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OF NORMAL AND MYCOPLASMA HYORHINIS INFECTED
SWINE ARTICULAR TISSUES AND SYNOVIAL FLUID.

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THE HISTOLOGIC AND BIOCHEMICAL CHARACTERIZATION
OF NORMAL AND MYCOPLASMA HYORHINIS INFECTED
SWINE ARTICULAR TISSUES AND SYNOVIAL FLUID

by

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TABLE OF CONTENTS

	Page
INTRODUCTION	1
REVIEW OF THE LITERATURE	4
Arthritis	4
Mycoplasma	13
Histology and Histochemistry	24
Clinical Chemistry	26
MATERIALS AND METHODS	29
Experimental Swine	29
Microbiological Methods	31
Inoculum and Inoculation Methods	31
Sample Collection	32
Clinical Chemistry Methods	35
Histologic and Histochemical Methods	42
Experimental Design	44
Data Analysis	47
RESULTS	49
Clinical and Gross Pathologic Observations	49
Clinical Chemistry	52
Normal Synovial Membrane	124
Normal Synovial Membrane-Acid Mucopolysaccharides	128
Normal Articular Cartilage-Morphology	141
Normal Articular Cartilage-Acid Mucopolysaccharides	150

	Page
Infected Synovial Membrane-Morphology	173
Infected Synovial Membrane-Acid Mucopolysaccharides	176
Infected Synovial Membrane-Enzyme Histochemistry	179
Infected Articular Cartilage-Morphology	183
Infected Articular Cartilage-Acid Mucopolysaccharides	188
Infected Articular Cartilage-Enzyme Histochemistry	191
DISCUSSION	
Clinical and Gross Pathologic Observations on Control and Infected Groups	195
Clinical Chemistry-LDH Activity	195
Clinical Chemistry-Protein	203
Clinical Chemistry-Glucose	209
Clinical Chemistry-Hyaluronic Acid	211
Histology and Histochemistry	212
SUMMARY	220
BIBLIOGRAPHY	224
ACKNOWLEDGMENTS	251

INTRODUCTION

Arthritis in swine is of agricultural importance as a major cause of economic loss. Effective control can only be brought about by understanding the pathogenesis of arthritis in swine. Once this understanding is obtained, economic losses may be reduced by effective control procedures.

The pathogenesis of any disease must be understood at the clinical, morphologic and biochemical levels. This understanding can only be obtained through the comparative study of normal and arthritic animals. Normal parameters must therefore be established since they are the basis for comparison.

It is within the scope of this work to establish normal parameters, particularly those pertaining to swine joints. The establishment of normal clinical, morphologic and biochemical parameters of swine joints can serve as a basis for the study of swine arthritis in general. In this investigation it will serve as the foundation for studies on M. hyorhinis arthritis in swine.

To date, the bulk of information held on M. hyorhinis infection in swine is of a microbiological and clinical nature. Information on morphologic alterations is infrequent, and on clinical biochemical values is virtually nonexistent. The revelation of these parameters may contribute

to a better understanding of the pathogenesis of M. hyorhinis arthritis in swine and hasten the development of effective control measures and a consequent reduction in economic loss.

Mycoplasmal arthritis in swine cannot be presented solely as an economic problem of agriculture however. With interest in mycoplasmas still mounting and a renewed search under way for an "etiologic agent" of human rheumatoid arthritis, mycoplasmal arthritis of swine is also attracting increased medical attention.

A number of workers in human and animal diseases have described mycoplasmal arthritis in swine as the closest animal model to human rheumatoid arthritis. Until a few years ago the position of "animal model of rheumatoid arthritis" was held by erysipelatous arthritis of swine. The basis for this recognition was largely a similarity between the histologic and morphologic parameters of the two diseases. More recently mycoplasmal arthritis of swine has also been recognized for its histologic and morphologic similarities to rheumatoid arthritis.

The long and arduous search for a microbiological etiology of rheumatoid arthritis has been abandoned and resumed on countless occasions. The elusiveness of an etiologic agent for this disease sparked the realization that the agent may still be of an infectious but extremely insidious nature. Mycoplasmas fit this description. With

the isolation of mycoplasmas from some cases of human rheumatoid arthritis a new excitement was created and the search for suitable animal models intensified.

It is intended, by means of this investigation, to extend the present knowledge of Mycoplasma hyorhinis induced arthritis of swine. The extension of this knowledge by histochemical and biochemical means is undertaken with a desire to better understand this disease in swine and also to realize the differences and/or similarities of it to human rheumatoid arthritis.

REVIEW OF THE LITERATURE

Arthritis

Arthritis was first described by Paracelsus in the 15th century during his study of human gout. Since then, the study of normal and diseased joints in man and animals has cumulatively added a wealth of information to many fields. Anatomists, for example, obtained the term synovia and some of the first anatomic descriptions of joints from Paracelsus' early studies on arthritis (Rodnan et al. (1966). More recently others such as Key (1932) and Gardner (1959) have made significant contributions to the terminology and classification of joints and joint tissue. Radin (1968) in his comprehensive summary on joint lubrication points to the unique lubricating properties of synovial fluid which are as yet unparalleled by any synthetic material. Hamerman and Schubert (1962) in their extremely coherent review on joints, point to the rapid accumulation of literature (10,000 references covering the period from 1946 through 1958) over only a 13 year period.

If the study of joints holds such fascination for so many, the study of various forms of arthritis must hold at least equal fascination. Arthritis can be classified by etiology into three forms: 1) infectious, 2) traumatic, and 3) degenerative.

Infectious arthritis results from a wide variety of

etiologic agents from which even the most highly developed species did not escape.

Arthritis in man has been associated with almost every evolutionary level of microorganism. Ubiquitous bacteria such as Escherichia coli, Staphylococcus aureus and many others have been frequently recovered from cases of human arthritis. Some of the more common bacterial arthritides in man were described in a concise review by Alicandri (1964). Other less commonly encountered arthritogenic organisms such as Pseudomonas pseudomallei (Diamond and Pastore, 1967) and Blastomyces dermatiditis (Sanders, 1967) have also been described in man.

Rheumatoid arthritis in man, whose specific etiology still escapes diligent rheumatologists, is the most widely studied of human arthritides. Many believe that rheumatoid arthritis occurs indirectly as a result of infection. Ford (1963) presents the case for an infectious etiology and states that although evidence is only indirect, it is plentiful. Hamerman (1968) discusses the nature of various initiating agents and events resulting in rheumatoid arthritis.

Since the "infectious agent" of rheumatoid arthritis has escaped detection for so long, many have searched for less obvious agents such as mycoplasma and viruses (Ford and Oh, 1965). Butas (1957) recovered pleuropneumonia-like organisms (PPLO) from recurrent poly-arthritis in man.

Bartholomew (1965 and 1967) has isolated and characterized mycoplasmas in several instances from cases of human rheumatoid arthritis.

Arthritides in other species are quite common also. Domesticated and laboratory animals are known to be affected by all three of the various forms of arthritis. The most intensely studied form of arthritis in animals however, is the infectious variety. Workers have isolated and characterized a host of infectious agents from many species of birds and mammals.

