Porcine suppurative arthritis and costochondral changes produced by Streptococcus equisimilis

Edgar Donald Roberts

Iowa State University

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ROBERTS, D.V.M., Edgar Donald, 1931–
PORCINE SUPPURATIVE ARTHRITIS AND COSTOCHONDRAL CHANGES PRODUCED BY STREPTOCOCCUS EQUISIMILES.

Iowa State University of Science and Technology
Ph.D., 1965
Health Sciences, pathology

University Microfilms, Inc., Ann Arbor, Michigan
Porcine Suppurative Arthritis and Costochondral Changes Produced by Streptococcus Equisimilis

by

Edgar Donald Roberts

A Dissertation Submitted to the Graduate Faculty in Partial Fulfillment of The Requirements for the Degree of Doctor of Philosophy

Major Subject: Veterinary Pathology

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

Head of Major Department

Signature was redacted for privacy.

Dean of Graduate College

Iowa State University
Of Science and Technology
Ames, Iowa

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INTRODUCTION

Arthritis results in a serious economic loss to the swine industry. In the 10-year period from 1942 to 1951 swine carcass condemnation from arthritis amounted to 24 million dollars annually in the U.S. (127). A survey conducted in 1963 by the Livestock Conservation Incorporated (110) has determined that pork processors assess and distribute a loss of 3.6 million dollars to suppurative processes other than cervical abscesses. This includes among other lesions, abscessation of peripheral lymph nodes draining suppurative joints. These surveys do not adequately reflect the actual loss. Many young infected pigs never reach market weight as they are either killed because they are poor gaining animals or die during the septicemic phase. The surviving animals make less efficient gains.

*Streptococcus equisimilis* infection in swine is confined primarily to suppurative joints. Collier (24) determined that of 13 beta-hemolytic cultures from arthritic lesions, 10 were *Str. equisimilis*. Switzer et al. (121) observed streptococcal arthritis from 18 percent of packing plant and 15 percent of diagnostic laboratory arthritic specimens. The most prevalent streptococcus by biochemical typing was *Str. equisimilis*.

Most of the information regarding *Str. equisimilis* concerns studies on the organism and its ability to produce disease. Little has been done to study the gross and microscopic
lesions of *Str. equisimilis* infection to provide information for the differential diagnosis of porcine arthritides.

It has been stated (114) that a rapid destruction of the articular surface is characteristic of suppurative arthritis. However, cartilage breakdown similar to that observed in naturally occurring cases has not been duplicated experimentally (29). The essential factors for the degradation of cartilage in the naturally occurring suppurative process are not known. Curtiss and Klein (29) recently concluded that while gross cartilage destruction in septic arthritis may be, in part, due to the action of leukocytic enzymes and plasmin, the exact mechanism is not known.

Recently, studies of the synovial cells from human rheumatoid arthritis by Barland et al. (10) indicated the prominence of a cell with abundant acid phosphatase-positive vacuoles, altered mitochondria and small Golgi apparatus. The etiologic factor of rheumatoid arthritis in man has not been determined. Thus to better understand the reaction of the synovial cells to a known infectious process, the cytochemistry and fine structural alterations of the *Str. equisimilis* infected joint were examined.

Attention has recently been focused on the costochondral junction lesions of swine as an aid in the diagnosis of chronic hog cholera (36). The mechanism of lesion formation was attributed to an imbalance in the calcium-phosphorus ratio with a later return of serum calcium to normal levels.
In the process of routine necropsy of swine at the Iowa Veterinary Diagnostic Laboratory (IVDL), Ames, Iowa, costochondral lesions were observed which were similar to those described for hog cholera. Evaluation of the herd history and the isolation of other infectious agents indicated that the disease was not hog cholera. Furthermore, suppurative arthritis was a significant finding in every case. A beta-hemolytic streptococci was isolated and proven to be \textit{Str. equisimilis} from these joints. The latter evidence strongly indicated that \textit{Str. equisimilis} could also cause visible alterations of costochondral junction. Serum calcium, phosphorus and alkaline phosphatase determinations accompanied the study so that the mechanism of lesion formation might better be compared with that of hog cholera.

This study was initiated to further elucidate the effects produced by \textit{Str. equisimilis} on the skeletal system of swine.
LITERATURE REVIEW

Introduction

The literature review was arranged by topics for the convenience of the reader. The topics include the main encompassed subject areas relative to this thesis and are: 1. streptococcal infections of swine 2. *Streptococcus equisimilis* 3. suppurative arthritis 4. streptococcal infections in man 5. rheumatoid arthritis in man 6. experimental models of streptococcal infection in the rabbit 7. rib lesion formation 8. fine structure of synovial membrane and 9. lysosomes in pathologic processes.

Streptococcal Infections of Swine

Streptococcal infections in swine have involved a wide variety of tissues in any age pig. The lesions may have resulted from localization of the organism following a septicemia (26) or as Collier (25) demonstrated in the case of cervical abscess in swine, from oral ingestion of the organism. The most frequent manifestations of streptococcal infection are arthritis (24, 41, 49 and 121), endocarditis (62, 64 and 70), meningio-encephalomyelitis (26, 41, 67, 86 and 113), cervical abscess (25, 33, 96, 118, 119 and 134), acute deaths (1, 18, 54 and 126) and diarrhea (46). A study of the incidence and causes of mortality of pigs in England from November, 1956, to October, 1959, indicated that streptococcal
meningitis, arthritis and septicemia were some of the more prominent factors producing a high baby pig mortality (91).

The literature on *Streptococcus sp.* arthritis in swine is very limited and largely confined to etiological factor surveys of arthritic joints. Field *et al.* (41) reported that streptococcal arthritis was prevalent in preweaning-age pigs. Infection might be confined to a single litter or in some herds in successive litters over a period of many months. The significance of the environment in the spread of the infection is not understood. The age of the pig involved suggested that the sow was the most probable source of infection.

Collier (24) studied 13 beta-hemolytic cultures obtained from arthritic lesions and found 10 of them to be *Str. equisimilis*. Field *et al.* (41), in 1954, stated that a Lancefield's type C beta-hemolytic (horse blood agar) streptococcus was isolated from an outbreak of suppurative arthritis. Switzer *et al.* (121) observed streptococcal arthritis in 18 percent of packing plant and 15 percent of diagnostic laboratory specimens. The majority of isolates were biochemically *Str. equisimilis*.

In a controlled field study, Helms (60) determined that pigs farrowed from healthy gilts, even in clean quarters, may show a high incidence of joint and navel involvement. It was later found that the apparently healthy gilts or sows had uterine infections.

The lesions of streptococcal arthritis were characterized
by a turbid, purulent exudate in the joint with inflammation of the surrounding tissues. It has been postulated that arthritis caused by this organism may persist for a long period of time if the pigs survive the septicemic phase (49).

Microscopically, in naturally occurring cases of streptococcal arthritis, there was rapid replacement of the synovial cell by connective tissue with subsequent neutrophilic infiltration of the surrounding tissue. In the subacutely involved joint, the synovial membrane was composed entirely of hyperplastic connective tissue. The articular surface had undergone rapid chondrolysis which resulted in ulceration of the articular surface or complete ankylosis of the joint had occurred (107). Thus suppurative arthritis was a cytolytic, chondrolytic process resulting in fibrosis of synovial membrane and destruction of cartilage.

A wide variety of *Streptococcus* spp. has been associated with endocarditis in swine. Kernkamp (70) reported that in 8 cases of streptococcal endocarditis 5 of the organisms were beta hemolytic and 2 non-hemolytic. Hont and Banks (64) described 2 cases of streptococcal endocarditis. One of the organisms was closely related to *Str. fecalis* and the other was related to the enterococci. Hofferber (62) reported that 5 baby pigs in 1 litter died 8 days to 3 weeks after birth from streptococcal endocarditis. He concluded that intrauterine infection occurred.

Kernkamp (70) observed that the valvular heart lesion
consisted of a thrombus with a proliferative reaction composed of connective tissue, leukocytes and small areas of hemorrhage of the bicuspid valve. Collections of platelets and fibrin were arranged in layers with a swirl-type arrangement on the outer edge of the proliferative zone. The proliferative response was most marked in the cusp of the valves with involvement of the adjacent myocardium. The cusp of the valve was thickened by connective tissue which extended deep into the thrombus. Increased vascularization of the area was evident. Large clumps of bacteria were found in the outer parts of the proliferative zone and the platelet area.

Endocarditis has been reproduced by Hont and Banks (64) in a young pig by intravenous injections of increasing doses of broth culture. Cotchin and Hayward (26) gave 2 pigs a single intravenous dose of an alpha-hemolytic streptococci recovered from a natural case of porcine endocarditis and 2 others were given repeated intravenous doses. Lesions of endocarditis were found macroscopically in the first and histologically in the second pig of the pair receiving the single dose. Arthritis developed in 1 pig of each pair. The 2 pigs given repeated injections showed no macroscopic valvular lesions.

Various species of streptococci have been isolated from meningio-encephalitis of swine (67, 86, 88 and 113). McNutt and Packer (88) studied several outbreaks of meningitis in baby pigs from which gamma- and beta-hemolytic streptococci
were isolated. The isolates were very heterogenous biochemically. No single species of organisms appeared to be involved in the disease condition. This condition was usually associated with a lowered resistance and none of the strains isolated were recognized as pathogenic species of streptococci. McErlean (86) reported an outbreak of meningio-encephalitis in a litter of pigs 4 weeks of age. A motile, pleomorphic, non-hemolytic streptococcus was isolated from the brain. The organism did not correspond to Lancefield's Groups A to G. The disease was reproduced in a young pig and a second pig died from a vegetative endocarditis produced by the organism. Schulte (113) observed an outbreak of diplococcal encephalitis in 15 pigs 3 to 4 weeks of age. Clinical signs consisted of contractions of the muscles of the back, disturbance of consciousness and generalized paresis. The lesions were an acute internal hydrocephalus with secondary flattening of the cerebral convolutions, suppurative leptomenigitis and a thickening of the ependyma.

Abscessation of the cervical lymph nodes of swine is an economic problem to the meat packing industry because of condemnation of the involved portion. Collier (25) quotes 1 Iowa packing plant estimate of an annual loss of $50,000. An Iowa packing company recently conducting a survey of losses caused by cervical abscesses estimated an annual loss of twelve million dollars to the meat packing industry. This included loss from condemnation, down-cutting time and clean up time in
packing plants\textsuperscript{1}.

Collier (25) found the disease to be enzootic on some farms where it affected pigs of all ages. However, the abscess was not usually detected until the pig was slaughtered (25).

Newsom (96) described the lesions of cervical abscesses in swine as ranging from the size of a walnut to that of a baseball and containing odorless, yellowish-green exudate under pressure. A beta-hemolytic streptococcus could be isolated from the exudate.

Stafseth and Clinton (119) first reported Lancefield's typing of the cervical abscess organism. A Group E beta-hemolytic streptococcus was isolated. Careless hog cholera vaccination was suggested as responsible for inducing the abscesses. Snoeyenbos et al. (118) doubted the possibility of vaccination transmission in his study of Group E streptococcus.

In a cultural survey of cervical abscesses in 492 swine in Iowa, Lancefield's Group E streptococcus was isolated from 85 percent of the specimens by Collier (25). Cervical abscesses were regularly induced in young swine given Group E streptococcus culture in the feed. Intranasal or intrapharyngeal inoculation of the above organism produced similar

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lesions but inoculation by various other routes failed to 
induce abscess formation.

Deibel et al. (33) recently studied 37 isolates of Group 
E streptococci. The physiologic characteristics were markedly 
homogenous. Many strains exhibited proteolytic capacities in 
litmus milk fortified with yeast extract. The majority hydro-
lyzed gelatin in agar plate cultures when incubated anaerobic-
ally. None of the common laboratory animals were susceptible 
to infection.

Yao et al. (134) determined the serotypes of 47 Group E 
streptococci isolated from swine cervical lymph node abscesses. 
Forty-two of the 47 strains belonged to his type IV as deter-
mined by precipitin and agglutination tests with type IV 
specific sera.

Between 1937 and 1940 Hare et al. (54) isolated strepto-
cocci from 27 pigs affected with various conditions such as 
fatal septicemias, exudative dermatitis and puerperal syn-
dromes. Hare et al. (54) described a beta-hemolytic strepto-
coccal septicemia in England that was characterized by a high 
mortality rate in unweaned pigs, and he observed an increased 
resistance in older pigs.

Agrimi (1) reported the occurrence of a subacute strepto-
coccal septicemia in week-old pigs. The organism isolated 
resembled Str. zooepidemicus but was impossible to type serol-
ogically.

Van Gils (128) isolated a pure culture of Lancefield's
Group L streptococci from a necrotic portion of the longissimus dorsi muscle of a pig.

**Streptococcus equisimilis**

Frost and Engelbrecht (43) proposed the name *Str. equisimilis* for strains which were serologically Lancefield's Group C but which were biochemically different from *Str. equi* and *Str. zooepidemicus*. Trehalose was fermented by *Str. equisimilis* but not *Str. equi* or *zooepidemicus* and sorbitol was fermented by *Str. zooepidemicus* but not *Str. equisimilis* or *Str. equi*.

*Str. equisimilis* is beta hemolytic (40 mm. zone) and forms discrete opaque colonies. In fluid media, it forms a heavy sediment. *Str. equisimilis* produces acid in glucose, maltose, sucrose, trehalose, glycerol and salicin. It does not hydrolyze starch or esculin and ammonia is produced in peptone medium (89). Evans (39) and Tillett and Garner (123) stated that human strains lyse human fibrin whereas animal strains do not.

Evans (39) studied 53 strains of *Str. equisimilis* to determine the typical and variant characters of the species. The type strain was isolated from a human pathologic process. It gave a positive reaction to Lancefield's type C, was sensitive to filtered phage B and fermented trehalose and salicin, but not sorbitol or lactose. Most strains agglutinated in 1 or more of the antiserums to Griffith's types 7, 20 and 21.
Evans stated that *Str. equisimilis* may occur on the mucous membrane of man and animals without producing lesions or it may cause infection in man and many species of animals. In man, *Str. equisimilis* has a tendency to infect other parts of the body more frequently than the pharynx.

Suppurative Arthritis

Luck (79) stated that the suppurative arthritis is manifested by a very turbid to purulent synovia, hyperemia and fibrosis of the synovial membrane and rapid destruction of the articular cartilage.

Hunter (66), as long ago as 1743, recorded one of the earliest observations of pyogenic arthritis.

It appears from Maceration, that the transverse fibrils are extremely tender and dissolable: and that the cohesion of the parts of the strait fibers is stronger than their cohesion with the bone. When a cartilage therefore is inflammed, and soaked in purulent matter, the transverse or connecting fibers will the sooner give way, and the cartilage becomes more or less red and soft. If the disorder goes on a little longer, the cartilage does not throw off a slough, but separates from the bone, where the force of cohesion is left, and where the disease soon arrives, by reason of the thinness of the cartilage. When the bone is thus exposed, the matter of the ulcer, or motion of the joint, corrodes or abrades the bony fibers. If the constitution is good there will shoot forth a callus; which either cements the opposite bones of the articulation, or fills up the cavity of the joint, and for the future prevents motion. But if, unfortunately, the patient labours under a bad habit of body, the malignancy, having got root in the bone, will daily gain ground, the carries will spread and at last the unhappy person must submit to extirpation.

Luck (79) noted that the earliest changes to appear in
the synovial membrane were hyperemia, edema and increased synovial secretion. Neutrophils concentrated at the site of the invading organism and soon "overflowed" into the joint cavity. This was referred to as the seropurulent stage. Healing at this stage might be prompt. If the organism remained active, the synovial fluid exudate may thicken to form pus within the joint.

The exact mechanism of articular surface breakdown is not known, but most workers agree that the process involves an enzymatic mechanism (29). In 1909, Nathan and Strang (95) stated that cartilage did not play an active part in pathological conditions of the joint, did not react by proliferation, did not form granulation tissue nor did it revert to connective tissue. The only morbid change was a passive one of degeneration produced by interference with its nutrition. These investigators concluded that if the bone was unaffected the cartilage resisted invasion of bacteria and granulation tissue. They emphasized that changes in septic arthritis were "passive" and not "active".

Phemister (104) in 1928 demonstrated that when hyaline cartilage was incubated at 55°C in broth culture or in exudates of non-pyogenic infections such as tuberculosis, no change occurred in the cartilage. If, however, the cartilage was incubated in pyogenic pus the cartilage was digested in 3 to 24 hours. This action was attributed to the release of proteolytic enzymes from the necrotic neutrophils.
Gives and Lindner (45) using leukocyte homogenates could not demonstrate a lytic action on collagen as suggested by Pheraister. Plasmin has been suggested as a factor in the breakdown of cartilage by Lack and Rogers (74) and Curtiss and Klein (29). This substance is a proteolytic enzyme derived from the blood and activated in synovial fluid by either trauma or a kinase produced by streptococci (73). Kopec (71) and Curtiss and Klein (29) reported that neither plasmin nor Staphylococcus aureus cultures or suspension of them had any significant gross or biochemical effect on collagen. No known mammalian enzyme is capable of destroying undenatured collagen similar to the collagenase produced by Clostridium welchii has been observed (82). Lysosomal enzymes in rat liver cells will hydrolyze acid-soluble collagen according to Frankland and Wynn (42) and they stated that the enzyme was most active at pH 4 which was unlikely to be present in an inflammatory process. Curtiss and Klein (29) evaluated the destruction of articular cartilage in septic arthritis and concluded that gross cartilage destruction in septic arthritis was not produced by leukocytic enzymes or plasmin on collagen alone. The removal of collagen by enzymatic or mechanical means was necessary for cartilage destruction. Thus the exact mechanism of in vivo collagen fibril degradation is not known.

Many proteolytic enzymes have been demonstrated to remove chondroitin sulphate from cartilage. Lack and Rogers (74) and Curtiss and Klein (29) demonstrated that plasmin will degrade
the chondromucoprotein of cartilage. Also Ziff et al. (137) demonstrated that leukocyte extracts and trypsin degraded chondromucoprotein of cartilage. Lucy et al. (80) reported that the lysosomal enzymes of the chondrocytes, when under the influence of hypervitaminosis A, will degrade the chondromucoprotein. These papers suggest that many factors will degrade the ground substance of cartilage; however, the significance of this material to physical breakdown of the cartilage is not known.

Curtiss and Klein (29) maintained that the enzymatic effect on matrix alone was not sufficient to account for cartilage destruction in septic arthritis. They suggested that the primary loss of the ground substance from cartilage could produce exposure of the collagen fibers and their destruction by mechanical rubbing produced by joint motion.

Streptococcal Infections in Man

Streptococcal joint infection in man has been associated with 2 types of infectious processes. In the active suppurative process, infection may spread from the bone into the joint as the result of an acute osteomyelitis or suppurative arthritis may result from metastasis of the streptococcal organism to the long capillary network within the synovial membrane (16). Bisgard (14) reported that in a series of 217 cases, more serious damage resulted when infection spread into the joint from the diaphysis.
A nonsuppurative, acute joint involvement is the type observed in rheumatic arthritis. The exact etiology of this type remains unknown. Boyd (16) stated that "although acute episodes develop 10 to 20 days after upper respiratory infections due to Group A beta-hemolytic streptococci, organisms cannot be recovered from involved tissues or body fluids other than the pharynx". Boyd (16) believed the streptococci appeared to initiate the process possibly by an antigen-antibody immune reaction involving connective tissue protein. The tissues (joints and heart) became sensitized by prosthetic groups of the organism uniting with connective tissue protein to create an antigen. This, in turn, elicited the formation of specific antibodies which resulted in antigen-antibody reaction and a focal allergic necrosis accompanied by a characteristic cellular response.

Boyd (16) concluded that streptococcal infection is associated with rheumatic fever. This is supported by "1. the demonstration of elevated titers of streptococcal antibodies, such as antistreptolysin and antistreptokinase, in patients with rheumatic fever, indicating recent contact with streptococci 2. the effect of the treatment of acute streptococcal infections with antibiotics and sulfonamides in reducing the incidence of rheumatic fever 3. the fact that acute streptococcal infections in children are not infrequently followed by an attack of rheumatic fever, and that persons who have had 1 attack are likely to have exacerbations after subsequent
streptococcal infections" (16).

Holbrook (63) listed 2 basic inflammatory changes present in rheumatic fever as exudative and proliferative. He felt that the exudative process was responsible for the swollen joints whereas the proliferative reaction was responsible for the Aschoff bodies and subcutaneous nodules. The joint process of rheumatic fever never resulted in suppuration of such a nature as to cause permanent damage to the joints. During the acute stage, joints were distended with a turbid, yellow fluid.

In a study of various tissues in fatal cases of hemolytic streptococcal infection beginning as scarlet fever, erysipelas, wound infection or puerperal infection, Mallory and Keefer (83) demonstrated that lesions were present in the heart, kidneys, liver, spleen, lungs and pancreas. In the heart, the most common lesion consisted of focal accumulations of lymphocytes and plasma cells. This lesion was most often found in patients who died between 6 and 15 days from onset of symptoms. The reactions were more conspicuous in patients with a bacteremia.

