs weighing between 450 and 550 Gm. was immobilized in an extended position in a plaster of paris cast with a technic similar to that used by Follis.[10] With this method the effect of weight and motion on the knee joint was removed. The animals were restricted to a scorbutic regimen for thirty days. Seven received no supplement, and 3 were controls. The diet used for this as well as for the succeeding group of guinea pigs was Rockland® rat pellets, supplemented by dry hay, yeast, rutin and fat-soluble vitamins. This diet was found later to contain ascorbic acid in amounts corresponding to an intake of approximately 0.2 to 0.4 mg. per day per animal. As a result the gross and the microscopic changes observed in this group of animals were those of "chronic" rather than of "acute" scurvy.

The extremity to which a cast had been applied showed at autopsy a varying but often considerable degree of muscular atrophy and in many cases slight to moderate subcutaneous edema, indicating a corresponding degree of circulatory impairment. Knee joints only were examined. Histologically, the results of Follis (i. e., considerably less severe or no scorbutic lesions in the bones of the immobilized leg) were fully confirmed in only 1 of 7 scorbutic animals studied. In a few animals the immobilized knee had less severe changes in the bones than the opposite, free extremity. In other cases, however, scorbutic changes

10. Follis, R. H.: Arch. Path. **35**:579, 1943.

were at least as severe in the immobilized as in the free extremity. This discrepancy of results can be explained in part by the fact that to 3 of the animals casts were applied ten days after the beginning of the scorbutic regimen. In all scorbutic animals, including those in which only minimal scorbutic changes were seen in the cast-encased legs, the incidence and the degree of the hemorrhagic manifestations were not modified. Hemorrhages, in fact, appeared to be more severe in the immobilized than in the free joint and to involve equally the flexor and the extensor aspect of the articulation. As a result, considerable fibroblastic proliferation had taken place in these joints. In most of the immobilized joints the intra-articular changes were at least as severe as, and did not differ considerably from, those found in the "free" joints of scorbutic animals. The periarticular fibrosis, however, was considerably less severe and more evenly distributed between the extensor and the flexor aspect of the cast-encased knee. In the control animals, hemorrhages were minimal or absent in both legs.

These results seem to indicate that hemorrhage plays a dominant role as far as the intra-articular lesions of scorbutic arthropathy are concerned. Mechanical stress, on the other hand, appears to be a determining factor in the considerably more abundant periarticular fibrosis, which is usually observed on the flexor aspect of the nonimmobilized joint. When the normal position of flexion is changed to one of forced extension and mechanical stress is removed, the periarticular fibrosis is distinctly reduced on the flexor aspect and slightly increased on the extensor aspect of the knee joint.

Effect of Ascorbic Acid Administered for Scorbutic Arthropathy.—A preliminary study to investigate the effect of administered ascorbic acid on preexistent articular lesions of scurvy was made on a group of 8 young adult guinea pigs. These animals were restricted to a scorbutogenic diet, including Rockland® rat pellets, for thirty days. At the end of this period 4 animals were given 0.4 mg. of ascorbic acid per day, and 4, 2 mg. per day for periods increasing from one to twenty-five days. Knees and ribs were examined histologically. The clinical and pathologic observations on this small group of animals indicated that much of the intra-articular and some of the periarticular fibrosis disappears after treatment. In the joint space, however, a small amount of fibrin may persist even after twenty-five days of rehabilitation. In addition, some of the periarticular fibrotic changes persisted throughout the experimental period, especially on the flexor aspect of the joint and in the adjacent muscles. This fibrous connective tissue became more mature and fairly abundant collagen was formed. The *Trümmerfeldzone* and the *Gerüstmark* were slowly reabsorbed and a limited number of new bone trabeculae were formed at the epiphysial line. Osteoblastic

activity was much greater in the subperiosteal space. In this area the osteoblasts which appeared as fibroblasts or immature mesenchymal cells in the scorbutic state reacquired their typical appearance and produced new bone at a remarkably fast rate. In this respect no difference was noted in the animals receiving either 0.4 or 2.0 mg. of ascorbic acid per day. No bone formation was observed in the masses of immature mesenchymal cells of the periarticular tissue. Osteoblastic activity and new bone formation were also limited in amount within the epiphyses.