Avian infectious arthritis involves both wild and domestic birds. Sokoloff (1963) stated that chronic hematogenous infectious arthritis is a fairly common cause of inanition and death in captive wild birds. A comprehensive review and summary of the more common causes of lameness in domestic poultry by Frazer (1965) discusses numerous etiologic agents found in poultry arthritis.

Etiologic agents of avian infectious arthritis form a wide spectrum of microorganisms and include at least one virus. Arthritis in poultry produced by salmonella, staphylococcus, pasteurella, erysipelothrix, streptobacillus and mycoplasma, although commonly encountered, usually occur as sequelae to septicemias and are not frequent primary arthritogenic agents (Jungherr, 1959).

Salmonella arthritis resulting from septicemia has been

recorded by Cosgrove and Lindenmaier (1961) and Ferguson et al. (1961) in broiler chickens with pullorum disease and by Rasmussen (1962) who, in studying over 60,000 ducks, isolated S. typhimurium most frequently from their arthritic joints.

Streptobacillus moniliformis has been isolated by Yamamoto and Clark (1966) and Mohamed et al. (1969) from turkeys with joint and sternal bursal lesions.

Lecce et al. (1955) reported an arthritis in chickens caused by an organism possessing many of the characteristics of a mycoplasma. Hayflick (1956) characterized a pleuropneumonia-like organism causing infectious tenovaginitis with arthritis in chickens. Adler (1959) isolated a mycoplasma from arthritic chicks. Kerr and Olson (1967) reported cardiac pathology associated with mycoplasmal (M. synoviae) arthritis and described pathologic similarities between mycoplasmal and viral arthritis. Olson and Solomon (1968) reported a natural outbreak of avian viral synovitis from which a virus but no mycoplasma was isolated.

Since chicken embryos are convenient for propagation of a variety of microorganisms, they have been widely utilized in studying mycoplasmas. The pathogenicity of mycoplasmas for chicken embryos was discussed by Casorso and Jungherr (1959), Chute (1960) and Hayflick and Stinebring (1960). Arthritic changes observed in chicken embryos were discussed by Moulton and Adler (1957).

Infectious ruminant arthritis may be attributed to, as in any other species, a wide variety of microorganisms. Van Pelt et al. (1966) described lesions of infectious arthritis in calves from which α -hemolytic streptococci and/or Corynebacterium pyogenes were isolated. Storz et al. (1966) reported polyarthritis in calves 1-3 weeks old from which he isolated and identified chlamydial agents. Mendlowski et al. (1960), Kater et al. (1962) and Shupe and Storz (1964) also reported an association of chlamydial agents with arthritis in sheep. Moulton et al. (1957), Simmons and Johnston (1963) and Hughes et al. (1966) isolated mycoplasmas from arthritic calves. Cordy et al. (1955) recovered mycoplasmas from a highly fatal septicemic and arthritic disease in goats.

All three forms of arthritis are seen in the canine and again a wide spectrum of etiologic agents are responsible. Hemophilia in the dog often results in joint disease as a sequel to intrasynovial hemorrhage (Swanton, 1959). McErlean (1966) and Clegg (1968) identified Brucella abortus as the etiologic agent of arthritis in dogs. Septic arthritis in a dog reported by Kavit (1958) was caused by Cryptococcus neoformans, normally a nonarthritogenic pathogen. Experimental arthritis has been produced in dogs inoculated with the chlamydial agent of ovine polyarthritis (Maierhofer and Storz, 1969).

Arthritis in laboratory animals is frequently of an experimental nature, but natural cases do occur. Spontaneous caseating arthritis was reported in rabbits by Arseculeratne (1962) who isolated an actinobacillus from affected joints. Nelson (1958) isolated mycoplasmas from joints and other tissue of breeding colony rats and mice with infectious catarrhal enteritis and synovitis. Howell et al. (1959) also reported the isolation of a mycoplasma (M. arthriditis) from polyarthritic joints and tumor tissue of mice with lymphosarcoma. Freundt (1959) reported frequent isolation of Streptobacillus moniliformis from mice with arthritis; pleuropneumonia-like organisms were also recovered from these mice.

Experimentally induced arthritis has been produced in a variety of laboratory animals by nutritional alterations (Silberberg and Silberberg, 1957), hormonal application (Jasmin and Bois, 1959), hypersensitivity reactions (Pearson and Wood, 1959; Dumonde and Glynn, 1962; Phillips et al., 1966; Rawson et al., 1969) and enzymes such as papain (Murray, 1964). Bacterial products (Jones and Carter, 1957; Pearson and Wood, 1959; Braude et al., 1963) and a variety of live bacteria (Cayeux et al., 1966 and Zaki, 1966) have also been used for production of arthritis in laboratory animals. Experimental mycoplasmal arthritis has been produced successfully by Ward and Jones (1962), Smith (1965a) and Barden and

Tully (1969) in small laboratory animals. Although numerous attempts at producing experimental models for human arthritides have ended in failure, Sokoloff (1960) wishes luck "to any of those who try." A complete review on experimental arthritis is given by Gardner (1960).

Arthritis in swine, as in any of the other species thus far described, can also be attributed to the same etiologic forms. A general discussion by Quin (1955) was given on the various swine arthritides encountered in swine practice.

Congenital and developmental lamenesses in pigs are not uncommon. Thurley et al. (1967) discussed observations on a splay leg condition in swine and attributed the condition to a myofibrillar deficiency of hereditary origin. Kowalczyk et al. (1958) presumed a hereditary basis for fibrous dysplasia of bones in pigs which were nutritionally adequate but highly inbred. Walker et al. (1966) described a developmental "leg weakness" in pigs and attributed it to joint stress with resultant cartilagenous alteration.

Other noninfectious arthritides such as nutritional and toxic forms are frequently observed. Whitehair (1958) described arthritis of pigs produced by calcium-phosphorus-Vitamin D imbalances and Swan (1949) described an arthritic condition in swine associated with nicotinic acid deficiency. Experimental zinc toxicity has been produced in weanling pigs with resultant arthritis and other lesions by

Brink et al. (1959).

Infectious arthritis in swine, as in man and other species, has a multitude of etiologic agents. Streptococcal arthritis has been reported by Sekeguchi and Irons (1917), Field et al. (1954) and Roberts et al. (1968) in swine. Brucella suis was isolated from spondylitic lesions in swine by Feldman and Olson (1933). Bierer (1956) relates the presence of arthritic lesions in baby pigs with pasteurellosis. Ward (1917) has isolated Bacterium (Corynebacterium) pyogenes from joints of swine with suppurative arthritis. Hemophilus suis has been recovered by Shanks (1939), Sutherland and Simmons (1947), Knott (1956) and Hjärre (1958) from a Glässer's-like disease of swine. Lecce (1960) described the interaction of H. suis and a pleuropneumonia-like organism in the production of a Glässer's-like disease in swine. Even organisms, as uncommonly associated with swine arthritis as Sporotrichum schenckii (Smith, 1965b) have been reported.

Probably one of the more interesting forms of arthritis is that produced in swine by Erysipelothrix rhusiopathiae. Some of the first reports of erysipelatous arthritis were made on swine brought to packing houses for slaughter (Ward, 1922 and Ducksberry, 1934). Erysipelatous arthritis as an important herd problem was brought to light by Harrington (1933) in his early description of field cases of the disease.