Rheumatoid Arthritis in Man

Rheumatoid arthritis is a clinicopathologic entity of unknown cause which may involve most if not all parts of the body but which is typically manifested by chronic progressive symmetrical inflammation of multiple joints (72). Chronic swine erysipelas arthritis has been reported to be similar to
rheumatoid arthritis in man (114).

Microscopic examination of articular tissues excised in the early stages of the disease revealed hyperemia, edema and inflammation in the synovial and subsynovial layers (4 and 79). The adjacent tendons and tendon sheaths were frequently affected in a similar manner. Initially, the cellular infiltration consisted of lymphocytes, plasma cells and mononuclear and neutrophilic leukocytes which were diffuse and evenly distributed (4). Later, focal collections of lymphocytes appeared and germinal follicles occasionally developed in the greatly enlarged and lengthened synovial villi (4 and 79). Many foci of lymphocytes were not perivascular. As the arthritis progressed, there was little evidence of tissue necrosis or suppuration. Granulation tissue extended from the synovial tissue at the perichondrial margins to cover the articular surfaces (4). Luck (79) suggested that chondroclasts and fibroblasts originating in the pannus may actively absorb the adjacent cartilage. Ossification of the granulation tissue leading to bony ankylosis may occur (4). Occasionally, a pannus destroyed a small quantity of cartilage and fused adjacent cartilage surfaces to produce a fibrous ankylosis (79). Fibrosis and focal lymphocytic infiltration of the subchondral marrow spaces were common (4).

The most characteristic single lesion associated with rheumatoid arthritis was the subcutaneous nodule. These firm swellings appeared over bony prominences, especially on the
olecranon process. Such lesions were firm and rubbery in consistency. When sectioned, they appeared greyish in color and frequently contained 1 or more yellowish-grey necrotic areas. Microscopically, the zones of necrosis appeared sharply outlined by large mononuclear cells in a radial and palisade arrangement. The surrounding connective tissue was both diffusely and focally infiltrated with lymphocytes and plasma cells (4).

Kulka (72) mentioned segmental vasculitis as a basic lesion of rheumatoid arthritis and other connective tissue diseases. There appeared to be a predilection for venules which tended to become obliterated by necrosis and fibrin impregnation. The venulitis was recognized as the primary lesion in the early developmental stages of both the articular and nodular lesions.

Experimental Models of Streptococcal Infection in the Rabbit

In an attempt to understand the pathologic processes of the rheumatoid joint, the rabbit has been used as an experimental animal. This animal has been used to demonstrate that an endocarditis and arthritis are lesions of the same organism and are not produced by different organisms (23). The changes are those of an active streptococcal infection (20). The response of the sensitized joint to intravenous injection of streptococci (5) has been studied.

Cole (23) reported that arthritis and endocarditis may be
produced by intravenous inoculation of rabbits with streptococci from various sources. The lesions were similar to those described as the result of the "micrococcus" or "Diplococcus rheumaticus" inoculation.

Cecil et al. (20) in their study of experimental arthritis in rabbits stated that small doses of various strains of streptococci injected intravenously produced acute arthritis in several joints and many became chronically involved. Experimental arthritis was produced by Str. hemolyticus and Str. viridans. Repeated injections increased the incidence of arthritis. Cecil also stated that the development of arthritis in these animals was related to the degree of bacterial invasion of the blood stream during the first 48 hours after injection rather than to a later dissemination of bacteria from an infected foci. The pathologic lesions of the synovial membrane in chronic streptococcal arthritis occurred after arthritis persisted for 3 or more weeks. In some instances after 12 weeks the synovial villi were prominent and visible with the naked eye.

After 4 to 6 weeks, mononuclear leukocytes, lymphocytes and plasma cells predominated, although neutrophils were still present in the synovial membrane. Considerable numbers of fibroblasts were also seen throughout the subsynovial tissue. Lymphocytes accumulated beneath the synovial membrane as well as about small capillaries in the granulation tissue. As they increased in number, they formed dense focal collections which
in some instances resembled lymph follicles although no definite germinal centers were observed. Similar collections of lymphocytes, frequently associated with fibrosis of bone marrow were seen in the epiphyses adjacent to the affected joints. The blood vessel walls were thicker than normal, and there was proliferation of the endothelium with narrowing of the lumen. In some sections, there was a pannus of fibrous connective tissue over the cartilage. Such a pannus was often associated with degeneration of both hyaline cartilage and bone.

Angevine et al. (5) evaluated the influence of streptococcal immunization on the genesis of experimental arthritis. Viable hemolytic streptococcal cultures were injected intravenously into normal rabbits and into rabbits previously immunized with a formalized vaccine. Arthritis was easily produced in rabbits previously immunized by the intravenous route, and seldom in those animals immunized by the intracutaneous route or in normal rabbits. Intraarticular sensitization by injection of heat-killed streptococci or streptococcal nucleoprotein into the carpal joint followed by intravenous injection of hemolytic streptococci has been studied. Arthritis developed more readily in the sensitized joint and was more obvious than in other joints. The inflammatory process was of a more chronic nature than that usually observed in nonsensitized animals.

Slide culture systems have recently been used by Lowry and Quinn (78) to study the effects of hemolytic streptococci,
Groups A and G, on various tissue culture cells. Cytopathic changes were similar to those produced by viruses. There appeared to be a thickening of the nuclear membrane. Cytoplasmic granulations were an early indication of an altered cell. Cytoplasmic blebs or outpouchings of clear blebs of the cytoplasm were seen early in the course of cell damage. For cell damage to occur, living streptococci had to be present. Growth products were not toxic for slide cultures of human tonsil, liver, monocytes and lymphocytes.

Rib Lesion Formation

Attention has recently been focused on the costochondral junction of swine as the result of the hog cholera eradication program. Hog cholera has been reported by Dunne (36) to produce characteristic lesions at this junction. The mechanism of this lesion formation has been attributed to an upset in calcium-phosphorus ratio, as the hog cholera virus drastically alters this ratio (36, 40 and 61). Dunne (36) mentioned that "a marked histological change occurs at the epiphysis of the ribs of infected pigs. The microscopic examination of the costochondral junction of a rib from a pig subacutely infected with hog cholera shows a markedly enlarged area of mature cartilage cells between the zone of cartilage cell multiplication and the irregular bone. The irregularity of this junction of trabecular bone and the zone of lacunar enlargement is evident upon gross examination of the infected rib".
Runnels et al. (111) attributed the basic lesion to a reduction in the invasion of the vesicular zone by the vascular endothelium. Groth (50) evaluated rib lesions from 47 Iowa swine which had an osteopathy characterized by increased pliability of the rib and enlargement of the distal rib epiphysis. Microscopically, he observed widening of the zone of columns and the vesicular zone of the cartilage, suppressed cartilage breakdown, thin distorted trabeculae in the primary spongiosa that contained necrotic and uncalcified cartilage and aplasia and hypoplasia of the osteoblasts. Hog cholera and/or swine influenza virus were suggested as possible etiologic agents. Groth (50) attributed the basic alteration to the endotheliotropic nature of the hog cholera virus and its interference with active vascular invasion of the vesicular zone. Thus an upset in calcium phosphorus ratio and suppression of vascular invasion (111) have been suggested as mechanisms of lesion formation of the costochondral junction.

The influence of inanition upon the transverse line formation must be considered as animals with an infectious process may go "off feed" for a period of time postinfection. Radioopaque transverse line formation has been associated with acute illnesses in man (57). These transverse lines were shown to be cancellous bony tissue that resulted from a cross-branching of the trabeculae in a horizontal plane. Park and Richter (103), in a study of the mechanism of development of the transverse line, produced such changes by feeding rats a
diet of only dextrose and thiamine chloride solution, thus simulating the natural condition of inanition. His description of this lesion was as follows:

The experimental diet produces a picture in the bones which includes resorbed trabecular and a greatly narrowed proliferative cartilage plate, encased in a thin layer of bone. This thin layer is the transverse line. After the basal state was reached (58 days) in which measurable growth of the proliferative cartilage ceased, the rats were returned to stock ration. After change to recovery procedures, osteoblasts settle on the bony film encasing the cartilage, and produce osteoid so that the line widens; the cartilage plate proliferates and capillaries and osteoblasts grow in through the line into the cartilage and the line. The line is later broken up by blood vessels and is resorbed. The entire process from the time of the formation of the line to its final dissolution requires slightly more than 25 days.

The line forms because the osteoblasts keep making bone after the cartilage growth has waned or vertically stopped. After the recovery diet is instituted, the line thickens because the osteoblasts make new bone on the pre-existing bony encasement without delay, whereas time is required for the cartilage cells to reestablish their cycle, a sequence beginning with division and ending in senescence. Thus cycle must be passed through before the osteoblasts and capillaries can penetrate the cartilage, destroy its cells, and form bone on the matrix frame. The thickening of the line ceases when the capillaries and osteoblasts have penetrated the line to form bone beyond. In the rat, the line is then resorbed.

Park's work demonstrated that the basic lesion of inanition is the cessation of cartilage production with a transverse line of bone formation in the metaphysis.
Fine Structure of Synovial Membrane

The electron microscopy of the normal synovial membrane has been described by several workers (10, 27, 75 and 77) and reviewed by Curtiss (28).

Most synovial cells are overlapped and intertwined (10). The synovial cells are oriented so that their cytoplasmic processes are arranged toward the surface of the membrane. Barland et al. (10) stated that the matrix of the membrane contained dense material but that no fibers with the periodicity of collagen were observed. Coulter (27) observed collagenous structures within the synovial membrane.

The lining cells of the synovial membrane have been divided into 2 types by Barland et al. (10), each supposedly with a different state of cellular activity as evidenced by their cytoplasmic components. The more numerous Barland type A cell had a prominent Golgi apparatus, numerous vacuoles (.4 to 1.5 micron) and contained varying amounts of a dense granular material, mitochondria, intracellular fibrils, micropinocytotic-like vesicles and many filopodia. Barland's type B cell contained large amounts of endoplasmic reticulum with a few large vacuoles, micropinocytotic-like vesicles and mitochondria. Barland et al. (10) suggested that the type A cell was active in uptake of material by phagocytosis and pinocytosis. The type B cell with an abundant endoplasmic reticulum was thought to be concerned with synthesis of proteins.
Active phagocytosis and pinocytosis of the synovial membrane has been demonstrated by Muirden (94). Ferritin, which has a distinctive fine structure appearance, was used to demonstrate absorption of macromolecules from the joint space and was used to demonstrate functional differences between the 2 cell types. Three minutes after injection the ferritin particles appeared in pinocytotic and larger vacuoles.

A basement membrane has not been observed separating the lining cells from the more basal capillaries. Direct continuity between the joint cavity and intercellular spaces was observed (10, 27 and 77). However, Langer and Huth (75), in a study of calf, dog and guinea pig synovial membranes, stated that the stratum synoviale always lined the joint cavity by a continuous layer of synovial cells which were separated from the deeper lying cells by a distinct basement membrane.

Mast cells have been observed in association with the synovial cell layer, especially in the light microscopy study of Asboe-Hansen (6). Castor (19) stated that the mast cells constitute nearly 3 percent of the synovial membrane cells and are usually located immediately beneath the intimal cell layer. These cells are characterized by large cytoplasmic granules and are thought by some to be involved in hyaluronate production (6).

The production of hyaluronate by synovial membrane cells has been demonstrated \textit{in vitro} by 2 experimental approaches. Yeilding \textit{et al.} (135) isolated radioactive hyaluronate after
incubating slices of synovial membrane in glucose C\textsuperscript{14}. Hedberg and Moritz (59) and Castor (19) identified hyaluronate in the supernatant fluid of the synovial membrane cells grown in tissue culture.

Curtiss (28) has summarized the known and unknown factors of the function of the synovial membrane cells.

The specific cell producing hyaluronate, however, has not been definitely identified. Its identification is obscured by the transformation of synovial cells into fibroblasts and even into epithelial cells after prolonged growth in tissue culture. Unknown at present are the ways in which hyaluronate is transported across the cell membrane, the molecular size that is transported, whether further polymerization occurs at the cell surface, and the manner in which the hyaluronate gets into the intercellular matrix. Several observations, however, are at least consistent with the suggestion that the intimal lining cells constitute the major site of synthesis: 1) The locations of the lining cells on the innermost part of the synovial membrane 2) The higher level of oxidative enzymes in the lining cells than in the other connective-tissue cells of the synovial membrane 3) The remarkable enzyme-containing protoplasmic processes of the lining cells.

Alteration of the synovial cell in the diseased state has been investigated by Barland et al. (9). In rheumatoid arthritis, prominent changes were observed in the type A cells. There were fewer filopodia, a smaller Golgi apparatus and altered mitochondria. Many cells contained large numbers of cytoplasmic granules not present in normal lining cells. These granules were characterized by variable electron-opaque content and were often membranous in appearance. The granules contained high levels of acid phosphatase activity and were
tentatively considered "residual bodies".

Hamerman et al. (51) summarized the significance of recent electron microscopic observations as follows:

Cytochemical studies and electron microscopy raise the possibility that the metabolic activity of the lining cells is altered in rheumatoid arthritis, 1) proliferation, and loss of orientation of the lining cells toward the synovial membrane surface may mean some change in the processes that control cell growth and orderly arrangement, 2) increased numbers of lysosomes may mean increased turnover of many extracellular components brought about by release of lysosomal hydrolytic enzymes, 3) many mitochondria appear abnormal, 4) the presence of many large vacuoles and of dense granules suggest enhanced synthesis of material, perhaps hyaluronate, for secretion; or delayed secretion; and/or increased uptake of some extracellular material. The concentration of two uridine coenzymes that might participate in hyaluronate synthesis was higher in rheumatoid synovial membrane than in synovial membrane from osteoarthritic joints.

Is the metabolism of the proliferating lining cells in the hyperplastic rheumatoid synovial membrane perhaps characterized by enhanced anaerobic and aerobic glycolysis? Does the abnormal appearance of the mitochondria in the rheumatoid lining cells indicate excessive oxidative "activity" or rather some abnormal exclusion from metabolic processes? This subject needs more study.

Recently Hamerman and Sandson (52), in a study of the hyaluronate-protein isolated from pathologic synovial fluids, discovered unusual properties that distinguished it from normal hyaluronate-protein. The hyaluronate-protein from pathologic effusions contained more protein, remained immobilized at the origin during zone electrophoresis at pH 4.5 and formed a gel during dialysis in acetate buffer at pH 4.5.
Lysosomes in Pathologic Processes

Lysosomes were first identified in rat liver cells in 1955 by de Duve et al. (32), and are known to occur in many, if not all, animal cells. "The lysosomes are tiny bags filled with a droplet of a powerful digestive juice capable of breaking down most of the constituents of living matter, much as these constituents are fragmented in the gastrointestinal tract of higher animals. In point of fact, the lysosomes function in many ways as the digestive system of the cell" (31). All biologically active compounds, including proteins, nucleic acids and polysaccharides are susceptible to the action of enzymes within the lysosome. Acid phosphatase, present within lysosomes, makes possible visual identification of the lysosomes (47).

In 1959 de Duve (30) noted the following properties of the lysosomes: 1. the dimensions correspond (in .25M sucrose) to a mean diameter of .4 micron 2. they consist of easily soluble hydrolases having further in common an acid pH optimum 3. the surrounding membrane of lipoprotein nature effectively prevents the enzymes from escaping, as well as their respective substrates from penetrating into the particles 4. simultaneous release of all internal enzymes in soluble and fully active form occurs following injury to the limiting membrane.

De Duve (30) stated that the particle was involved in
acid hydrolysis of foreign material engulfed by pinocytosis or phagocytosis, participates in physiologic autolysis and in specialized processes of involution, metamorphosis, holocrine secretion and necrosis. Since the enzymes within these particles were capable of destroying any cellular component, the principal defense of the cell was the integrity of the lysosomal membrane.

The integrity of the membrane depended on maintenance of some of its components in an oxidized state and rupture of the lysosomal membrane occurred rapidly in the absence of oxygen. During autolysis the particle membrane integrity was pH dependent. Thus acidosis may rupture the particle membrane (30). Berthet et al. (13) determined that acid phosphatase bearing granules are osmotic systems and must, therefore, possess a semipermeable membrane and an osmotically active internal medium. Recent work by Trump and Janigan (125) and Novikoff and Essner (98) demonstrated the degenerative cloudy swelling or hydropic degenerative changes of sucrose nephrosis and hydronephrosis to be changes in the lysosomal particles.

Luscombe (81) and Smith and Hamerman (116) found an elevation of acid phosphatase in the synovial tissues of rheumatoid synovial membrane and synovia. This rise in enzyme content was thought to be due to leukocytes in the effusion and to an increase in lysosomes of synovial membrane. West et al. (132) observed a positive correlation between the number of leukocytes in the synovia and the amount of enzyme present.
Dingle (34) demonstrated the ability of lysosomal enzymes to breakdown cartilage matrix with resultant loss of metachromasia. This mechanism was observed in vitamin A toxicity where a loss of metachromasia occurred (34 and 80).

Cohn and Hirsch (22) studied the cytoplasmic granules of the rabbit polymorphonuclear leukocytes. Approximately 70 to 80 percent of the total cellular antimicrobial agent, phagocytin, was present in the lysosomal granule fraction. This material was liberated from the granule by an environment of pH 5 or lower.

Weissmann et al. (131) reported that leukocyte granules were similar to lysosomes isolated from other tissues and that they share common surface properties with erythrocytes. Keiser et al. (69) demonstrated that it was possible to dissociate the effects on mitochondria and lysosomes, less streptolysin being necessary to damage lysosomes than mitochondria.

Thus it is evident that these cytoplasmic particles referred to as lysosomes are involved in many processes of inflammation and autolysis.
METHODS OF PROCEDURE

Source of Pigs

All pigs used in these experiments came from the disease-controlled herd maintained in isolation at the Veterinary Medical Research Institute (VMRI), Ames, Iowa. This herd has been in existence for 13 years, during which time new genetic stock has been added from Caesarean-derived pigs raised in isolation.

Pigs 1 day of age and 3 to 4 weeks of age were used for the experiments. The 3 week old pigs had received 2 ml. of injectable iron dextran\textsuperscript{1} intramuscularly during the first week of life and were weaned at 3 weeks of age. The 1 day old pigs received colostrum and were maintained on SPF-lac\textsuperscript{2}.

Source of Isolates

\textit{Str. equisimilis} isolates used as inoculums were recovered from naturally occurring cases of suppurative arthritis submitted to the IVDL for diagnosis. Various joints were involved. The isolates were from pigs younger than 2 weeks of age and were from farms where suppurative arthritis in young pigs was a problem. The isolates used in the various

\textsuperscript{1}Produced by Armour Veterinary Laboratories, Kankakee, Illinois.

\textsuperscript{2}Borden Company, New York, New York.
experiments included IVDL 1168 (isolate 1), IVDL 1154 (isolate 2) and IVDL 1131 (isolate 3). They were beta hemolytic on 5 percent horse-blood agar, fermented lactose and trehalose and produced no change in mannite, salicin, raffinose, inulin or sorbitol. Acid formation and reduction were observed in litmus milk. All organisms were Lancefield's type C as determined by the autoclave extraction method (106).

Stock cultures were maintained either on sealed blood agar slants maintained at room temperature or were lyophilized. *Streptococcus equisimilis* isolates stored on the blood agar slants were transferred to fresh blood agar slants every 2 months. A 24-hour culture of *Streptococcus equisimilis* (isolate 1) in 10 percent calf serum nutrient broth was lyophilized and stored at 5°C until used.

**Housing of Pigs**

All of the 3 week old pigs were maintained in isolation. Control animals were housed in the same building in adjacent pens. All units were cement floored with adequate self feeders and waterers available.

The 1 day old pigs were raised in cardboard boxes in isolation rooms in the Department of Veterinary Pathology, Iowa State University, Ames, Iowa.
Ration Fed

All pigs weaned at 3 weeks of age were self fed a complete 19 percent protein pig starter devoid of antibiotics. This was fed until the pigs were 6 to 7 weeks of age. The longer duration groups were fed a complete 16 percent protein pig grower ration. The feed of the 6-month group was changed to a 15 percent protein, low energy ration at approximately 3 months postinoculation.

Clinical Observations

All animals were observed daily for lameness or other deviations from normal. Daily temperatures were taken for the first 10 days.

Blood Chemistry

Blood was collected immediately after electrocution in acid-cleaned tubes from the brachial vessels for serum calcium, phosphorus, alkaline phosphatase and vitamin A determinations. The blood samples were allowed to clot and then were centrifuged at 400 G for 30 minutes. Serum was drawn off, pipetted into separate screw cap test tubes and frozen at -5°C until vitamin A determinations were made. Serum calcium, phosphorus and alkaline phosphatase determinations were made the same day as collection using acid-cleaned glassware.
Serum samples were submitted to Pharmatox Laboratories\(^1\) for vitamin A determinations.

Serum calcium determinations were made by the murexide method (55). The test makes use of the reaction which changes the color of a murexide solution from purple to pink when calcium is present. This method was used to detect microgram quantities of calcium in aqueous solution in the presence of magnesium. A calcium standard was used with each test. Colorimetric determinations were made with a junior Coleman Spectrophotometer Model 6A\(^2\) operated at 500 \(\text{m} \mu\) wave length.