COMMENT

The results of these experiments clearly indicate that a severe arthropathy develops in guinea pigs subjected to a condition of total or severe partial ascorbic acid deficiency. This disturbance cannot be considered to be a separate entity but is a part of the general manifestations of scurvy. The fundamental features of scurvy, namely, hemorrhages, active proliferation and failure of maturation of mesenchymal cells, and production of scanty ground substance and of immature collagen are all present at one stage or another of scorbutic arthropathy, this disease being obviously dependent on these manifestations.

The observations of other investigators [11] have been in general confirmed and extended in this study. Hemorrhages obviously play a more important role, especially in the earlier stages of the disease, than is admitted by some.[12] It is evident that unless the animals are killed and examined during the hemorrhagic phase of scurvy, the blood extravasated into the joint cavity and in adjacent areas will have been at least partially reabsorbed or organized by actively proliferating mesenchymal cells, and the importance of hemorrhages in initiating and maintaining the arthritic changes may then be underestimated. The importance of this factor is also proved by the fact that the condition of the joints may improve considerably after the hemorrhagic phase of the disease is over. Fibrous ankylosis persists in the chronically scorbutic animals because of the periarticular rather than the intra-articular lesions.

The fact that guinea pigs will adjust to a chronic severe partial ascorbic acid deficiency, probably on the basis of a sharp reduction of metabolism and therefore of ascorbic acid requirements, must be kept in mind in evaluating some of the pathologic changes. This phenomenon had been observed by Mouriquand in "chronic" scorbutic guinea pigs and called *paravitaminose*. According to this investigator, many of the lesions of these animals do not respond to ascorbic acid therapy and can be considered irreversible as measured by clinical observation and roentgenologic studies.

11. Wolbach and Howe.[1d] Rinehart.[4a] Rinehart and others.[4b] Schultz.[5a] Ham and Elliott.[5b] Mouriquand and Dauvergne.[5c,d] Mouriquand and others.[5e]

12. Meyer and McCormick.[1f] Rinehart.[4a]

The fact that there was no significant inflammatory cell infiltration within and around the joint at all stages of the disease must be stressed. This was also true of 8 guinea pigs in which a severe infectious process, such as bronchopneumonia, was found at autopsy. In these animals, however, the lesions of the joints appeared to be somewhat more severe than in comparable animals at the same stage of scurvy, probably indicating an aggravation of the scorbutic state. It is interesting to note that macrophages were seen only occasionally even around the areas of recent and old hemorrhage. The same applies to osteoclasts in spite of the considerable reabsorption of bone which takes place in chronically scorbutic animals.

Mechanical force (tension and compression) is without doubt important in determining the site and the severity of the articular and periarticular lesions of acute and chronic scurvy. The results obtained in the guinea pigs which had a leg immobilized in a cast are, however, somewhat different and less convincing than those reported by Follis.[16] A difference of experimental technics may have been responsible for this discrepancy. We feel, however, that although mechanical stress is important and is responsible for many of the features of scurvy, its importance should not be emphasized to the exclusion of other factors, particularly hemorrhage.