With the experimental production of erysipelatous polyarthrititis in swine by Collins and Goldie (1940), this form of arthritis established a foothold in arthritis research. A report by Doyle (1951) describing erysipelatous arthritis as a "rheumatoid disease" of swine, produced considerable excitement in the search for an animal model of human rheumatoid arthritis. Connell et al. (1952) established the pathogenesis while Usdin et al. (1952) and Neher et al. (1958) proposed a possible "hypersensitivity" mechanism for the disease serving to further establish swine erysipelatous arthritis as a potential model for human rheumatoid disease. Hughes (1953) and Sikes et al. (1956) reviewed the literature on swine erysipelas concentrating most of the knowledge held to that time. Sikes (1959) drew a close parallel between the histopathologic lesions of erysipelatous arthritis and human rheumatoid arthritis. With the report by Fletcher et al. (1966) of arthritis production in swine repeatedly given whole blood from arthritic donors, erysipelatous arthritis in swine was firmly established as an animal model for human rheumatoid arthritis.

With the excitement of swine erysipelas at its apex, a new organism began to gain recognition as a serious arthritogen in swine. McNutt et al. (1945) was one of the first workers to recognize the association of a pleuropneumonia-like organism (PPLo) with arthritis in swine. The

role of the PPLO in swine arthritis was further established by Cole (1957). McNutt (1959) finally characterized an arthritogenic mycoplasma from swine and called it the "B0" strain. Not only did the "B0" strain produce arthritis, but it produced a polyserositis typical of that observed in Mycoplasma hyorhinis (Switzer, 1955) infection of young swine. Roberts et al. (1963a, b) recorded the pathogenesis of experimental M. hyorhinis infection of swine. Switzer (1964) described yet another arthritogenic mycoplasma (M. granularum) which was found to be quite distinct from M. hyorhinis. A new arthritogenic mycoplasma (M. hyoarthrinosa) was described by Moore et al. (1966b) and its pathogenesis characterized by Robinson et al. (1967). More recently, parallels have been drawn between mycoplasmal arthritis in swine and human rheumatoid arthritis (Ross and Switzer, 1968). Lack (1968) in discussing experimental models for rheumatoid arthritis described mycoplasmal arthritis in swine as one of the closest parallels to human rheumatoid arthritis. One of the best reviews on mycoplasmas as causes of joint infections in animals is presented by Sharp (1964).

Mycoplasma

According to Hayflick and Chanock (1965), mycoplasmas can be defined in the following manner. The mycoplasmas are a group of microorganisms that can reproduce in cell-

free media, exhibit a characteristic colonial morphology and the smallest forms are in the size range of 125 to 150 mu. They are also highly pleomorphic due to their lack of a rigid cell wall, most require sterol and native protein for growth, are penicillin resistant and their growth is inhibited by specific antibody.

Mycoplasmas were first introduced by Nocard and Roux's paper "Le microbe de la péripneumonie" published together with their collaborators in 1898. The paper provided a good description of bovine pleuropneumonia and its transmissibility in cattle. Soon after, it was found that the contagious pleuropneumonia organism (Mycoplasma mycoides) could be grown in artificial media enriched with serum. Thereafter, many workers developed an interest in pleuropneumonia-like organisms and knowledge about them grew rapidly. Edward (1954), Klieneberger-Nobel (1954 and 1961) and Pekhov (1957) in their detailed summaries on the mycoplasmataceae present the bulk of knowledge held to that time. More recently, Klieneberger-Nobel (1967) presented a historical review discussing the key discoveries and milestones in the development of mycoplasmology.

A host of other workers have since contributed a vast amount of knowledge on mycoplasmas to the literature. Distinction of the mycoplasmas from bacteria has been attained by many methods. Chemical composition (Lynn and Smith, 1960),

nutritional requirements (Smith, 1960) and analysis of metabolic products (Neimarle and Rickett, 1960) have all assisted in the characterization of the mycoplasmataceae. Today a number of important features serve to distinguish mycoplasmas from other organisms. Madden and McCullough (1967) discuss many of these characteristics. One of the more recent methods of mycoplasma characterization, electrophoresis of cell proteins (Razin, 1968) has yielded considerable information in the taxonomical study of these organisms. Visual characterization of the mycoplasmataceae has been accomplished through electron microscopy (Edwards and Fosh, 1960; Maniloff et al., 1963; and Domermuth et al., 1964) and has enabled workers to distinguish the mycoplasmataceae from other similar organisms (Anderson, et al., 1965 and Tanaka et al., 1965).

Although mycoplasmas were introduced as pleuropneumonia producing agents in cattle, they have been described in man and many other species of animals (Ito, 1960). Many of the mycoplasmas of man are discussed in a review by Hayflick and Chanock (1965).

Mycoplasmas have been associated with a number of human diseases. Klieneberger-Nobel (1959) frequently isolated mycoplasmas from human genital infections. Chanock et al. (1962) characterized the etiologic agent isolated from human atypical pneumonia as a mycoplasma (M. pneumoniae).

Bartholomew (1965) isolated and characterized a mycoplasma from human patients with rheumatoid arthritis, systemic lupus erythematosus and Reiter's syndrome.

Mycoplasmas have also been reported on several occasions (Dmochowski, 1965; Dmochowski et al., 1965; Girardi et al., 1965 and Hummeler et al., 1965) in patients with leukemia and other forms of cancer. Although the relationship of mycoplasmas with neoplastic diseases such as leukemia is poorly understood, Barile (1967) suggested that a possible autoimmune mechanism may be involved. Others (Paton et al., 1965 and Macpherson and Russell, 1966) have described chromosomal alterations and cellular transformations in mycoplasma infected tissue culture cells suggesting mycoplasmal influence on genetic control.

Mycoplasmas are not only troublesome as agents of disease, but also create problems for the research worker. Tissue cultures are often unknowingly contaminated with these organisms (Hayflick, 1965) and are not readily removed (Jasmin and Richer, 1959).

An increasing awareness of subhuman animal mycoplasmoses has led to a better understanding of the mycoplasmataceae, but our knowledge of this group of organisms is still incomplete. With expanding knowledge, it is presumed that they will assume an even greater significance in veterinary medicine.

The nutrition, metabolism and pathogenicity of a number of animal mycoplasmas has been discussed by Adler and Shifrine (1960). Adler (1965) reviewed the large variety of mycoplasmas found in the various animal species.

A large number of pathologic mycoplasmal isolates from domestic poultry have been characterized (Dierks et al., 1967). Adler and Yamamoto (1956), Grumbles et al., (1958) and Edward and Kanarek (1960) discussed a number of mycoplasmas responsible for respiratory infections in poultry. Yamamoto and Adler (1958) discussed several avian strains and their capability for producing nervous signs, joint lesions and air-sac disease. Encephalitis has been described in turkeys infected with mycoplasmas by Cordy and Adler (1957) and Thomas et al. (1966). Chalquest (1962) reported on the cultivation of a pleuropneumonia-like organism (M. synoviae) responsible for producing avian infectious synovitis. The essential role of mycoplasmas in avian air-sac disease and the venereal implications of the organism are discussed by Johnson and Domermuth (1956) and Bigland and Benson (1968). Domermuth (1962) has produced presternal bursal synovitis ("breast blisters") in birds by inoculating them with a mycoplasma. The pathogenicity of multiple respiratory infections including mycoplasmal (M. gallisepticum) infection have been studied by Corstvet and Sadler (1966).