Serum inorganic phosphorus and alkaline phosphatase determinations were made using a lumetron colorimeter Model 401A\(^3\) (red filter 650 \(\text{m} \mu\) wave length). Both tests are dependent upon the presence of inorganic phosphorus. The solutions containing phosphate are treated with ammonium molybdate, whereby phosphomolybdic acid is formed from any inorganic phosphate present. When the reducing agent aminonaphthol sulphonic acid is added, the phosphomolybdic acid is reduced to yield a blue color called "molybdenum blue". This color is used to measure the amount of phosphate present (58).

Serum alkaline phosphatase catalyzes the liberation of

\(^{1}\text{Pharmatox Laboratories, 2006 Grand Avenue, Ames, Iowa.}\)

\(^{2}\text{Coleman Instrument Incorporated, Maywood, Illinois.}\)

\(^{3}\text{Photovolt Corporation, 1115 Broadway, New York 10, New York.}\)
inorganic phosphate from phosphate esters such as glycerophosphate solutions in which the serum is incubated. The phosphate liberated from the glycerophosphate solution is used as an index of phosphatase activity. One Bodansky unit corresponds to the liberation of 1 mg. of inorganic phosphate per 100 ml. of serum during a 1-hour period of incubation (58).

Necropsy Procedures

A complete necropsy was performed on all 30 pigs of Experiment 6 and all pigs killed in Experiments 1 through 5. All were killed by electrocution and the blood samples were collected immediately from the incised brachial blood vessels.

All joints were opened without searing the exterior or subcutaneous portion of the joint as this would have interfered with fixation of the synovial cells for electron microscopy.

Tissues were routinely collected from the costochondral junction of ribs 6 and 7, and synovial membrane and articular surface from all involved joints. If no joint lesions were observed, tissues were collected from the right femoro-tibial, tibio-tibial tarsal and humero-radial articulations. For light microscopic and histochemical studies, tissues were fixed in 10 percent buffered (pH 7.0) formalin (84).
Reisolation of Organism

Samples were routinely collected from the involved joint on sterile cotton swabs and cultured on 5 percent horse blood agar plates which were incubated at 37°C. In some instances where the visceral and joint lesions suggested either Mycoplasma sp or Erysipelothrix insidiosa infection, beef heart infusion-turkey serum medium plus 2000 units penicillin per ml, and sufficient thallous acetate to give a final concentration of 1:4000 (PPLO medium) (109) or sodium azide crystal violet medium was used (101).

Following isolation of the organism on horse blood agar, biochemical characterization of the organism was performed using mannite, lactose, salicin, raffinose, trehalose, inulin, sorbitol and litmus milk.

Tissue Preparation

After the bone specimens were fixed, they were decalcified overnight in a formic acid-citrate mixture (84) under a vacuum of 18 to 20 cm. of mercury. After 8 to 24 hours in the formic acid, the tissues were transferred to a 5 percent ammonium hydroxide solution and placed under a vacuum of 18 to 20 cm. of mercury for 1 to 2 hours. The bone was washed in running tap water for 6 hours, trimmed and processed with the remaining soft tissues, dehydrated with graded ethyl alcohol series, and cleared with chloroform. All tissues were
infiltrated and embedded in an Altmann's paraffin mixture (11) (56°C melting point) and were sectioned at 6 microns.

Three different stains were used. All sections were routinely stained with Harris hematoxylin and eosin (H and E) (84). Selected slides of the synovial membrane were stained with periodic acid Schiff (PAS) and Gram stain (84). The PAS was used to detect fibrinoid within the synovial membrane and the Gram stain was used to detect the presence of bacteria. Bone and cartilage were stained with toluidine blue to evaluate changes of the cartilage ground substance. The technique of Belanger (12) requires toluidine blue stain at pH 4.3 and tertiary butyl alcohol as a dehydrant.

All joint tissues (3 day, 30 day and 6 months postinoculation) which were examined by electron microscopy were collected immediately after the joint was opened; usually only the first 2 joints opened were fixed. Thin pieces (1 to 2 mm.) of synovial membrane and articular surface were minced in a Petri dish with a beeswax layer on the bottom. The tissues were minced in chilled 3 percent buffered glutaraldehyde, (pH 7.4), using either Millonig's phosphate (90), Hank's balanced saline (53) or veronal acetate (102) as a buffer. Mincing was followed by the transfer of tissue to 3 vials. One contained 1 percent buffered osmium tetroxide, and 2 vials contained 3 percent buffered glutaraldehyde using the various buffers previously described. One vial of glutaraldehyde fixed tissues was used for acid phosphatase procedures. Thus tissues were
fixed in 3 vials: 1 for osmium tetroxide, 1 for glutaraldehyde fixation and storage, and 1 for glutaraldehyde fixation and acid phosphatase procedures.

Tissues fixed in osmium tetroxide for 1 hour were dehydrated using a graded ethyl alcohol series and processed as follows: 1. 1 change 50 percent alcohol for 5 minutes 2. 1 change 70 percent alcohol for 5 minutes 3. 1 change 95 percent alcohol for 10 minutes 4. 2 changes of 100 percent alcohol for 15 minutes each 5. infiltrated with a 3:2 butyl-ethyl methacrylate mixture containing final concentration of 1 percent divinyl benzene (130) - 3 changes for 1 hour each 6. placed in 00 gelatin capsules and polymerized overnight at 58° to 60°C. One percent benzoyl peroxide was added to the methacrylate mixture as an initiator and the mixture then filtered through anhydrous sodium sulfate to remove excessive moisture. Blocks were trimmed and then sectioned at approximately 80 to 100 mμ with an LKB ultratome.¹

Sections were mounted on parlodion-coated copper grids, allowed to dry and stained with 1 percent uranyl acetate (129) for 30 minutes, 1 percent potassium permanganate (76) for 1 hour or saturated solution lead acetate² for 10 minutes. All

¹LKB-Produkter A. B., Box 12220, Stockholm 12, Sweden.

sections were examined at 50 KV on an Hitachi HU-11A\textsuperscript{1} electron microscope with condenser aperture of 300\,\mu m and objective aperture 50\,\mu m diameters. Kodak contrast projection plates\textsuperscript{2} (3 1/4 x 4 1/4) were used as negative material in the microscope.

Acid phosphatase staining was used according to Gomori's technique\textsuperscript{(87)} as modified by Sabatini \textit{et al.} (112) as follows: 1. fix tissues at 5\,\degree C in 3 percent glutaraldehyde for 30 minutes 2. incubate them at 37\,\degree C in substrate solution for 2 to 4 hours 3. incubate duplicate tissues at 37\,\degree C in substrate solution plus sodium fluoride (.05 percent) 4. rinse them in 1 percent acetic acid 5. precipitate phosphate by adding 5 percent ammonium sulfide for 2 minutes and 6. rinse tissues in buffer solution and postfix in 1 percent osmium tetroxide for 1 hour. Then the tissues were dehydrated, infiltrated and embedded as previously described for electron microscopy.

**Design of Experiments**

**Experiment 1**

This experiment was initiated to study the pathogenesis of streptococcal arthritis. Twelve 3 week-old pigs were randomized into 4 groups. The \textit{Str. equisimilis} inoculum was prepared by 8 daily yolk sac passages of isolate 1 in 6- to

\textsuperscript{1}Hitachi, L. T. D., Tokyo, Japan.

\textsuperscript{2}Eastman Kodak Company, Rochester, New York.
10-day chicken embryos and once in PPLO medium (109). A 24-hour culture was used, the turbidity of which was adjusted to number 3 on the McFarland's nephelometer tube scale (approximately $9 \times 10^8$ organisms per ml.). One ml. of culture was injected into the right stifle joint after the skin had been scrubbed with surgical soap and 70 percent ethyl alcohol applied 3 times. An 18-gauge needle was directed inward just medial to the patella until cartilage was entered. Then the needle was withdrawn slightly and the inoculum introduced into the joint. Three weeks later the same procedure was used for the introduction of 3 ml. of inoculum into the left stifle joint. Ten days later 3 animals were killed and a complete necropsy was performed. The remaining animals were not killed as lameness was not evident.

Experiment 2

To compare the pathogenicity of various field isolates, four 4-week old pigs were inoculated by various routes as shown below. In each instance, the organism was grown in PPLO medium for 24 hours. This culture had a turbidity corresponding to a number 3 McFarland's nephelometer tube. The isolate used, amount of inoculum and route of injection are summarized as follows:

<table>
<thead>
<tr>
<th>Pig Number</th>
<th>Isolate</th>
<th>Amount of Inoculum</th>
<th>Route of Injection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>5 ml.</td>
<td>Intraperitoneal</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>5 ml.</td>
<td>Intraperitoneal</td>
</tr>
<tr>
<td>Pig Number</td>
<td>Isolate</td>
<td>Amount of Inoculum</td>
<td>Route of Injection</td>
</tr>
<tr>
<td>------------</td>
<td>---------</td>
<td>-------------------</td>
<td>-------------------</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>5 ml.</td>
<td>Intraperitoneal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 ml.</td>
<td>1 week later intraarticular</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>5 ml.</td>
<td>Intraarticular</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 ml.</td>
<td>5 days later intraperitoneal</td>
</tr>
</tbody>
</table>

**Experiment 2**

This experiment was initiated to determine whether the virulence of the organism could be increased by daily yolk sac passage in 6- to 7-day old chicken embryos.

Two isolates (1 and 2) were passaged at daily intervals through chicken embryos for 10 passages. This was accomplished by the aspiration of one-tenth ml. of yolk material from an infected embryo and then injecting this immediately into another chicken embryo yolk sac. Each pig was injected intraperitoneally with 3 ml. of isolate 1. One animal served as a control. The animals were maintained on experiment approximately 10 days. No lameness was observed. The pigs were killed and a complete necropsy was performed.

**Experiment 4**

To determine the age factor of the pigs' susceptibility to *Str. equisimilis*, 2 pigs 1 day of age, that had received colostrum, were weaned and maintained on SPF-lac. Pig 1 at 2 days of age received intraarticularly 0.5 ml. of a 24-hour culture of isolate 1. Pig 2 at 2 days of age received intraperitoneally 0.5 ml. of the same organism which had been
propagated in the chicken embryo yolk sac for 10 passages. Pigs were maintained on experiment for 10 days, killed and a complete necropsy performed.

Experiment 5

Four 1-day old pigs that had received colostrum were used to elucidate the pathogenicity of _Str. equisimilis_ (isolate 1) which was administered in the feed and by intravenous inoculation. Two pigs were fed orally by adding 5 ml. of a 24-hour PPLO medium culture to the SPF-lac on 3 consecutive days. Two pigs were injected in the anterior vena cava with 0.5 ml. of a 24-hour serum broth culture.

Experiment 6

The inoculums used in Experiment 6 were prepared by passing a lyophilized culture of isolate 1 in a 10 percent serum broth. After 24 hours incubation the culture was streaked on horse blood agar plates and incubated for 36 hours. Smooth colonies were selected and transferred to serum broth. In each instance, a 24-hour broth culture (turbidity of number 3 McFarland's nephelometer tube) was used as inoculum. A Gram stained smear of each tube of inoculum was checked for any obvious contamination.

Thirty pigs 3 weeks of age were randomized into 5 groups and necropsied 3 days, 15 days, 30 days, 3 months and 6 months postinoculation. Two pigs in each group were used as uninoculated controls. Four pigs in each group were inoculated by
injecting 3 ml. of a 24-hour serum broth culture into the anterior vena cava.

Field Studies

Swine joint tissues were collected from 30 different cases of suppurative arthritis from which *Str. equisimilis* had been isolated. Costochondral junctions of ribs 6 and 7 were collected from 18 of the 30 cases submitted to the IVDL. In each instance, the streptococcal organism isolated was identified biochemically. Synovial membrane, articular surface with the adjacent bone and costochondral junctions were fixed in 10 percent buffered (pH 7.0) formalin and processed by the paraffin technique previously described. All blocks were sectioned at 6 microns and stained with H and E.
RESULTS

Experimental Production of Streptococcal Arthritis

Experiment 1

The results of intraarticular injection of broth culture of *Str. equisimilis* were evaluated in 12 pigs 3 weeks of age and randomized into 4 groups. Volume of inoculum was also evaluated. All 12 inoculated pigs developed marked lameness for the first 3 to 4 days after injection of the right stifle joint with 1 ml. of culture. After that period, lameness was not evident in any of the infected pigs. The opposite stifle joint was injected 3 weeks later with 3 ml. of the culture because the possibility existed that too small an amount of inoculum had been used for the first injection. Following injection of the second joint, the infected animals were markedly lame the first day. However, within 3 days no lameness was evident in any of the pigs. Six days after the second injection 3 pigs of 1 group were necropsied. The remaining inoculated pigs were not necropsied. All injected pigs developed a transient febrile response of 1° to 3°F following the first injection and a similar type response after the second injection but of shorter duration.

No gross alteration of the injected joints was observed. The microscopic changes of the right stifle receiving 1 ml. of inoculum were confined to the synovial cell layer. Degenerative changes characterized by marked pyknosis to necrosis were
observed (Figure 1). The joints receiving 3 ml. of inoculum were characterized by a mild proliferative response of the synovial membrane. There was a prominent synovial cell layer with varying stages of cellular degeneration and necrosis (Figure 2). Perivascular accumulation of lymphocytes and macrophages was present around adjacent blood vessels. No evidence of fibrosis was present in any of the joints examined.

Thus from Experiment 1 it was evident that intraarticular injection and the volume of inoculum injected were not adequate for the production of joint lesions which resembled those observed in naturally occurring cases.

Experiment 2

The ability of 3 field isolates of Str. equisimilis administered by various routes to produce clinical lameness was evaluated in 4 pigs.

The intraperitoneally and intraarticularly injected pigs developed a mild febrile response (1° to 2°F). Two of 3 intraperitoneally infected pigs developed lameness at about 3 days, but by 1 week postinoculation lameness was not evident and periarticular fibrosis was not present.

Following intraarticular injection, pigs were markedly lame but by the third day the pigs were walking normally. A slight elevation in temperature followed intraarticular injection.

Thus it appeared that the experimental production of
Figure 1. Synovial cell layer 4 weeks after intraarticular injection (1 ml.) of Str. equisimilis. Notice absence of fibrosis and pyknosis of synovial cells (arrow). Hematoxylin and eosin stain. X 256.

Figure 2. Synovial membrane 6 days after intraarticular injection (3 ml.) of Str. equisimilis. Observe perivascular accumulations of macrophages, lymphocytes and cellular debris. Hematoxylin and eosin stain. X 160.
streptococcal arthritis was not dependent upon a specific strain or isolate and that simultaneous intraperitoneal and intraarticular injections were not factors necessary for the development of the experimental disease.

Experiment 3

Two pigs were inoculated intraperitoneally with 3 ml. of culture from tenth chicken embryo yolk sac passage. A different isolate was used in each pig.

Each pig injected intraperitoneally developed a mild elevated temperature (1°F) and failed to develop lameness in 10 days. No gross or microscopic lesions were observed.

Experiment 4

Two pigs 1 day of age were inoculated intraarticularly and intraperitoneally, respectively, to determine the effect of age upon susceptibility to streptococcal organisms.

The pig injected intraarticularly developed a marked lameness and elevated temperature (3°F rise). By 3 days post-inoculation the temperature had returned to normal and lameness was not evident. The pig infected intraperitoneally failed to develop lameness and had a 1°F rise in temperature. The animals were not necropsied.

Experiment 5

To further evaluate the route of inoculation on experimental production of lameness, 2 pigs were fed Str. equisimilis
cultures and 2 were inoculated intravenously with the same organism.

Both pigs fed the streptococcal organisms developed a diarrhea and a mild febrile response (1°C to 2°F). By 5 days after the last streptococcal feeding, the temperature had returned to normal and the diarrhea had ceased.

The pigs receiving the intravenous injection developed a febrile response (2°C to 3°C) by the first day and lameness by the second day. The involved stifle and hock joints evidenced a local hyperthermia and periarticular swelling. By 10 days postinoculation marked periarticular fibrosis was observed and the pigs were very lame.

Thus in the 5 preliminary experiments it was determined that streptococcal arthritis could be established by intravenous injection, but could not be reproduced by intraarticular, intraperitoneal plus intraarticular, intraperitoneal or oral routes of inoculation. Rapid yolk sac passage of Str. equisimilis in chicken embryos did not enhance the pathogenicity so that intraarticular injection produced clinical arthritis.

Experiment 6

After it was established that intravenous injection of Str. equisimilis would reproduce joint lesions similar to the naturally occurring disease, 30 pigs 3 weeks of age were randomized into 5 groups. The groups were inoculated intravenously with Str. equisimilis and were killed 3 days, 15 days,
30 days, 3 months and 6 months postinoculation. Two pigs in each group were used as uninoculated controls. The gross joint lesions and the reisolation of streptococcus for each arthritic joint are given in Table 1.

Clinical Observations

Eighteen of 20 inoculated pigs developed lameness by 3 days postinoculation. All pigs developed a 3° to 4°F temperature elevation. By 15 days postinoculation 15 of 16 remaining pigs were lame and an intermittent temperature elevation was observed (maximum 4°F) in all pigs. Marked muscular atrophy was observed by 1 month postinoculation in the involved limbs of several pigs. Lameness was evident in all 12 of the pigs still on trial.

In pig 23 (Table 1) lameness persisted for 6 weeks after which definite lameness was not evident. Pig 24 (Table 1) developed a marked lameness in the pelvic limbs. This pig (24) died 2 months postinoculation as a result of fibrous pericarditis and valvular endocarditis. Str. equisimilis was isolated from the liver, spleen, kidney and heart lesions.

At 3 months postinoculation only 7 pigs were still on trial and lameness was observed in 6 of 7 pigs. In 2 lame animals at 2 months postinoculation, the periartricular fibrosis was not as pronounced as that of 1 month. By 6 months postinoculation, no evidence of fibrosis was observed. However, arrested growth of the long bones was evident in 2 pigs
Table 1. Summary of gross joint lesions in pigs inoculated intravenously with *Str. equisimilis* and in uninoculated control pigs

<table>
<thead>
<tr>
<th>Time Post-inoculation</th>
<th>Pig Isolation No.</th>
<th>Lesions Observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 days 1</td>
<td>1</td>
<td>Uninoculated control. None.</td>
</tr>
<tr>
<td>3 days 2</td>
<td>2</td>
<td>Uninoculated control. None.</td>
</tr>
<tr>
<td>3 days 3</td>
<td>+</td>
<td>R. tarsal joints: Slight increase turbid synovia, periarticular edema, congestion synovial membrane, no articular surface change.</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>L. tarsal joints: Increase clotted synovia.</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>L. humero-radial joint: Focal edematous area proximal to joint, increase turbid synovia, congestion to whitish coloration of synovial membrane, no articular surface change.</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>Atlanto-occipital joint: Abundance purulent exudate within joint, edema around joint.</td>
</tr>
<tr>
<td>3 days 4</td>
<td>+</td>
<td>L. femoro-tibial joint: Marked congestion synovial membrane, increase very turbid thin synovia, no articular surface change.</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>R. tarsal joints: Periarticular edema, increased clotted synovia, congestion to whitish coloration of synovial membrane.</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>L. humero-radial joint: Marked congestion of membrane, increase turbid synovia, periarticular edema, no articular surface change.</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>R. humero-radial joint: Increase clotted synovia, slight congestion synovial membrane.</td>
</tr>
<tr>
<td>3 days 5</td>
<td>+</td>
<td>R. tarsal joints: Periarticular fibrosis, marked increase turbid synovia, hyperemia synovial membrane, hyperemia and edema subcutaneous tissues surrounding joint.</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>L. tarsal joints: Increase turbid synovia (5 ml.).</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>R. humero-radial joint: Increase turbid synovia (10 ml.), foci purulent-fibrin material within joint, hyperemia synovial membrane.</td>
</tr>
<tr>
<td>Time Post-inoculation</td>
<td>Str. Isolation No.</td>
<td>Lesions Observed</td>
</tr>
<tr>
<td>-----------------------</td>
<td>-------------------</td>
<td>------------------</td>
</tr>
<tr>
<td>3 days</td>
<td>5</td>
<td>- L. humero-radial joint: Slight increase turbid synovia.</td>
</tr>
</tbody>
</table>
| 3 days                | 6                 | - R. humero-radial joint: Increase turbid synovia.  
|                       |                   | + L. tarsal joints: Marked distention of synovial cavity very turbid synovia, membrane appeared congested, beginning periarticular fibrosis.  
|                       |                   | + R. tarsal joints: Distention synovial cavity turbid synovia, hyperemia synovial membrane not marked.  
|                       |                   | - L. humero-radial joint: Increase turbid synovia.  
|                       |                   | - R. carpal joints: Increase turbid synovia.  
|                       |                   | - L. carpal joints: Increase turbid synovia.  
| 15 days               | 7                 | Uninoculated control. None. |
| 15 days               | 8                 | Uninoculated control. None.  
| 15 days               | 9                 | No gross lesion.  
| 15 days               | 10                | + L. lateral digit (2nd phalangeal joint): Marked periarticular fibrosis, slight increase synovia.  
| 15 days               | 11                | + R. tarsal joints: Marked periarticular fibrosis, no increase synovia, ulceration tibial tarsal articular surface, white discoloration synovial membrane.  
|                       |                   | + L. tarsal joints: Marked periarticular fibrosis. No increase synovia. White discoloration of synovial membrane.  
|                       |                   | + R. humero-radial joint: Increase turbid synovia.  
|                       |                   | + L. humero-radial joint: Increase turbid synovia.  
| 15 days               | 12                | + R. tarsal joints: Marked periarticular fibrosis, white discoloration of synovial membrane with hyperemia near tip villi.  