Some of the features of scorbutic arthropathy bear a resemblance to those of rheumatoid arthritis. We were not impressed, however, by any striking similarity of the two articular disorders when the over-all pathologic picture was considered. Hemorrhagic manifestations are definitely more severe in scurvy, as are the osseous changes. It is true that in occasional animals with chronic scurvy it is difficult to detect changes that are pathognomonic for scurvy. However, other lesions, such as small areas of fibrosis in the marrow and thickening of the periosteal bone, were present in most of these cases. To the best of our knowledge, no such changes have been mentioned as occurring in rheumatoid arthritis in any stage of the disease. Disuse atrophy is generally considered to be the main factor in causing osteoporosis in rheumatoid arthritis and possibly is partially responsible, together with inanition, for the osteoporosis of chronic scurvy. Synovial and subsynovial fibroblastic proliferation with pannus formation is present in both diseases and may lead to fibrous ankylosis. This condition, however, is spontaneously reversible, at least in part, in advanced chronic scurvy and never progresses to bony ankylosis. The fibroblastic proliferation of scurvy, therefore, does not seem to have the destructive properties which, as a rule, are observed in infectious processes and in rheumatoid arthritis. It is probably for this reason that in chronic scurvy with few exceptions the articular cartilage shows mild lesions of a regressive rather than a destructive nature. Although the severity of

scorbutic arthropathy may vary considerably in different joints, the lesions always appear to be in approximately the same stage, contrary to what is generally found in rheumatoid arthritis.

Changes consistent morphologically with fibrinoid degeneration were not uncommonly observed in the areas of mesenchymal cell proliferation especially in acute and chronic scurvy. This lesion was general in distribution and the adjacent cells were never arranged to form granulomatous nodules. The subcutaneous periarticular nodules that are occasionally found in chronic scurvy were also, in our opinion, unlike similarly placed lesions of human patients with either rheumatic fever or rheumatoid arthritis. The similarity of scorbutic arthropathy and rheumatoid arthritis is therefore only superficial. The differences between the two diseases become more apparent when the chronic lesions of scorbutic arthropathy are studied serially and when the entire course of this scorbutic process is reconstructed. Despite these differences, the possibility that partial ascorbic acid deficiency may be a predisposing or a contributing factor in certain forms of human arthritis cannot be ruled out entirely. The recent reports of striking effects of an adrenal cortex steroid (compound E [17-hydroxy-11-dehydrocorticosterone]) on the courses of rheumatoid arthritis and rheumatic fever [13] are of interest in this regard, and since ascorbic acid has been implicated as playing a role in the synthesis of adrenocortical hormones,[14] it would appear possible that ascorbic acid deficiency may play an indirect role in the genesis of these two diseases. Additional work to investigate this possibility is indicated.

SUMMARY

The articular lesions of acute and chronic scurvy were studied in 82 guinea pigs.

Scorbutic arthropathy involves in different degrees all the joints of the body. It is characterized by hemorrhages and fibroblastic proliferation, minor changes of the articular cartilage and striking absence of inflammatory cells. Hemorrhages and mechanical stress are the all-important factors in determining the general picture of scorbutic arthropathy. The incidence and the severity of the hemorrhagic manifestations decline considerably as chronic scurvy advances. Spontaneous improvement of the intra-articular lesions (true ankylosis) also occurs. Periarticular lesions (pseudoankylosis) are less likely to improve spon-

13. Hench, P. S.; Kendall, E. C.; Slocumb, C. H., and Polley, H. F.: Proc. Staff Meet., Mayo Clin. **24**:181, 1949. Hench, P. S.; Slocumb, C. H.; Barnes, A. R.; Smith, H. L.; Polley, H. F., and Kendall, E. C.: Ibid. **24**:277, 1949.

14. Giroud, A.; Santa, N.; Martinet, M., and Bellon, M. T.: Compt. rend. Soc. de biol. **134**:100, 1940. Sayers, G.; Sayers, M. A.; Lewis, H. L., and Long, C. N. H.: Proc. Soc. Exper. Biol. & Med. **55**:238, 1944.

taneously or even after therapy. Under the conditions of the experiment the periarticular fibrosis appears to be, for the most part, irreversible and responsible for the persistent functional impairment.

There is only a superficial morphologic similarity between the articular lesions of scurvy and those of either rheumatoid arthritis or rheumatic fever. It is possible, however, that partial ascorbic acid deficiency may be a predisposing or a contributing factor in certain forms of human arthritis.

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