Birds other than domesticated poultry are also affected by mycoplasmas. Mycoplasmas have been isolated from a mild respiratory disease in pigeons (Mathey et al., 1956), air sacculitis in a parakeet (Adler, 1957) and from ducks (M. anatis) with sinusitis (Roberts, 1964).

Since the first description of contagious bovine pleuropneumonia (CBPP) in 1898, interest in the pleuropneumonia-like organisms of ruminants has never waned. Mycoplasma mycoides remains the type species and CBPP remains the classical mycoplasmal disease. A number of other pathogenic mycoplasmas have since been isolated from a variety of ruminant diseases.

Recently, genital mycoplasmosis has received considerable attention. Mycoplasma bovigenitalium has been isolated from cattle with granular vulvovaginitis (Afshar et al., 1966), but its role in this disease is still not clear. At least one association of a mycoplasma with an aborted bovine fetus has been reported (O'Berry et al., 1966). An experimental metritis has been produced in cattle by intrauterine inoculation of a pathogenic mycoplasma originally isolated from mastitic cattle (Hartman et al., 1964). Berchtold and Bogel (1962) reported isolating a PPL0 from bulls with azoospermia. Hirth et al. (1967) reported the survival of M. agalactiae var. bovis in frozen bovine semen suggesting a possible role in some bovine venereal diseases. A

mycoplasma has also been incriminated in orchitis of sheep (McIlwain and Bolin, 1967).

Research on ruminant mastitis has also yielded several mycoplasmas. Hale et al. (1962) recovered a mycoplasma from cattle with severe mastitis and named the organism M. agalactiae var. bovis. The pathogenesis of mycoplasmal mastitis was studied by Jasper et al. (1966). Jasper (1967) suggested that a more appropriate name for the bovine mastitis organism is M. bovimastritis. Fincher (1964) related the economic severity of losses due to mycoplasmal mastitis in cattle.

Ruminant respiratory problems have also yielded several mycoplasmas. Mycoplasmas have been isolated from shipping fever in cattle (Hamdy et al., 1958) and ovine pneumonia (Boidin et al., 1958). Mackay et al. (1963) isolated pleuropneumonia-like organisms from sheep with pulmonary adenomatosis. Nonpathogenic mycoplasmas have also been isolated from the bovine respiratory tract. Hudson and Ethridge (1963) and Harbourne et al. (1965) isolated non-pathogenic respiratory mycoplasmas from the nasal cavity and lungs of normal cattle. The latter isolation proved to be M. laidlawii a saprophytic mycoplasma commonly found in normal animals, sewage, soil and water.

Mycoplasmas have also been associated with a variety of other conditions in ruminants. Laws (1956) isolated a

pleuropneumonia-like organism from peritonitis in goats. Cordy (1959) isolated a mycoplasma from goats with a fibrinopurulent synovitis. Surman (1968) recovered a mycoplasma from sheep with "pinkeye", a severe keratoconjunctivitis seen in Australia.

Several mycoplasmas have been recovered from dogs and cats. Gutekunst (1959) isolated five distinct types from dogs with respiratory illness. None of these organisms proved to be pathogenic however. Carmichael et al. (1964) isolated organisms with characteristics of mycoplasmas from puppies with a fatal septicemia. Mycoplasma (M. canis) has been associated with canine leukemia, although it was felt the organism was not actually responsible for the neoplasia (Chapman et al., 1967). Mycoplasma canis and M. spumans have been repeatedly isolated from the external genitalia and nasal cavities of dogs (Skalka and Krejčíř, 1968). The characterization of two mycoplasmas (M. felis and M. gatae) from cats has been reported (Cole et al., 1967). More recently, a third mycoplasma (M. leonis) has been added to the feline group (Heyward et al., 1969). None of the feline mycoplasmas isolated have been associated with disease.

Mycoplasmosis of laboratory animals has frequently been associated with disease. Sabin (1941) described a rheumatoid-like arthritis in laboratory rodents from which

mycoplasma was isolated. Mycoplasmas are frequently isolated from respiratory disease in laboratory rodents. Organick et al. (1966) and Kohn and Kirk (1969) described the pathogenicity of M. pulmonis in pneumonia of rats and mice. Brennan et al. (1969) reported that murine pneumonia may be a multiple infection involving both Pasteurella pneumotropica and M. pulmonis. Chronic respiratory disease in rats characterized by chronic bronchopneumonia and bronchiectasis has frequently been associated with mycoplasma infection (Gay, 1969 and Ventura and Domarodzki, 1967). Mycoplasmas have also been isolated from labyrinthitis (Vasenius and Tiainen, 1966) and otitis media (Olson and McCune, 1968) in laboratory rodents. Some workers (Gilbey and Pollard, 1967) believe mycoplasmas play a significant role in mouse leukemia.

The first report of transmission of a mycoplasmal infection in swine was most likely presented by McNutt et al. (1945). Switzer (1953a, b) isolated and characterized a filterable agent from swine with turbinate atrophy. This organism, which was very similar to Mycoplasma gallisepticum was named Mycoplasma hyorhinis (Switzer, 1955). The organism, although incapable of producing either turbinate atrophy or pneumonia in swine (Carter, 1954) was capable of producing a polyserositis and arthritis in pigs (Roberts et al., 1963a, b). A similar condition in swine was described by Heinze et al. (1963). After the recognition of the arthritis-

polyserositis caused by M. hyorhinis in young pigs, it became apparent that a somewhat similar arthritis occurred in older swine. The disease was not characterized by polyserositis and could not be isolated in the same manner used for isolation of M. hyorhinis. The development of a new medium containing swine gastric mucin enabled the isolation and identification of M. granularum (Switzer, 1964). Ross and Karmon (1970) detected significant differences between some strains of M. granularum and proposed a new species, M. hyosynoviae also an arthritogenic mycoplasma. A widely distributed chronic porcine pneumonia having little tendency for resolution or healing exists in the United States. The etiologic agent, suspected of being a mycoplasma (Carter and Schroder, 1955) was finally propagated, characterized and named M. hyopneumoniae by Maré and Switzer (1966). A mycoplasma has also been isolated from a metritis-mastitis syndrome in sows; the name proposed for this organism was M. hyogenitalium (Moore et al., 1966a). More recently another arthritogenic mycoplasma has been characterized and named M. hyoarthrinosa by Robinson et al. (1967). In England and Europe, another swine disease, "swine enzootic pneumonia" (SEP) was being studied and a mycoplasmal agent detected and named M. suis pneumoniae (Goodwin et al., 1965). This organism showed a marked similarity to M. hyorhinis (Dinter et al., 1965) and many consider M. suis pneumoniae to actually be M.

hyorhinis (Goiš et al., 1968) or M. hyopneumoniae. Others believe SEP is produced by a combination of M. hyopneumoniae and "other organisms" (Hodges et al., 1969). The controversy over SEP in England and Europe still exists. Other mycoplasmas have been isolated from swine. Taylor-Robinson and Dinter (1968) identified mycoplasmas isolated from swine as M. laidlawii, M. gallinarum and M. iners and suggested "maybe some PPL0's are not as host specific as we think."