<table>
<thead>
<tr>
<th>Time Post-inoculation</th>
<th>Str. Isolation</th>
<th>Lesions Observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 days</td>
<td>12'</td>
<td>L. tarsal joints: Marked periarticular fibrosis, white discoloration of synovial membrane with hyperemia near tip villi.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R. femoro-tibial joint: Periarticular fibrosis, hyperemia of synovial membrane, increase turbid synovia.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L. femoro-tibial joint: Periarticular fibrosis, hyperemia of synovial membrane, increase turbid synovia.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Atlanto-occipital joint: Marked increase turbid synovia, hyperemia synovial membrane.</td>
</tr>
<tr>
<td>1 month</td>
<td>13</td>
<td>Uninoculated control. None.</td>
</tr>
<tr>
<td>1 month</td>
<td>14</td>
<td>Uninoculated control. None.</td>
</tr>
<tr>
<td>1 month</td>
<td>15</td>
<td>L. tarsal joints: Slight periarticular fibrosis, fibrosis of synovial membrane.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R. tarsal joints: Slight increase synovia, small area ulceration of medial condyle tibial tarsal.</td>
</tr>
<tr>
<td>1 month</td>
<td>16</td>
<td>L. tarsal joints: Periarticular fibrosis, fibrosis synovial membrane.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R. scapulo-humeral joint: Slight increase turbid synovia, slight proliferation synovial membrane.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R. coxo-femoral joint: Increase turbid synovia, hyperemia synovial membrane, small focal ulcerated area near margin of acetabulum.</td>
</tr>
<tr>
<td>1 month</td>
<td>17</td>
<td>R. femoro-tibial joint: Hyperemia and proliferation synovial membrane, approximately 20 ml. synovia, focal areas congestion of articular surfaces.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R. tarsal joints: Slight periarticular fibrosis, slight turbid synovia with tags of fibrin, fibrosis synovial membranes.</td>
</tr>
</tbody>
</table>
Table 1 (Continued)

<table>
<thead>
<tr>
<th>Time Post-inoculation</th>
<th>Pig Str. Isolation No.</th>
<th>Lesions Observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 month 17</td>
<td>-</td>
<td>L. tarsal joints: Marked periarticular fibrosis, proliferation, fibrosis and hyperemia synovial membrane.</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>R. humero-radial joint: Ulceration lateral, distal condyle humerus, proliferation and fibrosis synovial membrane, slight increase synovia.</td>
</tr>
<tr>
<td>1 month 18</td>
<td>-</td>
<td>L. tarsal joints: Increase turbid synovia.</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>R. tarsal joints: Slight periarticular fibrosis, fibrosis synovial membrane slight increase turbid synovia. Small ulcerated foci on lateral condyle tibial tarsal bone.</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>R. scapulo-humeral joint: Yellow discoloration articular surface, increase thickened synovia, slight proliferation synovial membrane.</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>R. humero-radial joint: Slight increase turbid synovia.</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>L. humero-radial joint: Clotted synovia.</td>
</tr>
<tr>
<td>3 months 19</td>
<td>Uninoculated control. None.</td>
<td></td>
</tr>
<tr>
<td>3 months 20</td>
<td>Uninoculated control. None.</td>
<td></td>
</tr>
<tr>
<td>3 months 21</td>
<td>-</td>
<td>R. coxo-femoral joint: Fibrous ankylosis of joint, marked periarticular fibrosis, no synovia.</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>L. Coxo-femoral joint: Marked periarticular fibrosis, inspissated exudate within joint.</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>R. tarsal joints: Marked periarticular fibrosis, periarticular abscess formation.</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>R. scapulo-humeral joint: Periarticular fibrosis, erosion proximal end humerus, dry inspissated material within joint, fibrosis synovial membrane.</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>L. scapulo-humeral joint: Marked periarticular fibrosis, no synovia, fibrosis synovial membrane.</td>
</tr>
</tbody>
</table>
Table 1 (Continued)

<table>
<thead>
<tr>
<th>Time Post-inoculation</th>
<th>Pig Str. No.</th>
<th>Isolation</th>
<th>Lesions Observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 months</td>
<td>21 +</td>
<td>R. carpal joints: Periarticular fibrosis, no synovia, fibrosis synovial membrane.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>+ L. carpal joints: Marked periarticular fibrosis, no synovia, fibrosis synovial membrane, slight periarticular fibrosis.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>+ R. tarsal joints: Periarticular abscess formation, marked periarticular fibrosis, ulceration tibial tarsal surface, hyperemia synovial membrane.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>+ L. tarsal joints: Marked periarticular abscess and fibrosis, hyperemia synovial membrane, increase turbid synovia.</td>
<td></td>
</tr>
<tr>
<td>3 months</td>
<td>22 -</td>
<td>R. coxo-femoral joint: Periarticular fibrosis, fibrous ankylosis, fibrosis synovial membrane, no synovia.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>+ L. tarsal joints: Mild periarticular fibrosis, slight hyperemia, fibrosis synovial membrane, ulceration tibial tarsal articular surface.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>+ L. carpal joints: Marked periarticular fibrosis, ulceration articular surface, proliferation synovial membrane, minimal synovia.</td>
<td></td>
</tr>
<tr>
<td>3 months</td>
<td>23 -</td>
<td>L. coxo-femoral joint: Mild periarticular fibrosis, roughening and yellowish coloration of articular surfaces.</td>
<td></td>
</tr>
<tr>
<td>3 months</td>
<td>24 +</td>
<td>R. humero-radial joint: Periarticular fibrosis, marked increase thin synovia.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>+ R. femoro-tibial joint: Increase turbid synovia, marked hyperemia synovial membrane.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>+ L. femoro-tibial joint: Increase turbid synovia, hyperemia synovial membrane.</td>
<td></td>
</tr>
</tbody>
</table>
Table 1 (Continued)

<table>
<thead>
<tr>
<th>Time Post-inoculation</th>
<th>Pig No.</th>
<th>Str. Isolation</th>
<th>Lesions Observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 months 25</td>
<td></td>
<td></td>
<td>Uninoculated control. None.</td>
</tr>
<tr>
<td>6 months 26</td>
<td></td>
<td></td>
<td>Uninoculated control. None.</td>
</tr>
<tr>
<td>6 months 27</td>
<td></td>
<td>L. scapulo-humeral joint: Proliferation and hyperemia of synovial membrane, increase turbid serosanguineous synovia, necrosis and ulceration of both articular surfaces.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>R. tarsal joints: Articular surface appears thin with focal areas of erosion.</td>
<td></td>
</tr>
<tr>
<td>6 months 28</td>
<td></td>
<td>R. scapulo-humeral joint: Marked erosion at periphery of humeral articular surface, slight proliferation of synovial membrane.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>L. coxo-femoral joint: Yellow coloration articular surface, flattening head of femur with focal ulcerative areas.</td>
<td></td>
</tr>
<tr>
<td>6 months 29</td>
<td></td>
<td>R. tarsal joints: Fibrosis of synovial membrane, flattening and degenerative changes lateral condyle of tibial tarsal, periarticular fibrosis not prominent.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>L. tarsal joints: Mottling articular surfaces.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>R. scapulo-humeral joint: Depressed area 1-2 cm. posterior aspect of proximal end humerus.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>R. humero-radial joint: Hyperemia and proliferation of synovial membrane, lateral concyle of humerus focal raised areas of cartilage with roughening and fissure formation.</td>
<td></td>
</tr>
<tr>
<td>6 months 30</td>
<td></td>
<td>L. tarsal joints: Erosion lateral tibial tarsal condyle, slight proliferation of synovial membrane.</td>
<td></td>
</tr>
</tbody>
</table>

+ Isolation of Str. ecuisimilis
- Str. ecuisimilis was not isolated
at 3 months postinoculation as noted by the disproportionate shortening of the limbs (Figure 3).

At 6 months postinoculation, in the 4 surviving pigs lameness was evident in 1 of 4 pigs with overextension of the fetlock and distal joints of the limbs. Clinical lameness had disappeared in 3 of the 4 pigs after 2 months postinoculation.

**Synovial Membrane Changes**

**Normal control**

The normal synovial membrane of all 8 control animals whose ages ranged from 3 weeks to 6 months appeared similar. Grossly, the membrane was homogenously pink-white with normal villi. At 6 months postinoculation villous projections were more apparent.

Microscopically, the synovial cell layer varied from a single cell at the bottom of the villous crypts to 3 to 4 cells at the tip of the low villi (Figure 4). Small capillaries were observed in the connective tissue spaces underlying the synovial cells.

The normal fine structure of the synovial cell layer was determined in tissues from 8 pigs varying in age from 1 day to 2 months. The lining cell layer of the synovial membrane varied in thickness from 1 to 3 cells. The cells were arranged without connecting structures between the individual cells and, in some areas, in a lamellar pattern. Each synovial cell was separated by varying numbers of fibrillar
Figure 3. Pigs 3 months postinoculation. Observe disproportionate shortening of the limbs of one severely affected pig (right).
Figure 4. Normal swine synovial membrane. The synovial cell layer is 2 to 3 cells thick at tips of the villi. Hematoxylin and eosin stain. X 256.
structures with a periodicity resembling collagen (85). Free collagen bundles were observed within the lumen of the joint. A granular material was also located between cells and was similar to that observed within adjacent blood vessels; membrane structures were also observed between synovial cells. The cell outline varied from smooth (Figure 5) to irregular (Figure 6); usually the cells with pinocytic vesicles and a poorly developed endoplasmic reticulum had the irregular outline, while the even cell outline was either associated with a well developed endoplasmic reticulum (Figure 8) or with an absence of cytoplasmic organelles. Those cells with well developed endoplasmic reticulum appeared to be fewer in number and located in areas where the synovial cells were several cells in thickness (Figure 8). The most frequently observed cell had pinocytic vesicles and was most prominent in areas composed of 1 to 2 synovial cells. Thus swine synovial cells were arranged as single or multiple layers separated by collagen fibers. A basement membrane was not observed.

Four types of cells were found within the synovial layer; mast cell (Figure 8), a cell with numerous vacuoles (Figure 6), a cell with well developed rough endoplasmic reticulum (Figure 8) and a cell with a minimal amount of cytoplasmic structures (Figure 5).

The mast cell nucleus was very lobulated with a wide area of condensation of nucleoplasm adjacent to the nuclear envelope. In most micrographs there was a clear zone approximately
Figure 5. Normal swine synovial cells surrounded by collagen fibers. Notice the nucleus (N), joint cavity (J), collagen fibers (C), collagen fiber bundles within the lumen (CF) and the absence of cytoplasmic organelles. Glutaraldehyde fixation. Uranyl acetate stain. X 15,000.
Figure 6. Normal swine synovial cell with irregular outline. Notice rough endoplasmic reticulum (EE), variable sized vacuoles (V), nucleus (N), electron dense body (O). Osmium tetroxide fixation. Lead acetate stain. X 21,000.
40 to 60 μm in size around the nucleus. The most prominent structure of the cytoplasm was an irregularly shaped electron dense granule 134 μm by 400 μm (Figure 8) in dimension which was negative for acid phosphatase activity (Figure 10). Variable shaped membrane-bound clear vesicles were observed interspersed between the electron dense granules. Occasionally, a large osmophilic granule was observed within the vesicle.

In the synovial cell containing numerous vesicles and vacuoles and a poorly developed endoplasmic reticulum (Figures 6 and 7), the outline of the nucleus was smooth with an occasional indentation of the envelope. The nucleoplasm was finely granular with irregularly shaped areas of condensation at the envelope. Nucleoli varied in number from 1 to 2 per cell. In the cytoplasm, numerous large vacuoles were observed and many had numerous pinocytic vesicles (Figure 7) which appeared to be pinching off the cytoplasmic membrane; small pinocytic vesicles 10 to 20 μm in diameter were numerous throughout the cytoplasm. Dense structures resembling a poorly developed rough endoplasmic reticulum were observed in the cytoplasm. Each dense irregular structure was marginated by particles resembling ribonucleoprotein (RNP) particles. At the marginal portion of the cytoplasm, electron dense structures with an absence of membrane structures were observed which resembled lysosomes or phagosomes (Figure 7). However, in the cytoplasm similar type structures were present which had membrane systems suggestive of mitochondria.
Figure 7. Normal swine synovial cell. The nucleus (N), numerous vacuoles (V), pinocytic vesicles (PV), poorly developed rough endoplasmic reticulum (ER) and collagen fibers (C) are evident. Glutaraldehyde fixation. Uranyl acetate stain. X 14, 160.
Figure 8. Normal synovial cell layer at tip of villus. Observe mast cell (MS), rough endoplasmic reticulum (ER), nucleus (N), membranous structures (M) and joint cavity (J). Glutaraldehyde fixation. Uranyl acetate stain. X 11,200.
Gomori's acid phosphatase technique demonstrated acid phosphatase granules (Figure 9) in some of the clear cytoplasmic vesicles adjacent to the margin of the cytoplasm. Not all vacuoles contained acid phosphatase particles. In addition, clumps of acid phosphatase granules were present in cytoplasm, many granules of which did not appear to be confined to a membrane-bound structure. At the margin of the nucleus, in the areas of condensation of the nucleoplasm, acid phosphatase particles were numerous (Figure 9).

The third type cell (Figure 8) was characterized by a few indentations in the outline of the finely granular nucleoplasm with a minimal amount of condensation at the nuclear envelope. In most instances, structures of the cytoplasm were confined to a well developed endoplasmic reticulum with numerous RNP particles attached to the endoplasmic reticulum membranes. Membrane-bound clear vacuoles were observed in the cytoplasm.

The fourth type of cell in the synovial cell layer had a relative absence of cytoplasmic organelles (Figure 5). In some instances, a poorly developed rough endoplasmic reticulum was observed with an occasional ballooning of the endoplasmic reticulum. The mitochondria were not numerous.

3 days postinoculation

At necropsy, 4 pigs had a total of 18 arthritic joints. *Str. equisimilis* was isolated from 12 of the 18 joints. Gross joint lesions are tabulated in Table 1.
Figure 9. Normal swine synovial cell with numerous cytoplasmic vacuoles. Acid phosphatase particles (AP) are within some of the vacuoles and the nucleus. Glutaraldehyde fixation. Uranyl acetate stain. X 17,250.
Figure 10. Normal swine synovial cells (A). Notice acid phosphatase particles (AP) within the cytoplasm. Lobulated nucleus (MS) of a cell resembling a mast cell. The electron dense particles of the mast cell shown in Figure 8 did not stain with acid phosphatase technique. Glutaraldehyde fixation. Uranyl acetate stain. X 27,860.
Slight joint enlargement had resulted primarily from areas of increased turbid synovia, periarticular hyperemia and edema were observed adjacent to synovial cavities. In 2 joints, fibrin clots were observed (Figure 11). Varying degrees of hyperemia and edema of the synovial membrane were observed.

Microscopic changes were confined to the synovial cell layer and tissue immediately adjacent to it. There was congestion of the blood vessels and a marked vasculitis in some joints. The exudation of neutrophils and a few lymphocytes extended into the synovial cell layer. There was an abundance of cellular debris (Figure 12), cytoplasmic blebbing, vacuolation of the cytoplasm and eccentric nuclei of most of the involved synovial cell layer. In 1 joint, a fibrin sheet was attached to the synovial cell layer.

Marked fine structural alteration of the synovial cell had occurred. Each cell was separated by a space occupied by collagen fibers, cellular debris and inflammatory cells composed of neutrophils and lymphocytes. All synovial cells had numerous cytoplasmic processes (Figure 13) which formed filopodia in all directions of the plane of section (Figure 13). Numerous oval membrane structures were observed within the joint lumen (Figure 13) and apparently had originated from a blebbing process of the cytoplasmic membrane.

The various types of synovial cells observed in the normal synovial membrane were not observed. All synovial cells
Figure 11. Humero-radial articulation of pig at 3 days postinoculation. Fibrin clot (arrow) is present within the joint cavity. Note the white synovial membrane.
Figure 12. Swine synovial membrane at 3 days postinoculation. Observe marked irregularity of synovial cell outline and the cellular debris accumulation and neutrophil infiltration. Hematoxylin and eosin stain. X 400.
Figure 13. Synovial cell at 3 days postinoculation. Note numerous filopodia (F), membranous structures (M), "blebbing" of cytoplasmic membrane (B) and rough endoplasmic reticulum (ER). Osmium tetroxide fixation. Uranyl acetate stain. X 13,000.
appeared similar and were characterized by an enlarged nucleus with fewer indentations. The nucleoplasm appeared to be clumped in areas other than at the envelope (Figure 13). In many instances, the nucleoli were enlarged.

The most constant feature of the cytoplasm was the well developed rough endoplasmic reticulum (Figures 13 and 14) which was more prominent and distended with an electron dense substance. The RNP particles remained attached to the endoplasmic reticulum membrane. An occasional mitochondria appeared darker than in the control micrographs, however, the membrane structures were intact and distributed normally. A prominent Golgi apparatus was observed in some cells (Figure 14). Oval shaped osmophilic granular bodies (140 μm in diameter) were present in the cytoplasm (Figure 14).

Degenerating synovial cells (Figure 15) had clumping of the nucleoplasm and large cytoplasmic vacuoles. They occupied a large portion of the cytoplasm. The membrane-bound vacuoles contained irregularly shaped membrane structures and a fine fibrillar-type material. These vacuoles corresponded to the large acid phosphatase particles demonstrated with Gomori's technique (Figure 16). The mitochondria appeared normal (Figure 16).

In 1 micrograph, chained structures .12 μm in diameter were observed adjacent to a neutrophil (Figure 14). Although definite identification could not be made, these objects morphologically resembled a chain of streptococci.
Figure 14. Synovial cell at 3 days postinoculation. Nucleus (N), rough endoplasmic reticulum (ER), mitochondria (M), Golgi apparatus (G), microbodies (arrow), neutrophil (NP) and chained structures suggestive of streptococci (S) are present. Osmium tetroxide fixation. Uranyl acetate stain. X 78,500.
Figure 15. Degenerating synovial cell at 3 days postinoculation. Observe dense homogenous nucleoplasm (N), mitochondria (M), electron dense body (O), ballooning of endoplasmic reticulum (B) and membrane structures (MS) within the endoplasmic reticulum. Osmium tetroxide fixation. Uranyl acetate stain. X 57,750.
Figure 16. Acid phosphatase particles within a synovial cell which contains numerous cytoplasmic vacuoles similar to those in Figure 15. A nucleus (N) with acid phosphatase positive vacuole (arrow) is evident. Glutaraldehyde fixation. Gomori's acid phosphatase test. Uranyl acetate stain. X 39,000.
Neutrophils had large acid phosphatase particles which appeared to have been lysed, as the individual granules were observed throughout the cell in some cases (Figure 17). In others, the acid phosphatase was still confined to a definite body (Figure 18). Acid phosphatase was abundant in the nucleus of many neutrophils. "Free" acid phosphatase particles were numerous within the joint lumen.

Bodies containing acid phosphatase were abundant in the degenerated synovial cells with numerous large vacuoles (Figure 16), whereas in the other synovial cells, 2 or 3 acid phosphatase granules per cell were present and corresponded in location and size to the electron dense bodies of the cytoplasm. Occasionally, an acid phosphatase particle located at the margin of the cell had lysed, dispersing the acid phosphatase granules over that portion of the cell (Figure 19).

15 days postinoculation

In this trial a total of 10 arthritic joints was found in the 4 inoculated pigs. *Str. equisimilis* was isolated from 9 of 10 involved joints. Marked periarticular fibrosis of the involved joints was noted. Varying degrees of synovial membrane hyperemia were observed and it corresponded to the degree of exudation into the joint. In cases where marked joint suppuration was present, a yellowish-white coloration of the synovial membrane was observed. The synovial membrane had a congested cyanotic appearance in most of the involved joints.
Figure 17. Inflammatory cells at 3 days postinoculation. Neutrophil with acid phosphatase activity within the lobulated nucleus (N) and confluence of cytoplasmic acid phosphatase vacuoles (AP). Acid phosphatase granules within joint space (AG) and diffuse acid phosphatase cytoplasmic activity in adjacent cell (arrow). Glutaraldehyde fixation. Gomori's acid phosphatase technique. Uranyl acetate stain. X 46,500.
Figure 18. Cytoplasmic granules of a neutrophil in synovial exudate at 3 days postinoculation. Observe the confluence of acid phosphatase positive vacuoles (arrow) and the extracellular acid phosphatase granules (AP). Glutaraldehyde fixation. Gomori’s acid phosphatase technique. Uranyl acetate stain. X 111,000.
Figure 19. Swine synovial cell at 3 days postinoculation, note dense acid phosphatase particle (arrow) and scattered acid phosphatase granules throughout cytoplasm and nucleus (N). Glutaraldehyde fixation. Gomori's acid phosphatase technique. Uranyl acetate stain. X 45,000.
Microscopically, variation in the degree of cellular response was observed. In most joints, the changes were characterized by a minimal amount of cellular exudate on the luminal surface of the membrane. The synovial cells were prominent with a frayed cytoplasm (Figures 20 and 21). In most sections neutrophils had infiltrated the synovial cell layer, subsynovial cell space and were present within adjacent blood vessels. Perivascular plasma cell and macrophage accumulations were prominent in the less extensively involved joints. Fibrosis of the subsynovial cell space was dependent upon the severity of the lesion. In 2 joints, periarticular abscess formation was present. In the adjacent synovial membrane, hypertrophy of the synovial cells and perivascular plasma cell cuffing were observed.