Comprehensive reviews on swine mycoplasmosis were presented by Switzer (1959 and 1967) and Hodges (1969).

From the study of mycoplasmataceae by many workers, it can be seen that several broad areas of inquiry have yielded results intensifying interest in this organism. The first area involves the realization by molecular biologists, that the mycoplasma, as the smallest free-living microorganisms, are important materials for the elucidation of genetic coding and packaging problems. The mycoplasma have also been of great interest because of their nuisance to investigators working with cell cultures. The demonstration of a mycoplasma species as the etiologic agent of a human disease (primary atypical pneumonia) can also be included among these observations. The encounter of mycoplasmas in what were first thought to be pure virus preparations obtained from human and animal leukemias or cancerous tissues has stirred considerable excitement. Finally, realization that mycoplasmas

are of increasing economic importance as causes of animal diseases throughout the world has been observed. In particular, the observation that, as in other domestic and laboratory animals, mycoplasmas are etiologically responsible for economically important diseases in swine, some of which closely parallel important diseases such as rheumatoid arthritis in man.

Histology and Histochemistry

The histology and histochemistry of normal and arthritic articular tissues have been characterized in some species and is lacking in others. Workers in rheumatoid arthritis of man have frequently characterized normal and arthritic articular tissues of man. Hamerman and Blum (1959), Castor (1960), Palmer (1967) and many others have described the histochemical and histologic appearance of normal human synovial tissue. The histologic and histochemical characterization of synovial tissue from various human arthritides was well described by Hamerman et al. (1961). Blau et al. (1965) located the possible site of origin of synovial fluid hyaluronate by means of an immunohistochemical technique. Technology and interest in this area have developed to such a level that a number of workers are now involved in the ultrastructural characterization of these tissues. Coulter (1962) has ultrastructurally characterized the human synovial membrane. Norton and Ziff (1966) and Wyllie et al.

(1966) have characterized ultrastructural alterations in human rheumatoid synovial membrane. Barland et al. (1968) have recently described the ultrastructural location of hyaluronate synthesis in human synovial lining cells.

Histologic and histochemical studies of normal and arthritic tissues in other species are less plentiful. Laboratory animals are the only exception. Histologic and histochemical studies on normal rodent articular cartilage are numerous. Follis and Berthrong (1949), Balogh et al. (1961) and Greenspan and Blackwood (1966) have histochemically studied enzyme localization in rodent articular cartilage. Mankin (1962a, b and 1963) has studied rabbit articular cartilage by means of radioautography. Articular cartilage blood supply in fetal lambs was studied by Mazhuga (1965). Histochemical and histologic studies of articular cartilage from normal large animals are not as numerous. Studies of synovial membranes from normal large domestic animals are even fewer in number.

The histologic characterization of various infectious arthritides in swine have been reported, but are not overly abundant. Probably the most frequently reported histological description of arthritis in swine is that given for swine erysipelas (Sikes et al., 1956 and Sikes, 1959). Less frequent are reports on light microscopic alterations in articular tissues of swine with mycoplasmal arthritis.

Roberts et al. (1963b) and Moore (1966b) have contributed the only comprehensive works to date in this area. Only one group (Duncan and Ross, 1969) has so far ventured into the ultrastructural characterization of mycoplasmal arthritis in swine. Histochemical characterization of articular tissues from swine with mycoplasmal arthritis to date, are nonexistent.

Clinical Chemistry

Clinical chemistry studies on blood from normal and diseased swine are nearly as plentiful as those conducted in man. Serum protein studies for example, have been conducted in normal swine by a host of workers. Moore (1945), Koenig and Hogness (1946), Havassy and Slanina (1956), Ashton (1957), Rutqvist (1958), Van Klinkenberg (1959), Pensinger et al. (1959), Lecce et al. (1961), Miller et al. (1961), Lötsch and Müller (1963), Scopes (1963), Cochrane et al. (1964), Brooks and Davis (1969), and Tumbleson et al. (1967 and 1969) have all studied blood protein fraction distribution in normal pigs of varying ages from birth to maturity.

Others have studied swine blood protein in various disease states. Vesselinovitch (1955) studied blood protein fractions in swine with edema disease. Perlasca (1956) studied protein fraction distribution in swine under various stresses including tuberculosis. Weide and King (1962)

studied blood protein fraction distribution in pigs vaccinated against hog cholera. A similar study was done by Poul (1963) in pigs vaccinated against swine fever.

Blood protein studies in arthritis of man are more numerous than those in swine. Shetlar et al. (1958), Sikes et al. (1966), Papp and Sikes (1964) and Carter and Neher (1968) have all studied serum protein fraction distribution in swine with erysipelatous arthritis. Serum protein studies on swine with other forms of arthritis such as mycoplasmal arthritis are infrequent. Synovial fluid protein studies in swine with mycoplasmal arthritis are, to date, nonexistent.

Clinical enzymology reports on normal and diseased swine are less frequent than protein studies. Lactic dehydrogenase activity in normal swine blood nevertheless, has been studied by Zimmerman et al. (1965), Tegeris et al. (1966), Hyldgaard-Jensen (1967), Hyldgaard-Jensen et al. (1968), Hessel-de-Heer (1968) and Götz and Coenen (1969). This enzyme has also been studied on a limited basis, in diseased swine. Šlesingr (1965) reported alterations in blood levels of lactic dehydrogenase in swine with influenza. Studies of this enzyme in the blood or synovial fluid of arthritic swine have not been reported to date.

Plasma/synovial fluid glucose ratios have not been reported in arthritic swine. Only two groups (Crimmins and Sikes, 1965 and Bollwahn, 1967) have reported glucose levels

for arthritic swine synovial fluid.

Synovial fluid hyaluronic acid levels, though frequently reported in man have never been reported in normal or arthritic swine.

MATERIALS AND METHODS

Experimental Swine

Experimental swine utilized in these studies were procured from three different sources.

Articulation study group (AS group)

Five clinically normal animals in each of four different weight classes were procured from the Department of Animal Science Swine Nutrition farms, Iowa State University. The weight classes selected were: 1) 8-10 kg, 2) 20-25 kg, 3) 40-50 kg, and 4) 90-95 kg groups.

Slaughter group

This group consisted of arbitrarily selected lots of market weight (85-100 kg) swine brought for slaughter to a local packing plant.¹ The animals were briefly observed for evidence of systemic or arthritic disease. Clinically normal animals were mechanically stunned and carefully examined during sampling for any abnormality of limb or articulation. Animals with grossly detectable abnormalities were not sampled. Samples which did not fit the criteria of selection for study were discarded. Samples from 50 animals were retained for study.

¹Oscar Mayer and Co., Perry, Iowa.

Control and infected groups

Animals weighing 14-28 kg were procured from the respiratory disease-free herd maintained at the Iowa State University Veterinary Medical Research Institute. This herd was established in 1951 with surgically derived breeding stock and all subsequent introductions into the herd have been similarly derived. Clinical, pathologic and bacteriologic examinations performed annually on swine from this herd have confirmed its continued respiratory disease-free status. The young experimental swine were naturally farrowed and raised with the sows until weaning.

Prior to experimentation all animals were again given clinical and bacteriologic examinations to confirm the continued absence of arthritic or systemic disease and the absence of Mycoplasma hyorhinis, Mycoplasma granularum or other possible arthritogenic microbiological agents. Management and isolation programs were strictly supervised and all rations were prepared free of antibiotics or other drug additives.

The experimental swine were housed in strict isolation for the duration of the experimental period and were in all cases allotted to units by a randomization procedure. Thirteen animals were designated as controls and 25 animals served as principals.

Microbiological Methods

Routine bacteriological examination of the samples collected was performed by inoculation of the following media: 1) horse-blood agar plates streaked with a micrococcal nurse colony and 2) beef heart infusion broth containing hemoglobin, swine gastric mucin, turkey serum, penicillin and thallium acetate (Ross and Switzer, 1963). In addition, 2, 3, 5 triphenyl 2H tetrazolium chloride was added as a growth indicator. Blood agar plates were incubated at 37° C and examined after 48 hours for growth. Broth tubes were examined at 72 hours for any indication of growth. Any organisms which were recovered were identified.

Inoculum and Inoculation Methods

The inoculum used was prepared by inoculation of sterile beef heart infusion broth growth media (without penicillin and thallium acetate) with a low passage (7-9) culture of Mycoplasma hyorhinis, strain SK-76. Routine purity checks were performed by centrifugation of a sample culture, Giemsa staining, and microscopic examination of the sediment. Three mls of a 36-48 hour culture were used to intraperitoneally inoculate the experimental principals. Controls were inoculated with 3 mls of sterile, beef heart infusion broth.

Sample Collection

AS group

Each member of the four different weight classes was electrocuted and exsanguinated. Following exsanguination, each articulation was opened and bacteriological samples were aseptically collected. Representative blocks of fresh synovial membrane and articular cartilage were collected from each of three joints in the following manner.

Cubital articulation Two adjacent blocks of synovial membrane from the anterior aspect of the cubital articulation were collected for fixation and further histologic evaluation. One block was placed in 10 percent neutral buffered formalin, and the other was placed in a vessel of isopentane and rapidly quenched in liquid nitrogen at approximately -170° C until completely frozen. The frozen tissue was stored in air tight containers in an ice chest filled with dry ice at approximately -70° C. Two adjacent blocks of articular cartilage from the proximal articular surface of the radius, the cartilage of the glenoid cavity, were collected and processed in a similar manner.

Femorotibial articulation Synovial membrane was collected from the anterior aspect of the articulation extending from the ventral point of the patella to the tibial crest. Cartilage samples from two locations were collected from this articulation. The first sample was taken from the

nonweight bearing surface of the distal femoral trochlea at a point just beneath the medial patellar ligament and the second from the proximal weight bearing surface of the tibia.

Tibiotarsal articulation Synovial membrane was collected from the anterior aspect of this articulation and weight bearing articular cartilage from the proximal articular surface of the tibiotarsal bone.

Slaughter (85-100 kg) group

Each of the mechanically stunned animals was exsanguinated via jugular excision routinely used in swine packing plants. During exsanguination, approximately 10 ml of blood were collected in a tube containing 0.2 ml (approximately 200 units) of heparin.¹ The red cells were sedimented by centrifugation and the plasma decanted. Another 10 ml of blood were collected, allowed to clot, and the serum removed.

Both tibiotarsal articulations were closely examined and synovial fluid samples were withdrawn only from swine with grossly normal articulations. The volume of synovial fluid withdrawn ranged from less than 0.5 ml up to 2 ml per articulation. Synovial fluid samples from the two articulations of an individual animal were pooled, placed into tubes containing approximately one half drop of an EDTA anticoagu-

¹Rugby Laboratories Inc., Inwood, Long Island, New York.

lant (Sequester-Sol¹), and inverted several times.

The presence of grossly detectable hemolysis in either the plasma or serum served as a basis for rejection of the sample from further study. Only synovial fluids which were clear and contained no blood were retained for clinical chemistry determinations.

Due to the restricted nature of sampling from packing house material, microbiological and histological examinations were precluded. Personal post-mortem examination of each animal was not possible.

Control and infected groups

Fourteen days after inoculation each animal was electrocuted and exsanguinated by severance of the axillary vasculature. Blood and synovial fluids were collected from each animal via the procedures described for the "Slaughter group."

Because of the paucity of synovial fluid in 14 to 28 kg Control (normal) swine, synovial fluid samples from any or all of the articulations of an individual under study were pooled and studied as a single sample. Synovial fluid from Infected animal articulations, being plentiful (2-4 mls per articulation) was readily collected.

The articulations were opened and bacteriologic samples

¹Cambridge Chemical Products, Inc., Detroit, Michigan.

were aseptically collected. The articulations were examined for gross pathologic change. Cartilage and synovial membrane from each inflamed articulation were collected, divided and fixed in accordance with the procedures described in the AS group. Normal tissues were collected from the Controls.

Synovial fluid from the diseased articulations was examined grossly. The description and amount were recorded and the fluid was utilized for clinical chemistry determinations. Data from arthritic principals were retained for later statistical evaluation upon confirmation of the presence of M. hyorhinis. Negative bacteriologic and histologic findings were the basis for retention of data from Control animals.

In addition to bacteriologic samples from the articulations, post-mortem samples were also collected from the nasal cavity and prepuce or vagina.

Clinical Chemistry Methods

Each body fluid sample (plasma, serum or synovial fluid) selected for evaluation was analyzed for the following constituents in the following manner.

Total lactic dehydrogenase (LDH)

All LDH determinations were performed within 24 hours and most often within 6 hours of sample collection. In light

of findings by Kreutzer and Fennis (1964), Chilson et al. (1965) and Latner (1967) samples were never refrigerated or cooled. If absolutely necessary, the samples were held at room temperature overnight.

Samples of plasma and synovial fluid were analyzed for total LDH activity via the colorimetric method of Berger and Broida (Sigma, 1967). Following a 5 minute color development period, the individual samples were placed in 12 mm cuvettes and optical density was read at 450 mμ in a spectrophotometer (Coleman Junior, Model 6A¹). The optical density, using water as a reference, was recorded. Enzyme activity in Berger-Broida units per ml was obtained for each sample from a previously constructed optical density calibration curve.

LDH isoenzyme distribution

Plasma samples were directly applied to Microzone cellulose acetate electrophoresis membranes (Beckman²). Before application to the membrane, it was necessary to digest synovial fluid with hyaluronidase to facilitate electrophoretic separation of the sample constituents. Digestion of synovial fluid was accomplished in the following manner. Phosphate buffer for use in the electrophoresis cell

¹Coleman Instruments Inc., Maywood, Illinois.