30 days postinoculation

In this trial these 4 inoculated pigs had a total of 14 arthritic joints. Str. equisimilis was isolated from 8 arthritic joints.

The degree of periarticular fibrosis regressed with the resolution of the joint lesion. In the markedly involved joints, the increased synovia (approximately 30 ml.) was thin and turbid, whereas in the less severely involved joints a slight increase (approximately 3 ml.) in turbid synovia was observed. Large masses of fibrino-purulent material were present in 4 joints.
Figure 20. Synovial villi 15 days postinoculation. There is diffuse plasma cell, lymphocyte and macrophage infiltration in the villus. Abundant cellular debris, synovial cells, fibrin and inflammatory cells form the synovial cell layer. Hematoxylin and eosin stain. X 160.

Figure 21. Higher magnification of synovial cell layer in Figure 20. Plasma cells, cellular debris and fibrin constitute the luminal surface. Hematoxylin and eosin stain. X 375.
The synovial membranes were hyperemic and hyperplastic. In most synovial membranes increased connective tissue was evident as whitish streaks in the hyperemic membrane (Figures 22 and 23).

The microscopic alterations consisted of villous hypertrophy and hyperplasia of the synovial membrane (Figures 24 and 25). Mild capillary proliferation and an increase in the connective tissue of the subsynovial cell space were evident. Many of the capillaries appeared to be thrombosed. The larger blood vessels of the enlarged villi had a lamellar-type adventitial fibrosis.

The changes of the synovial cell layer varied from a ragged appearance of the basophilic cytoplasm to cellular hypertrophy. In most involved joints, there was deposition of a PAS positive fibrinoid in the synovial villi (Figures 26 and 27). Usually the fibrinoid was adjacent to the overlying synovial cells which were pyknotic and, in many instances, absent. Areas of organized fibrin on the surface of the synovial cells stained a homogenous pink with H and E and were PAS positive (Figure 28). This suggested that the fibrinoid accumulations in both locations were derived from the organization of fibrin. Plasma cell and mild neutrophil infiltration were observed throughout the synovial membrane and were particularly prominent around larger blood vessels. Macrophage infiltration of the subsynovial cell space was observed in some sections.
Figure 22. Synovial membrane changes of femoro-patellar articulation with an absence of regularly arranged, well developed villi at 30 days post-inoculation.

Figure 23. Swine humero-radial articulation 30 days post-inoculation with focal hyperplasia of synovial villi (arrow).
Figure 24. Synovial membrane 30 days postinoculation with marked fibroplasia and minimal vascularization in the villi. Focal lymphocyte accumulations within villi. Hematoxylin and eosin stain. X 116.

Figure 25. Higher magnification of Figure 24. Observe the frayed appearance of the synovial cell cytoplasm (arrow). Hematoxylin and eosin stain. X 256.
Figure 26. Synovial villus at 30 days postinoculation. Observe dense connective tissue, numerous small capillaries and fibrinoid (arrow). Hematoxylin and eosin stain. X 160.

Figure 27. Higher magnification of Figure 26. Fibrinoid within the villus (arrow) is associated with thinning of the adjacent synovial cells. Hematoxylin and eosin stain. X 400.
Figure 28. Diffuse fibrosis of synovial membrane at 30 days postinoculation. Note the increase in capillaries and fibrinoid (arrow) at luminal surface. Hematoxylin and eosin stain. X 116.
The fine structure of the synovial cells appeared uniform, however, they were different from those observed at 3 days postinoculation. The cells appeared more round with a persistence of the cytoplasmic processes (Figure 29) observed earlier. Each cell was surrounded by a granular material containing membrane particles (Figures 29 and 30). The nucleus was large and regular in outline. The nucleoplasm was homogeneously granular and was not condensed at the envelope.

The cytoplasmic structures were characterized by an increase in a finely segmented rough endoplasmic reticulum which had clumps of 3 to 4 RNP particles attached together (Figure 31) and widely distributed. Mitochondria and pinoctytic vesicle formation could not definitely be identified (Figure 31).

2 months postinoculation

A total of 17 arthritic joints was found in the 3 pigs killed 3 months postinoculation and in 1 that died 2 months postinoculation. Str. equisimilis was isolated from 14 joints. In 2 pigs (22 and 24, Table 1) marked periarticular fibrosis was observed, whereas another pig (23, Table 1) that had recovered clinically, evidenced a minimal amount of periarticular fibroplasia. At 2 months postinoculation numerous periarticular ulcerations of both the right and left tibial tarsal articulations had occurred. (Figure 32).

When the grossly involved joints were opened, small
Figure 29. Fine structural synovial cell changes 30 days postinoculation. Note nucleus with minimal concentration of nucleoplasm (N), short portions of endoplasmic reticulum with clumped RNP particles (arrow), cellular debris (C) and collagen fibers (CF). Osmium tetroxide fixation. Uranyl acetate stain. X 25,500.
Figure 30. Synovial cell at 30 days postinoculation with prominent nucleus (N), mitochondria (M) and small segments of endoplasmic reticulum (arrow) with clumped RNP particles. Osmium tetroxide fixation. Uranyl acetate stain. X 34,500.
Figure 31. Synovial cell fine structural changes at 30 days postinoculation. Note nucleus (N), electron dense structures with membrane components resembling mitochondria (M) and short segments of endoplasmic reticulum with clumped RNP particles (arrow). Osmium tetroxide fixation. Uranyl acetate stain. X 22,200.
Figure 32. Firm periarticular enlargements of tarsal joints 3 months postinoculation. Observe ulcerated foci on lateral surface of right tarsal joint.
periarticular abscesses were observed adjacent to the ulcerated areas of the skin. The synovia was scant to nonexistent with the exudate appearing inspissated. However, in the less severely involved joint there was a slight increase in synovia.

The synovial membrane from all pigs failed to evidence a regular arrangement of hyperplastic villi. In some areas, the membrane appeared light in color and contracted as one would expect in areas of chronic fibrosis.

Variation in the microscopic alteration was noted in the 3-month group. The variation was dependent upon the severity of joint involvement. The most marked lesions consisted of extensive periarticular fibrosis with irregularly placed polyp-like structures composed of dense connective tissue (Figure 33). In some areas of such joints, there was an absence of synovial cells whereas in others there was a definite increase (Figure 34). The synovial cell changes ranged from pyknosis and cytoplasmic blebbing to hypertrophy of the cytoplasm and nuclei. Fibrin was attached to the membrane and was associated with a decrease in the number of the synovial cells in the vicinity (Figures 34 and 35). Neutrophil, plasma cell and lymphocyte infiltration were prominent in most areas.

In the less severely involved joint, periarticular fibrosis was not prominent. Inflammatory changes were confined primarily to the synovial membrane. A mild degree of villous hypertrophy was observed. The variation of the synovial cell layer was dependent upon the degree of fibrin formation within
Figure 33. Fibrosis of synovial membrane with fibrin and cellular debris on synovial surface. Observe capillary hyperemia (arrow). Hematoxylin and eosin stain. X 64.

Figure 34. Villous hyperplasia of the synovial membrane at 3 months postinoculation. Notice diffuse fibrosis of the synovial membrane and variable thickness of synovial cell layer. Lymphocytes and plasma cells are numerous. Hematoxylin and eosin stain. X 64.

Figure 35. Higher magnification of tissue in circle of Figure 33. Congestion of blood vessels (A) and inflammatory infiltrate composed of lymphocytes, plasma cells and a few neutrophils. Fibrin on luminal surface (arrow). Hematoxylin and eosin stain. X 160.
the layer. Cellular debris and cytoplasmic processes of the synovial cells were prominent in most sections. Congestion of the blood vessels (Figure 35) and an occasional homogenous eosinophilic staining connective tissue focus occurred within the subsynovial cell layer (Figures 36 and 37). These deposits were nodular in outline with a stellate type cell within the pink staining material which resembled and was in a similar location as the fibrinoid masses observed at 30 days post-inoculation.

The changes in the mildly involved joints were confined to the synovial membrane and consisted of a mild hyperemia and a neutrophil infiltration.

In general, the severely involved joints had a minimal number of synovial cells and a marked fibrosis of the synovial membrane. In less severe joint involvement, villous hypertrophy was prominent with an increase in thickness of the synovial cell layer accompanied by plasma cell and lymphocyte infiltrations. Neutrophils were prominent in all cases. Fibrinoid connective tissue foci contained stellate cells.

6 months postinoculation

Nine arthritic joints were observed in the 4 pigs of this group (Table 1). The *Str. equisimilis* organism was not isolated from any of the involved joints.

Slight fibrosis of the periarticular tissues was observed. The synovial membrane changes varied from slight fibrosis (R.
Figure 36. Fibrosis of the synovial membrane at 3 months postinoculation. Focal areas of fibrosis (arrow) suggestive of organization of the fibrinoid seen in Figure 26 at 30 days postinoculation. Hematoxylin and eosin stain. X 100.

Figure 37. Higher magnification of Figure 36. A definite synovial cell layer is not present. Focal areas of homogenous material suggestive of organized fibrinoid (arrow). Congestion of blood vessels and lymphocyte infiltration. Hematoxylin and eosin stain. X 319.
tarsal, pig 29, Table 1) to long villous formations of vascularized connective tissue (L. scapulo-humeral, pig 27, Table 1) (Figure 38).

In joints where marked periarticular fibrosis had occurred earlier with later organization, the synovial membrane appeared more white than the control pigs' joints. Areas of connective tissue contraction were evident. Regularly placed villi were not present. Synovial fluid was usually very scant in such joints. The most extensively involved joint (R. scapulo-humeral, pig 27) had mild periarticular fibrosis with hyperemia and villous proliferation of the synovial membrane (Figure 38). The villi, however, were not arranged in a papillary type arrangement. An abundance of synovia (approximately 10 ml.) was observed within the joint. Variation in the synovial membrane changes was dependent upon the degree of recovery of the joint. Microscopic changes were observed in the joints from 3 of the 4 inoculated animals. In some joints, marked periarticular fibrosis had been evident for the first 2 months postinoculation. However, by 6 months postinoculation no evidence of gross involvement was present.

The microscopic changes of the recovered joints were characterized by alterations confined primarily to the synovial cell layer and the connective tissue spaces immediately adjacent and by the relative absence of synovial cells. There were areas of pyknosis of the synovial cells which were embedded in collagen fibers. In some areas the synovial cells
Figure 38. Scapulo-humeral articulation of a pig 6 months postinoculation. Observe ulceration, fibrillation and necrosis of articular surface.
appeared as simple squamous type cells (Figure 39). In sections where the synovial cells were observed, the cytoplasm appeared shrunken and stellate in morphology. Fibrosis of the small villi as well as the spaces immediately adjacent to the luminal surface had occurred. Many of the small capillaries had a homogenous pink material within the lumen. Lamellar type fibrosis of the adventitial fibers was present around the larger blood vessels.

In joints that evidenced current clinical lameness, the changes were characterized by marked villous proliferation of the synovial membrane (Figures 40 and 41). Some villi were composed entirely of connective tissue with an absence of a synovial cell layer. Other enlarged villi (Figures 40 and 41) (L. scapulo-humeral, pig 27, Table 1) had a continuous and a hyperplastic layer of synovial cells. They were enlarged with an abundant dark blue staining cytoplasm that had numerous cytoplasmic processes. Lesions of the villi consisted of an occasional perivascular accumulation of plasma cells and lymphocytes, an abundance of neutrophils (Figure 42A) within the vessels and mild adventitial fibrosis. There was a slight increased vascularization of the enlarged villi in each case.

Electron microscopic examination revealed that the synovial cells were few in number and separated by an abundance of mature collagen. Light microscopy evaluation demonstrated the cells to be very sparse and pyknotic. This was particularly evident in joints of the recovered animals.
Figure 39. Swine synovial membrane 6 months postinoculation. This particular joint had been severely involved for about 2 months. Minimal involvement was evident at 6 months. Fibrosis is confined to luminal portion of synovial membrane and the synovial cells are flattened and pyknotic. Hematoxylin and eosin stain. X 256.
Figure 40. Hyperplastic synovial villi with vascular connective tissue and minimal inflammatory cells. Prominent synovial cell layer. Hematoxylin and eosin stain. X 64.

Figure 41. Higher magnification of Figure 40. Note inflammatory cells confined to blood vessels of villi and prominent synovial cell layer. Hematoxylin and eosin stain. X 400.
Figure 42A. Synovial cell at 6 months postinoculation with marked fraying of the cytoplasm. This synovial cell is similar to those in Figure 39. Nucleus (N). Osmium tetroxide fixation. Uranyl acetate stain. X 16,650.
The fine structure alterations consisted of a small irregularly shaped nucleus with condensation of nucleoplasm at the envelope. The cytoplasm had contracted to thin filaments (Figure 42A) in some areas. The only recognizable organelle was an occasional mitochondria. Cells similar to this were negative for acid phosphatase.

Costochondral Junction Changes

Normal costochondral junction

The costochondral junction was even in outline in 10 control animals ranging in age from 3 weeks to 6 months. The central portion of the vesicular zone was 5 to 8 cells in thickness, whereas toward the periphery, the zone gradually thickened to approximately 20 cells. Large vascular canals periodically penetrated the cartilage (Figure 42B). The cellular components around blood vessels within the canals consisted of endothelial cells, cellular debris, osteoclasts and hematogenous pigments. The cellular components of the metaphyseal region consisted primarily of osteoblasts adjacent to the calcified cartilage-matrix trabeculae.

3 days postinoculation

Only 1 of 4 pigs had gross lesions of the costochondral junction even though all 4 developed arthritis. Lesions of the costochondral junction were confined primarily to the vesicular and metaphyseal zones. The costochondral junction
Figure 42B. Normal costochondral junction of pig 6 weeks of age. Vesicular zone (A), cartilage matrix trabeculae (B), metaphysis (C) and osteoblasts (D) are noted. Hematoxylin and eosin stain. X 162.
of 1 animal had a focal osteomyelitis (Figures 43, 44 and 45) which resulted in a decrease of the matrix trabeculae of the area. The vesicular zone adjacent to the inflammatory tissue was increased (Figure 44) to approximately 60 cells in width and the cartilage matrix of this zone stained eosinophilic. At the periphery of the metaphyseal lesion, active osteoblastic activity was evidenced by numerous mitotic figures and an abundance of osteoid in the region. The vascular canals within the cartilage had perivascular accumulations of neutrophils and cellular debris.

Gross alterations of the costochondral junction of 1 pig consisted of a focal irregularity in the costochondral junction (Figure 43). The adjacent metaphyseal region was lighter in color than the surrounding tissue. Similar alteration was not observed in the other 3 animals.

Microscopic lesions of the costochondral junction of the other 3 arthritic pigs were minimal and consisted of shrinkage of the osteoblasts. Decreased activity of the osteoblasts in the metaphysis resulted in a reduction of osteoid formation.

15 days postinoculation

All 4 pigs developed arthritis (Table 1). No gross or microscopic alteration of the costochondral junction was noted. The number of cells in the vesicular zone varied from 5 to 20 cells in thickness.
Figure 43. Focal undulation of the costochondral junction (arrow) at 3 days postinoculation. See Figures 44 and 45 for photomicrographs of this zone.
Figure 44. Costochondral junction lesion 3 days postinoculation. Focal arrest of vascular invasion has resulted in a broadening of vesicular zone (A). Observe the less intense staining in the involved area. Hematoxylin and eosin stain. X 64.

Figure 45. Higher magnification of circled area in Figure 44. Focal osteomyelitis at costochondral junction (arrow). Observe absence of active osteoblasts around cartilaginous matrix spicules. Hematoxylin and eosin. X 336.
30 days postinoculation

All 4 pigs developed arthritis as tabulated in Table 1. Gross costochondral lesions were observed in 1 and microscopic alterations in 2 animals. The gross change was characterized by a transverse secondary line of cartilage (Figure 46) approximately 1 cm. from the active area of vascular invasion in ribs 6 and 7.

Microscopically, the changes consisted of small areas of arrest of vascular invasion which had resulted in a broadening of the vesicular zone to approximately 50 cells. In another pig there was an increase in osteoclasts of the metaphyseal region.

3 months postinoculation

All 4 pigs developed extensive polyarthritis. Gross costochondral lesions were observed in 1 pig and microscopic alterations in 2 pigs. The change consisted of extreme irregularity in outline of the costochondral junction (Figure 47) and numerous light colored areas adjacent to the periosteum and within the vesicular zone of the cartilage.

Microscopically, the changes were characterized by cessation of cartilage production and by large poorly stained areas of matrix. Replacement fibrosis of some of the poorly stained areas of the cartilage had occurred.

In the metaphyseal region, there was a general thinning of the matrix trabeculae with an absence of osteoblasts in the
Figure 46. Costochondral junction 30 days postinoculation with a transverse cartilaginous line in the metaphysis.

Figure 47. Costochondral junction 3 months postinoculation with osteomyelitis of the metaphysis and penetrating vascular canals in the cartilage.
entire area. Basophilic staining granules of the osteocytes were observed suggesting early stages of necrosis. Many necrotic osteocytes were adjacent to or within osteoclasts. Neutrophils were not prominent in this area.

Another microscopic change was a focal arrest of vascular invasion resulting in an increase, approximately 60 cells in width, in the vesicular zone. The inflammatory tissue of the metaphysis was partially isolated by a transverse zone of new bone formation.

6 months postinoculation

Arthritis had been noted in all 4 animals. However, by 6 months postinoculation progressive joint lesions were present in only 2 pigs and were of a secondary osteoarthritis type. Gross or microscopic alterations were not observed in the costochondral junctions.

Naturally Occurring Streptococcal Costochondral Lesions

The costochondral lesions are grouped according to the age of the animal. The data have been tabulated to help classify the lesions even though it was not known that all animals were infected at birth. Microscopic changes are summarized in Table 2. In general, the most severe rib lesion was found in animals that had a severe osteomyelitis in other areas of the skeletal system.
**Pigs younger than 1 week**

The vesicular zone (Figure 48) was increased in all 3 animals and varied from 30 to 60 cells in width. The cartilage columns were unevenly arranged which resulted in a variation of the number of cells in this zone. In some areas, a new vesicular zone had developed (Figure 49) and was revascularized apparently from the vascular canals that normally penetrate the cartilage. This change, however, was more prominent in pigs 2 weeks of age.

Lesions of the metaphyseal region consisted of degenerative changes of the osteoblasts (Figures 50 and 51). In most instances, they were very spindle shaped, whereas in other areas the osteoblasts were morphologically normal and were not actively secreting osteoid as evidenced by an absence of pink staining material on the calcified trabeculae. An increase in neutrophils was not prominent in this region.

**Pigs 1 to 2 weeks of age**

In 7 pigs, the vesicular zone varied from 10 to 70 cells in thickness. The vascular canals that penetrated the cartilage contained abundant neutrophils and cellular debris.

The metaphyseal region consistently evidenced changes of the osteoblasts which varied from pyknosis to complete absence of the osteoblasts. In areas devoid of osteoblasts, there was a reticular type cell present in the adjacent marrow spaces. Many involved areas had disintegration of the cartilage matrix.
Figure 48. Costochondral junction of naturally occurring case of *Str. equisimilis* infection with broadening of the vesicular zone (arrow). Hematoxylin and eosin stain. X 26.

Figure 49. Costal cartilage of a naturally occurring case of *Str. equisimilis* infection. Observe development of a "new" vesicular zone (A) with revascularization from the vascular canals that penetrate the cartilage (B). Hematoxylin and eosin stain. X 16.
Figure 50. Costochondral junction of naturally occurring *Str. equisimilis* infection. Note the absence of osteoblast in the metaphysis as evidenced by "bare" cartilaginous matrix trabeculae (arrow). Hematoxylin and eosin stain. X 100.
Figure 51. Higher magnification of Figure 50. Observe shrinkage of osteoblasts in metaphysis (arrow). Hematoxylin and eosin stain. X 336.
columns as evidenced by its granular appearance and eosinophlic staining. Mitotic figures were frequently observed in the metaphyseal region which was in contrast to the same area from control pigs where only an occasional mitotic figure was seen.

In 3 animals, secondary transverse cartilaginous line formation was evident grossly. This secondary zone was composed entirely of mature, degenerate cartilage cells (Figure 52) similar to those of the vesicular zone. In the metaphyseal side of this secondary zone, there was marked to mild fibrosis and osteolysis. No evidence of an orderly vascular invasion from the metaphyseal side of the "old" vesicular zone was noted. Isolated foci of neutrophils and osteoclasts were evident on both sides of the transverse line of cartilage. A lack of staining affinity of the zone of provisional calcification was apparent in both the primary and secondary vesicular zones.

**Pigs 2 to 4 weeks of age**

The 5 pigs of the 3- to 4-week age group evidenced changes in both the vesicular zone and metaphyseal region. The vesicular zone was broadened to 15 to 50 cartilage cells. There was a tendency for several columns of cartilage cells to clump. The matrix of the cartilage cell was associated with a poorly stained zone of provisional calcification.

In the metaphyseal region, the osteoblasts were markedly
Figure 52. Transverse cartilaginous line formation (A) of costochondral junction of a naturally occurring case of *Str. equisimilis* infection. "Old" metaphysis (B) and "new" metaphysis (C) resulting from revascularization. Hematoxylin and eosin stain. X 16.
degenerated or completely absent (Figures 50 and 51). A reticular type cell remained in the mildly fibrotic marrow space. One pig had isolated islands of cartilage cells within the metaphyseal region, the arrangement of which suggested a previous transverse line formation. In another pig, the area of initial damage was evident by a transverse area of fibrosis. Numerous osteoclasts were present in this zone. Neutrophils were generally increased in the marrow spaces. Thus changes in the metaphysis were similar to the younger group with the exceptions of loss of osteoblasts and of organization of the cartilaginous transverse line.

**Pigs 4 weeks of age and older**

In the 3 pigs of this group the vesicular zone evidenced marked variation in thickness. In 1 animal, the vesicular zone was approximately 10 cells in thickness whereas in the remaining animals the zone was up to 30 cells in thickness. A secondary transverse line of cartilage was not observed.

The most consistent lesion in these naturally infected pigs was the absence or minimal activity of the osteoblasts in the metaphyseal region. In many instances, the calcified trabeculae were not rimmed by osteoblasts. There was a reduction in the number of trabeculae in the metaphyseal region. Osteoclasts with a very foamy appearing cytoplasm were numerous. These observations are summarized in Table 2.
Table 2. The main costochondral changes observed in naturally occurring streptococcal infected pigs

<table>
<thead>
<tr>
<th>Age of Groups</th>
<th>Number of Pigs Affected</th>
<th>Transverse Line</th>
<th>Pyknosis Osteoblasts</th>
<th>Necrosis Osteoblasts</th>
<th>Hemorrhage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 week</td>
<td>30-60 cells</td>
<td>0/3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3/3</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>2 weeks</td>
<td>10-70 cells</td>
<td>3/7</td>
<td>7/7</td>
<td>0/7</td>
<td>0/7</td>
</tr>
<tr>
<td>3 to 4 weeks</td>
<td>15-50 cells</td>
<td>1/5</td>
<td>5/5</td>
<td>3/5</td>
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<td>Over 4 weeks</td>
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<td>0/3</td>
<td>3/3</td>
<td>2/3</td>
<td>0/3</td>
</tr>
</tbody>
</table>

<sup>a</sup> Numerator - number of animals with lesion  
Denominator - number of animals observed

Bone and Articular Surface Lesions

3 days postinoculation

Eighteen arthritic joints were observed from 4 pigs. The lesions of the articular surface and bone of all 4 infected animals were confined to focal microscopic areas of osteomyelitis of the metaphysis and epiphysis. Gross articular surface lesions were not observed.

Involvement of the epiphysis and metaphysis appeared to produce changes in the epiphyseal plate somewhat differently. Focal osteomyelitis on the epiphyseal side of the epiphyseal plate resulted in a lack of staining of the entire segment of the epiphyseal plate (Figures 53 and 54), in degenerative
Figure 53. Proximal tibial epiphyseal plate 3 days postinoculation with a focus of less intense staining. Focal osteomyelitis on epiphyseal side (arrow) and vacuolization of matrix of central portion (A). Hematoxylin and eosin stain. X 64.

Figure 54. Higher magnification of the poorly stained zone of Figure 53. Observe the vacuolization of the matrix of central portion (arrow). Hematoxylin and eosin stain. X 160.
changes of the chondroblasts as evidenced by cytoplasmic vacu­
olization, and in a decrease in maturation of the cells in the
vesicular zone. In some areas, the degenerative changes were
manifested by a decrease in cartilage production resulting in
a focal thinning of the epiphyseal plate. In 2 pigs, involve­
ment of the epiphyseal side resulted in the development of
vacuoles of the cartilage matrix (Figure 54) in the zone of
mature cartilage cells.

Focal accumulation of neutrophils in the metaphysis
resulted in a lack of staining of the adjacent zone of provi­
sional calcification. The small foci of neutrophils were
associated with an increase in connective tissue, decrease of
cartilage trabeculae in the primary spongiosa, increase of
osteoclasts at the periphery of the lesion and a broadening of
the vesicular zone. Osteoblastic activity was minimal in such
focal areas.

In all pigs, small foci of neutrophils were observed
throughout the marrow spaces. These foci were encapsulated
with connective tissue and osteoclasts. A reduction in bony
spicules has occurred.

15 days postinoculation

Ten arthritic joints were observed in these 4 pigs.
Gross lesions were observed in the bone and articular surfaces
in 3 of 4 animals. Gross articular changes were confined to
the condyles of the tibial-tarsal bone and consisted of small
ulcerated areas (approximately 1 cm. in diameter) and diffuse involvement of the lateral condyle (Figure 55). In 2 animals hemorrhage of fibrotic foci approximately 3 cm. in diameter had occurred in the lumbar spinal region (Figure 56), involving an intervertebral disc and adjacent epiphyseal plates.

Microscopically, in each instance where gross alteration was observed, the articular surface lesion originated from the vascular canals at the peripheral portions of the cartilage (Figure 57). Changes within the cartilage consisted of focal accumulations of neutrophils and cellular debris surrounded by a fibroblast type cell (Figure 58). Cartilage immediately adjacent to the vascular lesions appeared hyperplastic as evidenced by numerous cartilage cells per lacunae. A less severe but similar type vascular lesion was observed in the arthritic joints from other pigs of this group. These consisted of metaplasia of the cartilage cells surrounding the vascular canals to fibroblasts and decreased metachromasia (Figure 59). Such lesions were manifested grossly as a focal "punched out" red area within the cartilage. It was minimal in the tangential zone where the exudate was confined to the joint. In each instance, regardless of the degree of suppuration within the joint, gross articular surface lesions were associated with perivascular alteration of the cartilage.

The lumbar vertebral lesions of 2 pigs consisted of suppuration, fibrosis and chondrolysis which involved the intervertebral disc, adjacent cartilage and bone marrow. In 1
Figure 55. Tibio-tibial tarsal articulation 15 days post-inoculation. Observe periarticular fibrosis, focal areas of hyperemia of synovial membrane and eroded areas on lateral condyle of tibial tarsal bone.

Figure 56. Lumbar vertebrae 15 days postinoculation. Note the lesion (arrow) of the cartilage and adjacent nucleus pulposus.
Figure 57. Vascular canals of the articular surface in a control pig. Note the numerous vessels in each canal. Hematoxylin and eosin stain. X 336.
Figure 58. Articular surface at 15 days postinoculation with perivascular metaplasia of cartilage to fibroblasts. Note reduced metachromasia (arrow). Toluidine blue stain. X 100.
case, the focal suppurative process appeared to have originated from intrachondral vascular canals adjacent to the intervertebral disc.

Lesions of the metaphyseal region consisted of a focal fibrosis surrounded by ossified cartilage (Figures 59 and 60) that stained pink with H and E but the lacunae were much larger than that of the osteocyte and contained several cells per lacunae. At the periphery of such lesions, abundant osteoclasts were present. The adjacent vesicular zone was greatly broadened (up to 40 cells) which suggested a focal arrest in vascular invasion. Revascularization appeared to have occurred at the peripheral portion of the broadened vesicular zone.

30 days postinoculation

The 4 pigs had 14 arthritic joints for observation. Gross and microscopic alterations were observed in the articular surface of all pigs killed. Articular surface lesions consisted of focal ulcerative areas in all 4 animals, yellow discoloration of the articular surface in 1 of the 4 pigs and reddish foci in 1 of 4 pigs. One lateral humeral condyle had a large ulcerative area with an abundance of connective tissue over it.

The small ulcerated foci were manifest microscopically by a perivascular lesion characterized by clumping and hyperplasia of chondrocytes (Figures 61, 62 and 63), metaplasia of
Figure 59. Epiphyseal plate of distal femur 15 days post-inoculation with focal fibrotic lesion (A) in metaphysis resulting in broadening of the vesicular zone (B). Revascularization has occurred at the periphery of the widened vesicular zone (arrow). Toluidine blue stain. X 16.

Figure 60. Higher magnification of Figure 59. Broadened vesicular zone (A), "old" metaphysis (B) and area of revascularization (C). Toluidine blue stain. X 64.
Figure 61. Tibio-tibial tarsal articulation 15 days post-inoculation. The gross lesion is shown in Figure 55. Notice the focal light stained perivascular areas within the articular surface (A) and an area of erosion (B) on the surface. Hematoxylin and eosin stain. X 16.

Figure 62. Higher magnification of Figure 61. Vascular penetration of articular surface (arrow) and hyperplasia of cartilage cells in tissue surrounding vascular canals are evident. Hematoxylin and eosin stain. X 60.

Figure 63. Higher magnification of Figure 61. Note marked hyperplasia of cartilage cells surrounding vascular canals (arrow). Hematoxylin and eosin stain. X 60.
cartilage cells to fibroblasts and a loss of metachromasia of the perivascular areas. Articular surfaces having a similar type change were manifest grossly by pitting and flattening.

The articular surfaces which appeared yellow and those having the focal reddened areas had microscopic changes confined primarily to the tangential (surface) and germinal (middle) zones of the cartilage. These microscopic changes consisted of multiplication of cartilage cells within each lacunae (Figures 65 and 67). Normally, in the tangential zone, the single cells per lacunae become flattened and parallel to the articular surface (Figures 64 and 66). In some areas of arthritic joints, there were 4 or 5 cells per lacunae with degenerative changes such as vacuolization of the cytoplasm and accumulation of cellular debris. Involvement of the zone of provisional calcification was observed in 1 instance as evidenced by marked enlargement of the cartilage lacunae with 15 to 20 cells. All articular surfaces of involved joints had varying degrees of cartilage cell hyperplasia with swelling of the individual cell and were associated with a decrease in metachromasia of the cartilage matrix.

Small microscopic lesions of the proximal tibia and distal femur epiphysis and metaphysis were observed in 2 pigs. There was a focal arrest of cartilage production, lack of a vesicular zone, lattice-work-like trabeculae in the primary spongiosa and pyknosis of the osteoblast in this area. Thus at 30 days postinoculation the articular surface changes were
Figure 64. Articular surface of the proximal radius in a 30 day control pig. Notice that the cells in the tangential zone (A) are arranged 1 per lacunae and flattened parallel to the articular surface. Hematoxylin and eosin stain. X 100.

Figure 65. Articular surface of the proximal radius 30 days postinoculation. Observe hypertrophy and hyperplasia of the cartilage cells of the germinal (A) and tangential zones (B). Hematoxylin and eosin stain. X 100.
Figure 66. Articular surface of the proximal radius of 30 day control pig. Higher magnification of Figure 64. Hematoxylin and eosin stain. X 336.

Figure 67. Articular surface of the proximal radius 30 days postinoculation with hypertrophy and hyperplasia of cartilage cells of tangential (A), germinal (B) and zone of hypertrophy (C). Hematoxylin and eosin stain. X 336.
produced by both a vascular intracartilaginous change and hyperplasia of the cartilage resulting in an altered matrix of the cartilage.

3 months postinoculation

Seventeen arthritic joints were observed in 3 of 4 pigs (Table 1). The gross lesion varied from a fibrous ankylosis to fibrillation and erosion of the articular surface (Figure 68).

Microscopically, the fibrous ankylosis of the joint was associated with broad perivascular areas of connective tissue within the cartilage (Figure 69). In many areas, the pannus appeared to have originated from these perivascular areas. The tissue forming the pannus had focal areas of suppuration. Periosteal new bone formation was prominent around such joints.

Other areas undergoing extensive fibrillation had nodular areas of cartilage hyperplasia in perivascular areas with a loss of metachromasia of the ground substance (Figure 70). Large fissures were developing between the enlarged lacunae. In areas adjacent to vascular canals, cartilage cells were undergoing metaplasia to fibroblasts.

The focal zones of erosion (Figure 71) had occurred in areas of cartilage cell hyperplasia. Lateral erosion of the germinal zone was observed from the eroded surface lesion (Figure 72).

In the articular surface of all involved joints with no
Figure 68. Secondary degenerative osteoarthritis of coxo-femoral articulation 3 months postinoculation. Notice the dull yellowish coloration of the cartilage and fissuring and erosion of the peripheral portion of the articular surface.
Figure 69. Pannus formation (A) over the articular surface (B) resulting in fibrous ankylosis at 3 months postinoculation. In many areas, connective tissue appears to have originated from the perivascular areas (arrow). Hematoxylin and eosin stain. X 64.

Figure 70. Secondary degenerative osteoarthritis at 3 months postinoculation. Observe clone formation of cartilage cells and developing fissures (arrow) between them. Hematoxylin and eosin stain. X 160.
Figure 71. Erosion (arrow) of the hypertrophic articular surface at 3 months postinoculation. Hematoxylin and eosin stain. X 64.

Figure 72. Lateral tunnelling from focal eroded areas similar to those in Figure 71. Hematoxylin and eosin stain. X 64.
apparent gross alteration, cartilage cell hyperplasia of the tangential and germinal zones was found and was more prominent than that of 30 days postinoculation.

Maturation of the cartilage cells was not observed adjacent to areas of epiphyseal fibrosis which was associated with an increased matrix between the cartilage cell columns and with a reduction of metachromasia.

Periosteal new bone formation was prominent in most sections. In 1 case, a periarticular abscess of the periosteum and adjacent epiphysis was observed. Colonies of streptococci were abundant in this lesion.

6 months postinoculation

Nine arthritic joints were observed in this group of 4 pigs (Table 1). Gross and microscopic articular surface lesions were found in all pigs. Marked ulceration and necrosis of the articular surface with yellowish discoloration of the remaining cartilage were observed in the most extensively involved joints (Figure 38). In the coxofemoral joint, the round ligament was completely eroded. Most involved joint cartilages were fissured. The lateral condyle of the tibial tarsal bone (pig 30, Table 1) was flattened and extensively fissured. Minimal articular surface lesions in 2 pigs consisted of roughening with an occasional fissure formation.

Microscopically, the surfaces with extensive fissures had marked hyperplasia of the cartilage cells into enlarged
lacunae (clone formation). Broad bands of matrix separated the cartilage lacunae (Figures 73 and 74). The cartilage cells within the lacunae were undergoing degenerative changes consisting of vacuolization of the cytoplasm and pyknosis of the nuclei. Loss of metachromasia was observed. The lesions of the head of the femur from 1 pig (pig 28, Table 1) were characterized by increased accumulation of cartilage cells at the junction of the tangential and germinal zones (Figures 73 and 74). The cells had large nuclei and abundant cytoplasm. The tangential zone was composed entirely of acellular matrix. Abnormal perpendicular orientation of the long axis of the cartilage cells to the articular surface was a striking feature.

Naturally Occurring Cases of Streptococcal Arthritis

The 30 naturally occurring cases of streptococcal arthritis had been submitted to IVDL during the Fall of 1963, and Winter, Spring and Summer of 1964. Twenty five of the pigs were younger than 3 weeks of age while 5 were older than 3 weeks. In all 30 cases of suppurative arthritis, marked periarticular fibrosis was evident with small abscess formation in the periarticular tissue in 5 of the 30 animals. Marked lameness was noted in the living animals presented for necropsy.

The synovia varied from a marked increase of a very turbid fluid to a thickened fibrino-purulent exudate. In 2 of the older animals scant amounts of synovia were present.
Figure 73. Osteoarthritis in scapulo-humeral joint at 6 months postinoculation. The animal was clinically lame. Observe the perpendicular orientation of the cartilage lacunae (A), hyperplasia of the cartilage cells within lacunae of the tangential zone and erosion and fibrillation of tangential zone (arrow). Hematoxylin and eosin stain. X 16.

Figure 74. Higher magnification of Figure 73 demonstrating the erosion and fibrillation of the cartilage of the tangential zone. Hematoxylin and eosin stain. X 400.
Mild degree of hyperemia was evident in joints where suppuration was minimal. Gross hyperplasia of the synovial villi was minimal to non-existent.

Microscopically, varying degrees of synovial villi enlargement was observed. In the more extensively involved joints, the membrane was composed entirely of an immature connective tissue. Purulent exudate suppuration and fibrin formation were found on the luminal surface (Figure 75). The synovial cell layer was usually absent in the severely affected joints.

Changes in the less extensively involved joint were synovial cell hypertrophy or, in some cases, atrophy. There was an abundance of cellular debris and neutrophils on the luminal surface as well as marked neutrophil, plasma cell and macrophage infiltration. Vascular thrombosis and necrosis were common. An increased amount of a mildly vascularized connective tissue was present in the villi. Mineralization of the connective tissue villi occurred in 3 cases.

In the 3 more chronic cases fibroplasia of connective tissue of the villi (Figures 76 and 77) was evident with perivascular infiltration of plasma cells, macrophages and neutrophils. Hyalinized fibrin material was found in subsynovial layer of the villi.

Thus the experimentally produced synovial membrane lesions of Str. equisimilis were similar to those naturally occurring cases from which Str. equisimilis was isolated.
Figure 75. Acute inflammatory changes in the synovial membrane of a naturally occurring case of Str. equisimilis infection. Notice the absence of synovial cell layer in areas of fibrin formation (A), plasma cell and neutrophil infiltration of the villus and fraying of synovial cells (arrow). Hematoxylin and eosin stain. X 160.
Figure 76. Chronic changes in the synovial membrane of a naturally occurring case of *Str. equisimilis* infection. Observe connective tissue formation within the villi (A), prominent synovial cell layer in some areas (B) and absent in others (C), and lymphocyte and plasma cell infiltration of the perivascular spaces. Hematoxylin and eosin stain. X 64.

Figure 77. Higher magnification of Figure 76. Note the frayed synovial cell layer (arrow) and perivascular plasma cell and lymphocyte infiltration. Hematoxylin and eosin stain. X 256.
Gross articular alteration was observed in 10 of the 30 field cases examined. Such changes were characterized by ulceration and erosion of the weight bearing portion of the joint surface.

Microscopically, the joints with no gross articular surface alteration had varying degrees of cartilage cell hyperplasia (Figure 78) primarily of the tangential and germinal cell zone, hypertrophy of the cartilage cells of the tangential zone in the more extensively involved joints and hyalinization of the cartilage matrix with desquamation of the adjacent cartilage cells. Perivascular areas of transition of cartilage cells to fibroblasts were evident (Figure 79). Widening and infiltration with inflammatory exudate of the vascular canals of the cartilage were prominent. One case had a small abscess formation within the articular surface (Figures 80 and 81). The abscess apparently originated within a vascular canal and was characterized by a focal accumulation of neutrophils and macrophages and encapsulated connective tissue. Hyalinization of the surrounding cartilage ground substance was observed.

Beneath the altered articular surfaces of some extensively involved joints there was an osteomyelitis of the adjacent epiphysis. There was lysis of all cellular elements including cartilage and bone. The cartilage ground substance had a loss of staining affinity as well as a loss of cellular detail within the lacunae.
Figure 78. Hyperplasia of the cartilage cells of the tangential and germinal zones and focal eosinophilic staining areas of tangential matrix (arrow) in naturally occurring case of *Str. equisimilis* arthritis. Area of erosion (A). Hematoxylin and eosin stain. X 160.

Figure 79. Articular surface of naturally occurring case of *Str. equisimilis* infection. Metaplasia of the perivascular cartilage cells to fibroblasts (arrow). Hematoxylin and eosin stain. X 256.
Figure 80. Vascular lesion in the articular surface of a naturally occurring case of *Str. equisimilis* infection. Notice the focal inflammatory process (arrow) and hyperplasia of the tangential and germinal zones. Hematoxylin and eosin stain. X 64.

Figure 81. Higher magnification of Figure 80. Focal inflammatory process originating in the vascular canals of the cartilage. Hematoxylin and eosin stain. X 160.
Gross lesions of the metaphysis, epiphysis and epiphyseal plate consisted of focal light colored areas and focal broadening of the epiphyseal plate. Marked periosteal new bone formation was prominent in each case. In areas of extensive osteomyelitis, an increased diameter of the diaphysis or epiphysis was observed. Microscopically, when focal areas of suppuration were observed on the epiphyseal side of the epiphyseal plate, a decrease in production and maturation of the cartilage was evident. The cartilage trabeculae in the metaphysis were lattice-work-like. Complete necrosis of the epiphyseal plate was observed in areas of marked suppuration of the epiphysis and metaphysis. Varying degrees of vesicular zone broadening were evident. In 1 case there was an extensive focal osteomyelitis of the metaphysis which resulted in a marked broadening of the vesicular zone (Figures 82 and 83). A secondary vesicular zone had developed and vascular invasion from the marginal areas had occurred resulting in a transverse line of cartilage in the metaphysis. Such a lesion was not evident across the entire epiphyseal plate.