²Beckman Instruments Inc., Fullerton, California.

and as a diluent was prepared as follows:

.03M Phosphate Buffer (pH 7.5)

Dibasic sodium phosphate	11.8 gm
Monobasic potassium phosphate	3.8 gm
Sucrose	4.5 gm
Distilled water	3000 ml

The ingredients were mixed and pH was checked on a pH meter (Leeds and Northrup¹).

Hyaluronidase enzyme preparation, using the phosphate buffer as a diluent was done in the following manner:

Testicular hyaluronidase (Sigma ²)	3.0 mg
Phosphate buffer (pH 7.5)	0.9 ml

The ingredients were thoroughly mixed until the enzyme was completely dissolved.

Synovial fluid digestion was accomplished as follows:

Enzyme preparation	0.1 ml
Synovial fluid	0.3 ml

The synovial fluid was digested at 37° C for 2 hours and then applied to the electrophoresis membrane.

Electrophoresis of the samples was accomplished in the following manner. A single drop from each of the samples was placed on a thin sheet of paraffin (Parafilm "M"³).

¹Leeds and Northrup, Philadelphia, Pennsylvania.

²Sigma Chemical Co., St. Louis, Missouri.

³Marathon Products Division of the American Can Co., Neenah, Wisconsin.

From the paraffin, uniform volumes of sample were applied to the pre-buffered and cell-positioned membranes with the aid of a 0.25 μ l applicator (Beckman Sample Applicator¹). For adequate isoenzyme resolution, 4 applications of plasma and 6 applications of synovial fluid digestate were necessary.

The electrophoresis cell (Beckman Microzone Cell¹) containing the .03M phosphate buffer (pH 7.5) was set in an ice bath to prevent heat denaturation of the enzyme during electrophoresis. The cell was connected to the power supply (Gelman, Model 38201²) and electrophoretic separation was carried out at 140 volts and 5-10 milliamperes for 90 minutes. After electrophoresis, the membrane was removed and incubated in the dark at 37^o C for 1 hour in contact with an agarose-reactant mixture (Wright et al., 1966). Upon completion of the incubation period, the membrane was washed in a stream of distilled water, dried between blotters, and stored in a plastic holder. The dried membrane was scanned densitometrically (Densicord Densitometer and Integrator³) using a 610 m μ filter and percent transmission was graphically recorded. Using integration marks, the relative percentage of each of the five isoenzymes was determined.

¹Beckman Instruments Inc., Fullerton, California.

²Gelman Instrument Co., Ann Arbor, Michigan.

³Photovolt Corp., New York, New York.

Total protein

Serum and synovial fluid samples were analyzed for total protein content by the Biuret method (Gornall et al., 1949). Following a 30 minute color development period the optical density of each sample was read in a spectrophotometer (Coleman Junior, Model 6A¹) using a 540 mμ filter and a reagent blank. Total protein content was calculated from the closest protein standard (Dade²).

Protein fraction distribution

Serum samples were directly applied to the electrophoresis membrane but again it was necessary to first digest synovial fluid with hyaluronidase to allow adequate electrophoretic migration. The digestion was accomplished in a manner similar to that described for LDH. Instead of a phosphate buffer however, barbital buffer (Beckman Buffer B-2³) at a pH of 8.6 was used as an enzyme diluent and for filling the electrophoresis cell. The digestion procedure was carried out in the same manner as described for LDH.

Samples were applied to the electrophoretic membrane as with LDH except only one application of serum was necessary for adequate resolution. Four applications of

¹Coleman Instruments Inc., Maywood, Illinois.

²Dade Reagents Inc., Miami, Florida.

³Beckman Instruments Inc., Fullerton, California.

synovial fluid digestate were required for adequate resolution.

The electrophoresis cell containing barbital buffer (Beckman Buffer B-2¹) at pH 8.6 was connected to the power supply and electrophoretic separation was carried out at 250 volts and 3-9 milliamperes for 50 minutes. Following electrophoresis, the membrane was treated with Ponceau-S fixative-dye solution and cleared in acetic acid ethanol (Beckman, 1965). The membrane was squeegeed onto a clean glass plate and dried for 10-15 minutes in a 100-115° C oven.

The dried membrane was scanned on a densitometer (Densicord Densitometer and Integrator²) using a 545 mμ filter and percent transmission was graphically recorded. The relative percentage of each of the protein fractions was determined by using integration marks.

Total glucose content

All glucose determinations were made within 6 hours after collection. Protein-free filtrates of plasma and synovial fluid were prepared by a modification of Folin and Wu's method (Van Pelt and Conner, 1963). The protein-free filtrate was analyzed for total glucose via the ortho-

¹Beckman Instruments Inc., Fullerton, California.

²Photovolt Corp., New York, New York.

toluidene method (Harleco¹). The optical density of the cooled samples was read on a spectrophotometer (Coleman Junior, Model 6A²) using a 630 mμ filter and a reagent blank.

Hyaluronic acid content of synovial fluid

Synovial fluid was analyzed for hyaluronic acid by first preparing a depolymerized, protein-free filtrate (Castor *et al.*, 1966). The protein-free filtrate was used in a modified uronic acid carbazole reaction (Bitter and Muir, 1962) for the determination of total uronic acid content. Glucuronolactone (Aldrich³) standards of 3, 5, 10 and 20 μgm per ml were used. Hydrolysis of the sample was accomplished by heating for a 20 minute period instead of the recommended 15 minutes to increase the yield of uronic acid (Castor⁴). Following a 2 hour cooling and color development period, optical density of the samples was read in a spectrophotometer (Coleman Jr., Model 6A²) using a 530 mμ filter and a reagent blank. Hyaluronic acid content of the samples was determined from a standard curve.

¹Hartman-Leddon Co. Inc., Philadelphia, Pennsylvania.

²Coleman Instruments Inc., Maywood, Illinois.

³Aldrich Chemical Co. Inc., Milwaukee, Wisconsin.

⁴Castor, C. W., Ann Arbor, Michigan. Hyaluronic acid determination by the carbazole method. Private communication. 1968.

Histologic and Histochemical Methods

Hematoxylin and Eosin (H + E)

Tissue samples for histologic study were removed from formalin, embedded in paraffin, cut at 5 μ and stained with Harris hematoxylin and eosin-Y by the procedure described in the Manual of Histologic and Special Staining Techniques (Armed Forces Institute of Pathology, 1960). Decalcification of cartilage was not necessary.

Acid mucopolysaccharide histochemistry

Paraffin sections were stained with alcian blue and alcian yellow (Carlo, 1964) and a modified safranin O staining procedure (Lillie, 1954). The safranin O procedure was accomplished in the following manner. Paraffin sections were rapidly deparaffinized to 80 percent ethanol. They were stained in Weigert's acid iron chloride hematoxylin (Lillie, 1954) for 6 minutes. Sections were washed in 3 rinses of distilled water with a final rinse in 1 percent acetic acid. Next, they were stained in 0.1 percent safranin O for 4 minutes and followed by 3 rinses in distilled water. The sections were stained with fast-green FCF for 3 minutes and dehydrated with one change of 95 percent and 2 changes of absolute ethanol. After clearing with 1 change of a 50 percent alcohol-xylene mixture and dehydrating through 2 changes of xylene, the sections were mounted in permanent mounting medium.