Blood Chemistry

The sampling period for serum calcium, phosphorus and alkaline phosphatase determinations are given in Tables 3, 4 and 5 and are summarized in Figure 84. Vitamin A determinations are noted in Table 7.

At 3 days postinoculation, the serum calcium of the
Figure 82. Transverse cartilaginous line formation in the proximal tibial epiphyseal plate in a naturally occurring case of Str. equisimilis infection. Observe focal fibrotic lesion in "old" metaphysis (A), broadening of vesicular zone (B) and revascularization forming a "new" metaphysis (C). Hematoxylin and eosin stain. X 16.

Figure 83. Higher magnification of Figure 82. Observe fibrotic lesion indicating area of initial damage (A), "old" vesicular zone (B) and revascularization (C). Hematoxylin and eosin stain. X 64.
inoculated pigs was lower than the controls. This trend was observed throughout the experiment. Animals severely infected evidenced the most marked reduction in serum calcium (6.8 ml, percent maximum depression). Streptococcal infection produced a statistically significant reduction in serum calcium and the factor of time postinoculation was significant when analyzed using randomized block design with analysis of variance. Infection produced a significant calcium reduction at the .01 level of confidence (F = 4.65 infection) whereas the time postinoculation was significant at the .05 level of confidence (F = 3.00 time postinoculation) (F.05 = 2.80, .01 = 4.20).

Analysis of variance data are given in Table 6.

Serum phosphorus levels remained similar to the control animals at 3 days postinoculation. At the 15- and 30-day samplings, inorganic phosphorus levels were increased. One severely involved pig had a phosphorus level of 13.8 mg. percent. The 3-month and 6-month determinations were similar to the control pigs. Streptococcal infection did not produce a statistically significant elevation in the serum phosphorus level.

Streptococcal infection produced a marked increase in alkaline phosphatase by 15 days postinoculation (maximum 25 Bodansky units). By the 3-month sampling, all serum levels had returned to the level of the control pigs. Both time postinoculation and infection were not statistically significant.
Even though the inorganic phosphorus and alkaline phosphatase values were not statistically significant, the individual degree of infection could not be quantitated. Considerable individual variation in degree of infection occurred. In most instances the depression of serum calcium and elevation of phosphorous and alkaline phosphatase were associated with extensive lesion formation.

Vitamin A values were not determined on all animals as this analysis factor was added after the experiment was in progress. The findings were variable. Infection in some instances produced a reduction in the serum vitamin A levels. The control mean was 33.3 International units whereas 20 International units were observed as a maximum depression in inoculated pigs. Vitamin determinations are given in Table 7.
Table 3. Serum calcium determinations (mg. percent) from swine infected with *Str. equisimilis*

<table>
<thead>
<tr>
<th>Sample Number</th>
<th>Days Postinoculation</th>
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<th>30 day</th>
<th>3 month</th>
<th>6 month</th>
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a - mg. percent
- each figure represents mean of paired sample determinations

Table 4. Serum phosphorus (mg. percent) determinations from swine infected with *Str. equisimilis*

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<th>15 day</th>
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a - mg. percent
- each figure represents mean of paired sample determinations
Table 5. Serum alkaline phosphatase (Bodansky units) determinations from swine infected with *Str. equisimilis*

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a - Bodansky units
- each figure represents mean of paired sample determinations

Table 6. Analysis of variance of blood chemistry values from *Str. equisimilis* infected swine using randomized block design

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F value .05 - 2.80  
.01 - 4.26  
* - significant at the .05 level  
** - significant at the .01 level

Table 7. Swine serum vitamin A determinations following Str. equisimilis infection

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a - specimen vial broken in freezer  
b - International units
Figure 84. Serum calcium, phosphorus and alkaline phosphatase determinations. Each value represents mean value at that particular sampling period.
CALCIUM

PHOSPHORUS

ALKALINE PHOSPHATASE

INOCULATED

CONTROL
DISCUSSION

Reproduction of Disease

The study of streptococcal arthritis conducted by Field et al. (41) indicated that it was principally a disease of the young pig. However, a study of agents isolated from the arthritic joints of swine by Switzer (121) revealed that streptococcal arthritis also occurred in older pigs.

Streptococcal arthritis is commonly observed in pigs under 2 weeks of age which suggests that they are commonly exposed to the organism at a very young age (60). It is generally assumed that such infections are the result of an umbilical infection (navel ill). Helms (60) determined that the most constant pathologic change encountered in young pigs was associated with infections of the navel. However, abscesses or inflammatory changes were seldom observed in the umbilical region of the young streptococcal infected pig observed in this field study.

An epidemiological survey to determine the route of entrance of the organism in naturally occurring streptococcal infections in young pigs was not determined in this study. It was demonstrated in Experiments 1 through 5 that a disease syndrome resembling the naturally occurring streptococcal arthritis could be reproduced only with intravenous inoculation of the organism. Repeated intraarticular injection with doses up to $4.5 \times 10^9$ organisms failed to produce gross or
microscopic lesions resembling those observed in the naturally occurring cases. It was further demonstrated that different methods of cultivation of the organism did not produce significant alterations in pathogenicity.

Intraarticular injection resulted in a cellular response at 6 days postinoculation which consisted of degenerative changes of the synovial cells, perivascular lymphocyte and macrophage accumulations and a lack of fibrosis. Fibrin formation was not observed. The joint injected 3 weeks previously had only pyknosis and necrosis of the synovial cells. No inflammatory cells or fibrosis of the synovial membrane were present. Thus it became evident that the septicemic phase was essential for the production of progressive joint lesions.

The exact mechanism of lesion formation was not determined in this study. With intraperitoneal and intraarticular injection generalized systemic effect of the organism did not occur. Also, cultures of joint fluid and peritoneal cavity were negative for Str. equisimilis from the animals killed following these routes of inoculation. This suggested that the proper environment was not present for streptococcal multiplication. Edlund (38), using radioactive Mycobacterium phlei, demonstrated that little systemic invasion occurred following intraarticular injection. They attributed this to some factor other than thrombosis of the joint capillaries because extra-articular dissemination failed to occur when
thrombosis was prevented with heparin. This would explain the very mild temperature elevation observed in most of the intra-articularly injected pigs.

Another mechanism of lesion formation to be considered is an immune reaction to the products of inflammation such as fibrin. The components of the inflammatory reaction are probably of considerable importance in such an immune phenomenon as inflammatory components may persist in the joint and serve as an antigen. The tissue response to intraarticular inoculation was characterized by mild round cell exudation and little tissue destruction. Conversely, the progressive arthritis which followed intravenous inoculation was characterized initially by marked cellular exudation, fibrin deposition and considerable tissue destruction. Dumonde and Glynn (35) stated that a progressive joint lesion suggested the presence of an endogenous antigen whose repeated advent could then be responsible both for maintaining the appropriate immunological state as well as eliciting the appropriate local reactions. He produced an experimental arthritis which resembled rheumatoid arthritis of man by the single injection of washed autogenous fibrin into the knee joints of rabbits. Astrup and Sjolin (8) demonstrated that human synovial tissue and fibrous capsular tissue contained no more than trace amounts of tissue thromboplastin. Their work suggests that the major portion of the fibrinolytic substance was of systemic origin and that fibrin persisted within the joint. The fibrinolysin deficiency
may explain the persistence of fibrin within the streptococcal arthritic joint.

Another possibility to consider in the genesis of streptococcal joint infection is the adsorption of an antigenic portion of the streptococcal organism by synovial cells. This would require partial degradation of the organism prior to adsorption. Following the production of antibodies, this would involve the synovial cell in the antigen antibody reaction. Stewart and Martin (120) have demonstrated the adsorption of a Str. pyogenes (human origin) red cell sensitizing antigen to various tissues of the body. The antigen is referred to as the Hickey antigen.

In view of the experimental results obtained in the present study it appears that some systemic inflammatory factor is essential for the production of progressive Str. equisimilis arthritis, the exact nature of which was not determined.

Synovial Cell Fine Structure

The fine structure of the normal swine synovial cell layer from 8 pigs was studied. This cell layer consisted of variously shaped cells separated by collagenous fibers and cellular debris. In general, the collagenous fibers tended to be more abundant in areas where the synovial cells were 1 cell in thickness. The synovial cell layer consisted of mast cells, cells with well developed rough endoplasmic reticulum, cells with numerous vacuoles and pinocytic vesicles and cells with
few cytoplasmic organelles and RNP particles. Cells intermediate in classification with a well developed rough endoplasmic reticulum, an occasional vacuole and prominent mitochondria were also observed.

Similar observations were made by Barland et al. (10) of the human synovial membrane. A basement membrane as described by Langer and Huth (75) was not detected in this study. A prominent Golgi apparatus was not present in these swine synovial cells in contrast to the observations of Wyllie et al. (133) on the guinea pig. Barland divided human synovial cells into type A which was characterized by pinocytic vesicles, numerous filopodia and large vacuoles and type B which was characterized by a well developed endoplasmic reticulum. Barland et al. (10) suggested that both cell types were probably different metabolic states of the same cell. The type A cell was concerned with phagocytosis and pinocytosis. Chapman et al. (21) observed that intraarticular injection of iron dextran resulted in phagocytosis of the particles by the type A cells. The present study indicates a wide variety of development of the cytoplasmic organelles which suggests a broad range in the metabolic activity of similar cells as noted by Barland et al (10).

Cytochemically, acid phosphatase particles were observed in the large clear vacuoles at the margin of the swine synovial cells. Amounts varied from a few granules to extensive acid phosphatase activity. The acid phosphatase vacuoles
suggest such cells to be involved in phagocytosis. These vacuoles corresponded to the phagosome observed in phagocytic cells by Chapman et al. (21). Muirden (94) recently detected active phagocytosis of ferritin particles injected into the joint by the lining synovial cells.

In addition to the phagocytic vacuoles, membrane-bound osmophilic bodies were observed in swine synovial cells. These bodies resembled the cytolysomes discussed by Novikoff and Essner (99) which have been described in physiologic and pathologic processes as membrane-bound cellular areas where various organelles may be confined for acid hydrolysis and subsequent utilization of the breakdown products.

The exact source of the acid phosphatase particles observed in the swine synovial cells is not known. Microbodies were mentioned by Ashford and Porter (7), ribosomes by de Duve (30) and Golgi apparatus by Brandes and Bertini (17) and Novikoff and Essner (98) as sites of production of acid phosphatase. It was difficult to explain the frequent occurrence of such particles within the nucleus, especially of the neutrophil where possible increased production of acid phosphatase is taking place as the result of the inflammatory process. The particles may indicate acid phosphatase production in the nucleus or, alternatively, non-specificity of the stain. Acid phosphatase activity was negative in the sections treated with sodium fluoride which inhibits acid phosphatase activity, thus suggesting that the staining reaction was specific for acid
phosphatase.

Streptococcal Arthritis

The streptococcal infected joints were enlarged and very firm thus resembling the external changes observed in swine erysipelas by Sikes (114). *Mycoplasma sp.* arthritis as reported by Roberts and Doyle (107) does not produce the same degree of periarticular enlargement or swelling. Also periarticular abscess formation observed in streptococcal infection was not seen with swine erysipelas by Sikes (114) or mycoplasmosis by Roberts and Doyle (107).

Experimentally, periarticular enlargement became apparent by 3 days postinoculation. At this time, the swelling was primarily caused by increased amount of synovia. By 7 to 10 days, periarticular fibrosis became increasingly present and was marked by 2 weeks postinoculation. The animal in the 3-month group which died 2 months postinoculation had extensive periarticular abscess formation of both hock joints. Recovery from extensive gross fibrosis was observed in 3 of 4 pigs by 6 months postinoculation. Hock joints that appeared markedly enlarged and firm at 2 months postinoculation, appeared grossly normal at 6 months postinoculation indicating that the connective tissue had regressed.

Depending upon the degree of suppuration and connective tissue formation, the synovial membrane changes varied from a faint hyperemia to a white discoloration. Changes at 30 days
postinoculation consisted of a disorganized pattern of villous proliferation. Areas of connective tissue contraction were evident. By 6 months postinoculation, large villi composed of dense connective tissue were evident in some joints and in those joints where healing had occurred, the synovial membrane was more fibrous-white in appearance. The synovial membrane changes of chronic mycoplasmosis consisted of yellowish velvet-like proliferation of the villi whereas with erysipelas a regularly arranged hypertrophied villi was observed (107). Thus *Str. equisimilis* produces a whitish discoloration of the chronically involved synovial membrane with an occasional polyp of connective tissue.

The acute inflammatory process of streptococcal arthritis may be confused with that of swine erysipelas or mycoplasmosis. However, in many instances the exudation was extensive enough to obscure the hyperemia. Hyperemia is prominent in both erysipelas and mycoplasma arthritis. Turbid synovia may be observed in all 3 conditions although the synovia is more likely to become thickened and purulent in streptococcal infection.

The subacute stage was characterized by a diffuse increase in connective tissue throughout the synovial membrane, with a lack of proliferation of the larger blood vessels. This has not been described in either mycoplasmosis or erysipelas.

Six months postinoculation the streptococcal synovial
membrane changes which consisted of long connective tissue villi formation might be confused with swine erysipelas. However, the villi in streptococcal arthritis were not regularly placed as was frequently observed in swine erysipelas.

Microscopically, the acute synovial membrane changes consisted of exudation of neutrophils, macrophages and plasma cells and fibrin formation. The synovial cell layer had considerable cellular debris, "blebbing" of the cytoplasm and a slight enlargement of the synovial cell. In areas of fibrin formation a synovial cell layer was absent. Varying degrees of thrombosis and vasculitis of the synovial vessels occurred suggesting the vascular endothelium as an important site of injury. The changes reported in acute mycoplasmosis by Roberts et al. (108) were very similar except that infiltration of lymphoid elements was consistently more extensive in mycoplasmosis.

By 30 days postinoculation the synovial villi were hypertrophic and were rich in connective tissue. There was hyperplasia of the synovial cells which were enlarged and exhibited increased cytoplasmic basophilia. These changes were closely correlated with the fine structural alterations. Cytoplasmic blebbing and cellular debris were not as extensive as observed earlier. In some areas, plasma cells and lymphocytes extensively infiltrated the synovial membrane, whereas in other joints of the same animal marked infiltration of neutrophils occurred. The differences in cellular pattern of the synovial
membranes within the same animal suggests the possibility that there are varying degrees of healing even though most of the joints were probably infected at the same time. Isolation of Str. equisimilis could not be correlated with the cell type in the inflammatory exudate. In most involved joints, hyalinized masses of fibrin were observed on the surface of the synovial cells. Homogeneous pink staining material was observed within the villi and in such areas synovial cells were absent or degenerate. This pink material closely resembled fibrinoid and stained positive with PAS stain.

One of the most characteristic lesions of the diffuse collagen diseases in man is fibrinoid formation. In this study, fibrinoid formation was a constant feature of the synovial membrane by 30 days postinoculation. Boyd (16) considered there to be 2 stages in fibrinoid formation. First, swelling of the ground substance and then loosening and separation of the collagen bundles.

The exact nature of fibrinoid is not known. Fibrinoid material stains bright pink with eosin, is positive for fibrin and is PAS positive. Altschuler and Angevine (2) stated that the lesion may be a depolymerization of the acid mucopolysaccharide of the ground substance with secondary changes in the fibers. However, Gitlin et al. (44) has shown, by the use of fluorescein labeled rabbit antiserum against human fibrin, that fibrinoid is, in part, composed of fibrin. Taylor and Shepherd (122), using the fluorescent technique demonstrated
that a component in areas of fibrinoid necrosis of the subcutaneous rheumatoid nodules gave specific fluorescence with the rheumatoid patient's own serum. They suggested the lesion resulted from a localized antigen antibody reaction. Movat and More (92) and Movat et al. (93) evaluated the significance of fibrinoid. Mechanical injury was followed by hemorrhage and fibrin formation along the edges of a wound. This conglomerate and inspissation of plasma protein and fibrin resulted in the formation of typical fibrinoid. Similar findings were observed in this study. Movat et al. (93) stated that "fibrinoid degeneration of collagen" did not occur in their experiments. The exudate furnished the matrix for the formation of a young, acid mucopolysaccharide-rich connective tissue which later was converted into a collagenous scar. In this study, eosinophilic staining material was found on the surface of the synovial cells and within the villi at 30 days postinoculation. The location of the fibrinoid in both areas is in agreement with Movat et al. (93) who attributed fibrinoid to an inspissation of plasma protein and fibrin. At 3 months postinoculation, circumscribed masses of connective tissue corresponding in location to the previously observed fibrinoid were observed. The location and appearance of the material suggested organization of fibrinoid into connective tissue.

At 6 months postinoculation, the joints which evidenced gross resolution had marked pyknosis of the synovial cells
with a decrease in periarticular fibrosis. A similar healing process has not been observed in mycoplasmosis or swine erysipelas. In other arthritic joints, the change consisted of the formation of a prominent irregular villi composed of dense connective tissue. The connective tissue was vascularized by small capillaries which is in contrast to vascularization by large blood vessels with fibrosis of the media observed with erysipelas (114). Chronic mycoplasmosis (107) was characterized by enlarged villi composed of lymphocytes, hyperplastic synovial cells and with little or no fibrosis.

The synovial membrane lesion in streptococcal arthritis progressed from a marked neutrophil and macrophage rich exudative process to fibrosis by 15 days postinoculation. As the time postinoculation progressed, fibrosis of the enlarged villi became prominent. Fibrinoid formation was evident by 30 days postinoculation and appeared to have become organized by 3 months. Synovial cells in the acute phase were very "frayed" and surrounded by an abundance of cellular debris. In the subacute phase, synovial cells became enlarged with a less "frayed" appearance and had a basophilic staining cytoplasm. By 6 months postinoculation, synovial cells were either pyknotic or prominent similar to that observed in the subacute stage.
Fine Structure of Infected Synovial Cell

Fine structural alterations of the acute streptococcal infected synovial cell consisted of marked filopodia formations with frequent cytoplasmic membrane blebs, focal areas of condensation of nucleoplasm, prominent Golgi apparatus and a very active endoplasmic reticulum with attached RNP granules. There was no apparent increase in number of the electron dense bodies of the cytoplasm. In most cells there was a broadening of the endoplasmic reticulum with an increase of material causing intermediate electron scattering within the endoplasmic reticulum. All synovial cells appeared similar except for an occasional degenerating synovial cell. These changes suggest that most of the normal synovial cell types differentiate into a cell with a well developed endoplasmic reticulum and Golgi apparatus. Such organelle changes indicate an active secretory cell which can be correlated grossly with an increased synovia.

Experimental *Str. equisimilis* infection produced degenerative changes of the endoplasmic reticulum, clumping of the nucleoplasm, extensive vacuolization of the endoplasmic reticulum with membrane structures within the vacuoles and relatively normal mitochondria were observed. Such vacuoles were associated cytochemically with marked acid phosphatase activity. The acid phosphatase activity of the synovial cell was confined to the degenerated cell with extensive "ballooning"
of the endoplasmic reticulum and to localized round structures within the cytoplasm.

Increased acid phosphatase activity in the cytoplasm and nuclei of the neutrophils during the acute exudative phase was observed. Acid phosphatase particles were also abundant in the extracellular spaces. In many instances, acid phosphatase staining granules were not entirely confined to the localized particles. They appeared to be lysed and to have discharged their enzyme into the cytoplasm. De Duve (30) stated that the integrity of the lysosomal membrane depended on the maintenance of some of its components in the oxidized state. Anoxia released 1 or more of the enzymes which breakdown the membrane. The autolysis of the particles was shown to be strongly pH dependent in vitro.

At 30 days postinoculation, all synovial cells appeared similar. They were enlarged with prominent oval nuclei and abundant cytoplasm. The synovial cells resembled the dedifferentiated cell observed from tissue culture preparations. Oberling and Bernhard (100) described cytoplasmic dedifferentiation in tumor cells. The degree of dedifferentiation was inversely related to the degree of development of the rough endoplasmic reticulum. In this study, the most obvious alteration of the synovial cell was in the short segments of the endoplasmic reticulum that were associated with clumped RNP particles. The RNP particles were not clumped in normal cells. The altered endoplasmic reticulum structures were evenly
distributed throughout the cytoplasm. There was no apparent increase in osmophilic bodies or vacuoles in the cytoplasm. These changes were associated grossly with a marked increase in a thin turbid synovia.

Information about the constituents of the synovial effusion in a streptococcal infected joint is not complete. In rheumatoid arthritis in man, Hamerman and Sandson (52) reported an abnormal hyaluronate-protein complex which suggests that the product of the diseased synovial cell is abnormal. Smuckler et al. (117) have shown a defective protein synthesis of the liver cell following administration of carbon tetrachloride; this abnormal protein synthesis was associated with dislocation of the RNP particles from the membranes of the rough endoplasmic reticulum. Whether the clumping of the RNP particles in the 30-day group indicated an altered protein synthesis and/or was associated with the dedifferentiation process is not known.

In general, the synovial cell changes progressed from a broadening of the abundant endoplasmic reticulum with a material of intermediate electron scattering to finely segmented endoplasmic reticulum with clumping of the RNP particles on the endoplasmic reticulum membrane. Structural changes observed in this study suggest that in the *Str. equisimilis* infected joint the primary cellular alteration concerns the endoplasmic reticulum and associated RNP particles with a later dedifferentiation of the synovial cell.
The changes of the endoplasmic reticulum observed in the synovial cell at 3 days postinoculation were associated with an increased thin turbid synovia. Barland et al. (9) recently reported prominent changes in the type A cells in rheumatoid arthritis. There were fewer filopodia, a smaller Golgi apparatus and altered mitochondria. An increase of cytoplasmic acid phosphatase granules considered to be residual bodies were observed in the rheumatoid synovial cell. Streptococcal infection in swine appears to produce a dedifferentiation of the synovial cells to Barland's type B cell in which there is a prominent rough endoplasmic reticulum and Golgi apparatus in the acute phase.

Horn et al. (65) demonstrated that phagocytosis of staphylococci by the rabbit heterophil was accompanied by configurations suggesting granule fusion with newly formed phagocytic vacuoles. Continued phagocytic activity resulted in the depletion of both acid and alkaline phosphatase containing granules. In streptococcal arthritis 3 days postinoculation, the acid phosphatase particles in some areas of the neutrophils appeared to be in clumps comparable to those seen in Horn's experiments. In addition, free acid phosphatase particles were observed extracellularly in most sections. Rheumatoid synovial fluid had high levels of acid phosphatase which Smith and Hamerman (116) correlated with an increased concentration of neutrophils within the synovia.
Articular Surface

Articular surface changes were first observed 15 days postinoculation and consisted of focal eroded areas of cartilage. In each instance where grossly evident changes were observed the lesion originated within vascular canals of the cartilage. The changes within the cartilage consisted of focal accumulations of neutrophils and cellular debris surrounded by fibroblast type cells that blended into hyperplastic cartilage. Several perivascular areas of cartilage cell fibroplasia were observed and a loss of metachromasia occurred only in the perivascular areas of cartilage cell alteration.

By 30 days postinoculation, hyperplasia of the cartilage cells of the germinal and tangential zones was observed in all involved joints. In areas of extensive erosion, marked proliferation of the cartilage cells within the lacunae was observed. There was a loss of metachromasia of the ground substance in these areas.

At 3 and 6 months postinoculation, the osteoarthritis was characterized by erosion of the articular surface, marked increase in cartilage cells per lacuna (clone formation) of germinal and tangential zones and fissuring along matrix areas between enlarged clones. Changes of osteoarthritis were associated with a loss of metachromasia.

Changes similar to those observed experimentally were noted in naturally occurring cases of *Str. equisimilis*. 
articular surface was noted in each instance where infection was confined to the joint. Where gross articular surface change was observed, the lesion appeared to have been initiated within the vascular canals of the cartilage. The zone of provisional calcification served as an adequate barrier against osteomyelitis of the epiphysis.

Since the classical experiments of Phemister (104) in 1928, various experimental models have been used to explain the mechanism of articular surface breakdown in septic arthritis. In most in vitro models, normal cartilage was used and various exudates and enzyme preparations were added. Usually conditions (pH, temperature) necessary to degrade the normal cartilage were such that, under natural conditions of infection, gross alteration of the cartilage was not possible or variable results obtained (28).

Two major components of the cartilage must be considered in articular surface breakdown. Collagen fibers form 1 component and the ground substance in which the collagen fibers are embedded forms the second component. Curtiss and Klein (29) evaluated destruction of cartilage in septic arthritis and concluded that leukocytic enzymes or plasmin had no effect on collagen. They stated that collagen degradation was essential for articular surface breakdown and that the only known mammalian enzyme capable of doing this was Clostridium welchii produced collagenase reported by MacLennan et al. (82). Such an enzyme does not exist in septic arthritis. Curtiss and Klein
(29) thus stated that the exact mechanism of \textit{in vivo} collagen fibril degradation was not known.

The ground substance is another component involved in breakdown of the articular surface. This material is a polymer of a substituted disaccharide which is composed of galactosamine, acetate, glucuronic acid and sulphate. The principal constituent is an acid mucopolysaccharide chondroitin sulphate. The matrix polymer exists as a protein containing complex of high molecular weight. The origin of this substance has been demonstrated by Gross \textit{et al.} (48) to be the chondrocyte.

Many proteolytic enzymes have been reported to remove chondroitin sulphate from the cartilage. Lack and Rogers (74) and Curtiss and Klein (29) demonstrated that plasmin will degrade the chondromucoprotein of cartilage. Ziff \textit{et al.} (137) demonstrated that leukocyte extracts and trypsin degrade chondromucoprotein. Johnson (68) mentioned the leaching of the ground substance by movement of water in and out of the tangential zone during exercise of a joint. Thus chondroitin sulphate is very susceptible to its environment and is unlikely to take part in the active breakdown of cartilage.

Curtiss and Klein (29) maintained that the enzymatic affect on the ground substance alone is not sufficient to account for cartilage destruction in septic arthritis. They suggest that the primary loss of the ground substance from cartilage could cause exposure of the collagen fibers and their destruction by mechanical means.
Metachromasia of the cartilage ground substance has been used to assess the changes of the ground substance. Dziewiatkowski et al. (37) using $^{35}$S autoradiographic technique, demonstrated that the sulphated compounds of the matrix are produced in the chondrocytes. Belanger (12) suggested that the degree of metachromasia can be correlated with the degree of sulphation of the matrix, thus indicating a relationship between the degree of metachromasia and the presence of chondroitin sulphate.

In the experimentally produced streptococcal arthritis, a loss of metachromasia of the articular cartilage was accompanied by an alteration in the cartilage cells. This was manifested by a metaplasia of the chondrocytes to fibroblasts and by hyperplasia of the cartilage cells surrounding the vascular canals. The grossly normal articular surface of involved joints evidenced hyperplasia and hypertrophy of the cartilage by 30 days postinoculation. Facts obtained from this research indicate that the cellular alteration precedes a marked loss of metachromasia. In involved joints of earlier sampling periods without the cellular alteration, the gross change was not evident and loss of metachromasia was not marked.

The hyperplasia and hypertrophy of cartilage cells also suggests the inability of the cartilage cell to maintain the matrix constituents. Anderson (3) believes that in the epiphyseal plate where an active maturation of the cartilage occurs, the greatest degree of metabolic activity was observed when
the chondrocytes underwent transition from the proliferating to the hypertrophic state. This intense metabolic activity was of short duration. As hypertrophy increased, the cell began to die and it displayed all of the nuclear and cytoplasmic changes associated with necrosis. Metabolic activity ceased and glycogen stores disappeared.

Thus the hypertrophy and hyperplasia of the cartilage probably indicated an attempt on the part of the animal to strengthen the surface against the abnormal joint environment. Instead of strengthening the articular surface, the modified cellular state resulted in an altered matrix which allowed fissuring and erosion.

In most of the experimental models used to explain cartilage breakdown, normal bovine nasal septum was used (28). This would explain the lack of in vitro duplication of cartilage breakdown, as normal instead of hypertrophic or hyperplastic cells were present. The hyperplasia and hypertrophy of the cartilage cells can be correlated with changes in the synovial cells in the same joint. These underwent dedifferentiation and had a poorly developed finely segmented endoplasmic reticulum and clumped RNP particles suggestive of altered synthesis.

Endochondral Ossification

Zonal changes in endochondral ossification were variable and dependent upon the degree of localized infection.
Involvement of the metaphyseal side of the endochondral ossification zones resulted in broadening of vesicular zone and a loss of staining affinity in the zone of provisional calcification. In one instance, formation of a secondary vesicular zone was observed. Focal areas of fibrosis were evident in the adjacent metaphysis. The secondary vesicular zone development was more prominent in the naturally occurring cases of streptococcal arthritis and was observed in the epiphyseal plate of the femur in 1 field case.

Lesions of the costochondral junction of swine are frequently used as an aid for the diagnosis of chronic hog cholera. The gross lesions observed at necropsy consist of a transverse line of cartilage formation which has been separated from the primary costochondral junction. Dunne (36) stated that "a marked histological change occurs at the epiphysis of the ribs of infected pigs. The microscopic examination of the costochondral junction of a rib from a pig subacutely infected with hog cholera shows a markedly enlarged area of mature cartilage cells between the zone of cartilage cell multiplication and irregular trabecular bone. The irregularity of this junction of trabecular bone and the zone of lacunar enlargement is quite evident upon gross examination of the infected rib". Dunne indicated that hemorrhage was a prominent feature of the costochondral junction.

Thus from Dunne's description the changes of hog cholera are similar to those observed with streptococcal infection.
Broadening of the vesicular zone, lack of cellular activity in the metaphyseal region and a transverse cartilage line formation in the subacute cases occur in both diseases. Prominent areas of hemorrhage were not consistently observed with streptococcal infection.

Changes of the metaphyseal zone differed slightly from that reported for hog cholera. With infection induced by *Str. equisimilis*, varying degrees of pyknosis and necrosis of the endothelial cells and osteoblasts were observed. Increased connective tissue elements were present in the marrow spaces and, occasionally, a focal increase in neutrophils was present. Hemorrhage was not prominent in this zone. Hog cholera virus produced marked hemorrhage in this zone with necrosis of most of the osteoblasts in the region resulting in "bare" cartilage trabeculae. Young (136) reported a disappearance of the cellular elements of the metaphysis by the production of metaphyseal ischemia produced surgically.

Thus with the infectious processes, the initial damage to the metaphysis was similar. Hog cholera virus produced hemorrhage and thrombosis of blood vessels whereas *Str. equisimilis* produced a focal vascular damage which resulted in concomitant focal osteomyelitis and in some instances fibrosis. The end result of both was probably metaphyseal ischemia.

The possible mechanisms of lesion production of the vesicular cartilage zone consisted of a loss or decrease of vascular invasion and/or a decrease in the maturation of the
cartilage cells. Trueta (124) demonstrated that physical blockage of the blood vessels by insertion of a plastic disc in the metaphyseal region resulted in a marked broadening of the vesicular zone. Young (136) described the changes produced in the metatarsal metaphysis of young rabbits by total infarction of this area. "Post-operatively, the chondrocytes of the growth cartilage thickened as a result of the accumulation of maturing cartilage cells. Three days post-operatively a zone of dead or dying cells appeared at the metaphyseal and at the growth cartilage columns. From the fifth day onward, vascular processes invaded the zone of maturing chondrocytes, some vessels reaching the bone from outside the epiphyseal vessels passing through the growth cartilage. Subsequently reorganization of the growth plate and a reestablishment of ossification in a metaphyseal direction occurred in the revascularized areas" (136). Thus lesions of ischemia in the metaphysis of both experiments resulted in alterations similar to those observed with streptococcal infection and those reported for hog cholera. The experiments of Trueta (124) and Young (136) appear to be the most logical explanation of broadening of the vesicular zone in these infectious conditions. Ischemia and endothelial cell damage appear to occur in both conditions.

A poorly stained zone of provisional calcification occurred in the ischemic lesions surgically produced by Young (136) and Trueta (124), in hog cholera lesions described by
Dunne (36) and lesions initiated by focal areas of streptococcal osteomyelitis. The poorly stained area suggested a focal demineralization. In vitro demineralization of bone may be readily produced by lowering the pH of the extracellular environment. Harris (56) has described a lowering of the pH in areas of inflammation. Whether the demineralization is the direct effect of a vascular alteration or a local acidosis, produced by a deficient blood supply, is not known.

The exact mechanism of the transverse line formation of the metaphysis is not known. Dunne (36) considers that the line formation is initiated by a return of the serum calcium and phosphorus level to normal. The results of this research support the hypothesis that the chronological steps of rib lesion formation are: 1. that the vascular invasion from the metaphysis that produces solution of the vesicular zone is obstructed physically by focal inflammatory tissues which, in turn, permit excessive accumulation of cartilage cells in the vesicular zone 2. that if the vascular damage is severe enough, a new vesicular zone develops 3. that the endothelial and/or adventitial cells of the large vascular canals within the cartilage then penetrate laterally into the new vesicular zone, thus establishing a new metaphysis and 4. that the establishment of a new metaphyseal zone produces a grossly visible transverse line formation. This lesion appears to be an attempt to slough the damaged tissue by a process of revascularization.
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The possibility of inanition causing the transverse line formation must be considered, as a radioopaque line has been associated with acute illnesses in man by Harris (57). In inanition the transverse line was bone. The basic alteration was a cessation of cartilage growth with subsequent "sealing off" of the metaphyseal side of the epiphyseal plate. With the infectious processes, cartilage production was not arrested and the transverse line was cartilage rather than bone.

Focal areas of osteomyelitis in the epiphyses resulted in marked alteration of the adjacent epiphyseal plates. Cessation of chondrocyte proliferation, thinning of the epiphyseal plate, loss of staining affinity and metachromasia of the matrix and vacuolization of the cartilage matrix at approximately the center of the epiphyseal plate were noted. Trueta (124) demonstrated that the blood supply to the epiphyseal plate was from the epiphyseal side. Physical blockage of the epiphyseal blood supply resulted in decreased staining intensity of the segment, inhibition of cartilage multiplication and vacuolization of the cartilage matrix in the center of the epiphyseal plate. Hence it is probable that the comparable lesion produced by Str. equisimilis is the result of ischemia.

Blood Chemistry

A Str. equisimilis infection produced a decrease in serum calcium and elevation of serum phosphorus and alkaline phosphatase. Only the calcium depression was statistically
significant (P > 0.01). However, evaluation by the randomized block design of analysis of variance did not take into consideration the degree of infection which would be difficult to evaluate and is highly subjective. Hewitt (61), Eveleth and Schwarte (40) and Dunne (36) reported that a depression of serum calcium and elevation of serum phosphorus and alkaline phosphatase occurred in hog cholera. Slein and Logan (115) studied the action of toxin produced by *Bacillus anthracis* which has 2 components, a protective antigen and a filter passing factor. Hyperphosphatasemia was produced by the injection of the protective antigen alone and was not inhibited by antiserum. Such a rise did not occur when only the filter passing factor was injected. Bilateral nephrectomy did not prevent the production of hyperphosphatasemia by the antigen component of the toxin. Bluger (15) reported that alkaline phosphatase concentration of serum is an indicator of the host reaction to infectious disease.

Boyd (16) states that alkaline phosphatase is produced by osteoblasts as an essential enzyme in the growth and repair of bone. Its level in the serum, in the absence of liver disease, reflects the degree of osteoblastic activity. Pritchard (105), in a study of phosphatase in rat femurs, found that areas of mesenchyme in which centers of ossification later appeared reflected activity before osteoblasts had differentiated. The enzyme first appeared in the nuclei of mesenchymal cells and as the cells enlarged and assumed the form of osteoblasts, the
activity became more intense and appeared in both nucleus and cytoplasm and extracellularly, in collagenous fibers. With the appearance of definitive osteoblasts, enzyme activity fell abruptly, especially in the nucleus.

It is apparent that the mechanism of production and exact significance of alkaline phosphatase is not known. It is doubtful if the elevated alkaline phosphatase was associated with an increased osteoblastic activity in *Str. equisimilis* infection since necrosis or degeneration of the osteoblasts of the metaphysis occurred.
SUMMARY

The establishment of *Str. equisimilis* organism in joints and bone was dependent upon a septicemic phase. The possible mechanisms have been discussed.

Localization of *Str. equisimilis* in the synovial membrane resulted in an effusion of fluid into the joint containing neutrophils and macrophages. The degree of grossly visible hyperemia was inversely related to the degree of neutrophil exudation. The neutrophils persisted in the synovial membrane, but plasma cells and lymphocytes were the most prominent cell at 15 days, 30 days, 3 months and 6 months postinoculation. Focal areas of suppuration were present in the synovial membrane of all joints. Areas of fibrinoid formation were evident within the synovial villi by 30 days and by 3 months; such areas were organized nodules of connective tissue. Fibrosis of synovial villi became prominent at 15 days postinoculation. Six months postinoculation the villi were hyperplastic and fibrotic in some animals.

The cytoplasm of the synovial cells at 3 days postinoculation appeared very frayed with an abundance of cellular debris. At 15 days postinoculation, there was still considerable cellular debris and the cell nuclei were more round accompanied by a frayed appearance of the cytoplasm. By 30 days postinoculation, considerable swelling of the entire cell was accompanied by basophilic staining reaction of cytoplasm.
In joints where healing had occurred, the synovial cells were flattened and pyknotic.

The fine structural study of the normal synovial cells revealed 3 cell types in different metabolic states. One was a cell with a well developed endoplasmic reticulum, an occasional Golgi apparatus and mitochondria. The second cell contained a poorly developed endoplasmic reticulum and numerous pinocytic vesicles and membrane-bound vacuoles. Another cell which was quite prominent had a relative absence of cytoplasmic organelles.

At 3 days postinoculation, the fine structure of all synovial cells appeared similar. An active, extensively formed endoplasmic reticulum with a more prominent Golgi apparatus was evident. Between the endoplasmic reticulum membranes was a material of intermediate electron scattering ability. Vesicle formation or pinocytic vesicles were not observed. Prominent filopodia had formed with frequent blebbing of the cytoplasmic membrane.

By 30 days postinoculation, all synovial cells appeared similar to the dedifferentiated tissue culture cell. They possessed large nuclei with little nucleoplasm and extensive, finely segmented endoplasmic reticulum with a clumping of RNP particles on the endoplasmic reticulum membrane. Mitochondria were evident, but a Golgi apparatus was not prominent in any of the cells.

Acid phosphatase activity of the normal synovial cell was
confined to the vacuoles at the periphery and to discrete foci within the cytoplasm. At 3 days postinoculation, there appeared to be a dispersion of the acid phosphatase particles within the cytoplasm of the synovial cell.

A well developed rough endoplasmic reticulum was present in most cells. Dedifferentiation of synovial cells and altered RNP endoplasmic reticulum relationship after 30 days postinoculation suggested altered synthesis by the synovial cell.

Gross changes of the articular surface before 30 days postinoculation were dependent upon a vascular change within the cartilage producing metaplasia of the cells to fibroblasts. In some instances, suppuration of the perivascular spaces of the vascular canal was evident. In all involved joints at 30 days postinoculation, the cartilage cells of the germinal and tangential zones were hypertrophic and hyperplastic and were associated with a loss of metachromasia. Before 30 days postinoculation, regardless of the degree of suppuration within the joint, the cartilage remained intact. A vascular change within the cartilage produced perivascular, cartilage-cell hyperplasia and, in some instances, metaplasia to fibroblasts. Marked clone formation, erosion and fibrillation were evident at later sampling periods.

The lesions observed and sequence of events suggested that breakdown of the articular surface is dependent upon an altered cell before the breakdown of the cartilage can occur.
Lesions of the bone marrow spaces resulted from localization of *Str. equisimilis* in the metaphyseal region or in the epiphysis. At 3 days postinoculation, discrete foci of neutrophils were observed throughout the bone marrow. Focal neutrophilic lesions of the metaphysis resulted in broadening of the adjacent vesicular zone. In areas where lesions were more extensive, a secondary vesicular zone had developed with lateral penetration of the blood vessels of the adjacent vascular canals of the cartilage. As the new metaphysis was formed, the "old" vesicular zone formed a transverse cartilage line that was evident grossly. These lesions were observed in both experimentally and naturally occurring cases of *Str. equisimilis* infection. The lesion resulted from a localized ischemia produced by vascular damage in the metaphyseal region.

Lesions of the epiphysis and the adjacent epiphyseal plate resulted in a focal arrest in growth of cartilage, degenerative changes of the chondrocytes and vacuolization of the cartilage matrix.

Swine *Str. equisimilis* infection resulted in a significant lowering of the serum calcium, an elevation of the phosphorus by 30 days postinoculation and elevation of alkaline phosphatase.
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ACKNOWLEDGMENTS

The counsel and patient guidance of Dr. F. K. Ramsey, Head, Department of Veterinary Pathology, Iowa State University and Dr. W. P. Switzer, Veterinary Medical Research Institute, Iowa State University, throughout this investigation are gratefully acknowledged.

The author is also grateful for the time and suggestions contributed by other members of the graduate committee who are: Dr. J. M. Layton, Pathologist, College of Medicine, State University of Iowa, Iowa City; Dr. R. A. Packer, Department of Veterinary Hygiene, Iowa State University; Dr. M. S. Hofstad, Veterinary Medical Research Institute, Iowa State University; and Dr. L. E. Roth, Department of Biophysics and Biochemistry, Iowa State University.

The technical assistance of Alvina Owenson, Grace Faber and Kay Pierce is acknowledged.

To my wife, thanks are especially due for encouragement and for carrying the major share of family responsibility during the completion of this work.