Enzyme histochemistry

Liquid nitrogen-isopentane fixed tissue samples were individually embedded in cryostat mounting media (Tissue-Tek O.C.T. Compound¹) and frozen sections 8-10 μ thick were cut on a cryostat (International-Harris Cryostat Model CT²) and mounted on gelatin-coated glass slides. They were stained for diaphorases and dehydrogenases by the nitro BT method (Thompson, 1966) using individual nitro BT (Sigma²) veronal buffer-substrate mixtures for each individual enzyme preparation. Following a 1 hour incubation at 37° C, the sections were removed from the substrate mixture and washed gently with 2 changes of distilled water. The sections were post-fixed in 1 percent acetic acid for one minute and 10 percent neutral buffered formalin for 10 minutes. They were counterstained with Kernechtrot's nuclear fast red prepared according to the Manual of Histologic and Special Staining Techniques (Armed Forces Institute of Pathology, 1960). Staining was accomplished in 3-5 minutes, and the slides were again gently washed and mounted in glycerin-jelly.

Cryostat sections were also utilized for acid and alkaline phosphatase preparations. These phosphatase preparations were made by a simultaneous diazo dye capture

¹Ames Company, Division of Miles Laboratories Inc., Elkhart, Indiana.

²International Equipment Co., Boston, Massachusetts.

procedure (Sigma, 1963) incubating the sections in alkaline phosphatase substrate for 30 minutes and in acid phosphatase substrate for 60 minutes. The sections were removed from the substrate after incubation, post-fixed and counter-stained in the same manner described above for the diaphorases and dehydrogenases. Mounting was done in glycerin-jelly.

Experimental Design

The overall objective of this study was the biochemical, histologic and histochemical characterization of selected body fluids and articular tissues from clinically normal and Mycoplasma hyorhinis infected, arthritic swine. Articular tissues were evaluated by means of histochemical localization of acid mucopolysaccharides and selected enzymes as well as by standard light microscopy. Blood and synovial fluids were analyzed for total lactic dehydrogenase activity and isoenzyme distribution, total protein content and fraction distribution, and glucose levels. Synovial fluid levels of hyaluronic acid were also determined.

In order to develop guidelines for study and a maximum return of information, this investigation was divided into two general studies, intragroup and intergroup comparisons.

Intragroup comparisons

Intragroup comparisons were made to determine the normal range of histologic parameters in tissues of the various articulations. Body fluids were compared with one another to determine if biochemical relationships exist between swine blood and synovial fluid from normal and infected swine.

The study of normal histologic and histochemical differences in various articulations of swine was accomplished in the following manner. The four weight classes of the AS group, being representative of several growth periods of swine from weanling through market weight, were utilized. Individual articulations were compared with homologous articulations from other members of the AS group. Synovial membrane and articular cartilage from each of three articulations (cubital, femorotibial and tibiotarsal) were compared and variations in morphology and histochemistry recorded.

Biochemical relationships between normal swine blood and synovial fluid were studied in two different weight groups in an attempt to parallel the histologic studies. Members of the Slaughter group (85-100 kg) were used to represent the heavy weight (90-95 kg) class of the AS group. Members of the Control (14-28 kg) group were used to represent another weight class (20-25 kg) of the AS group. After collection, samples from individuals of both groups were

analyzed for the biochemical parameters discussed above, data were collected and statistically evaluated. Blood values were compared with synovial fluid values for each of the parameters.

Biochemical relationships between blood and synovial fluid have also been studied in Mycoplasma hyorhinis infected swine. Samples were collected from 18 infected (14-28 kg) animals at necropsy and analyzed. Data were evaluated and the two fluids of Infected animals compared.

Intergroup comparisons

Intergroup comparisons were made to determine the influence of weight and Mycoplasma hyorhinis infection on these histologic, histochemical and biochemical parameters.

The effect of weight on histologic and histochemical parameters was studied in the following manner. Histologic and histochemical preparations used in the intragroup comparisons were reorganized and re-evaluated, comparing analogous articulations from the various weight classes of animals of the AS group. Weight class constituents of the AS group were compared and evaluated for differences attributable to weight. The 20-25 kg constituent of the AS group was also compared with the Control (14-28 kg) group.

Weight effect on biochemical parameters was studied by utilizing data obtained from the Slaughter and Control groups. Slaughter data were statistically evaluated and

compared with Control data.

The effect of infection on histologic and histochemical parameters was obtained by comparing preparations from the Control group with those from the Infected group.

The influence of infection on biochemical parameters was studied by statistically evaluating and comparing data from the Control group with that of the Infected group.

Data Analysis

All data from clinical chemistry determinations were programmed into a digital computer and analyses conducted in the following manner.

Group means, standard deviations, and standard errors were established for each clinical parameter. Several factorial analyses of variance were computed to study group-fluid-fraction interactions. From these analyses of variance tables also came the estimates of variance for each of the groups compared and the "F" tests which were used for pointing out significant clinical parameter interactions and population characteristics.

Results obtained from population characteristic studies and analyses of variance supported the use of group mean comparisons in the remainder of the study. Response profile analysis curves were utilized in graphically portraying group mean relationships. Group mean comparisons were made via Student's "t" test, utilizing the group means and group

estimates of variance described above.

Summary tables and graphs were constructed for graphic visualization of the concentrated data and their comparisons from the statistical calculations.

RESULTS

Clinical and Gross Pathologic Observations

Control group

Control animals remained clinically normal throughout the experiment. No lesions was observed at necropsy.

Infected group

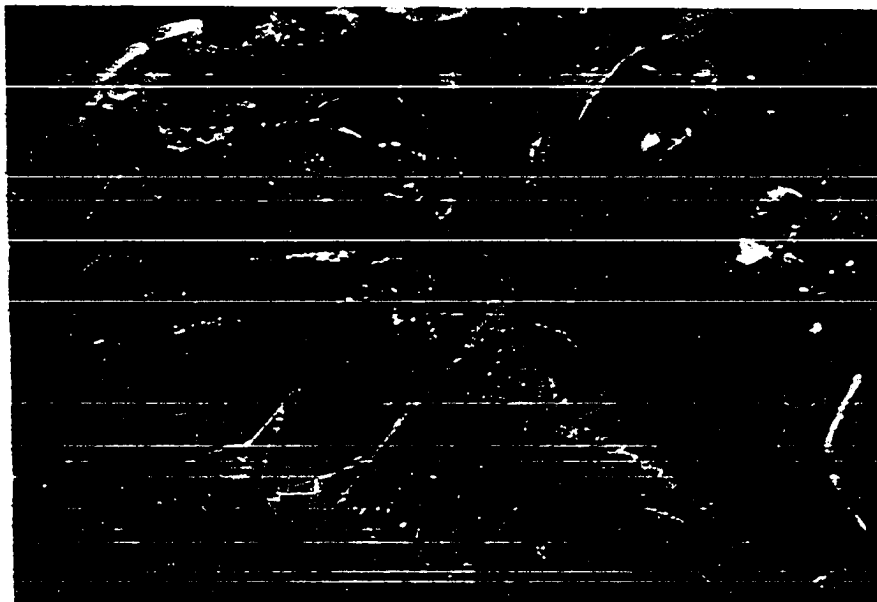
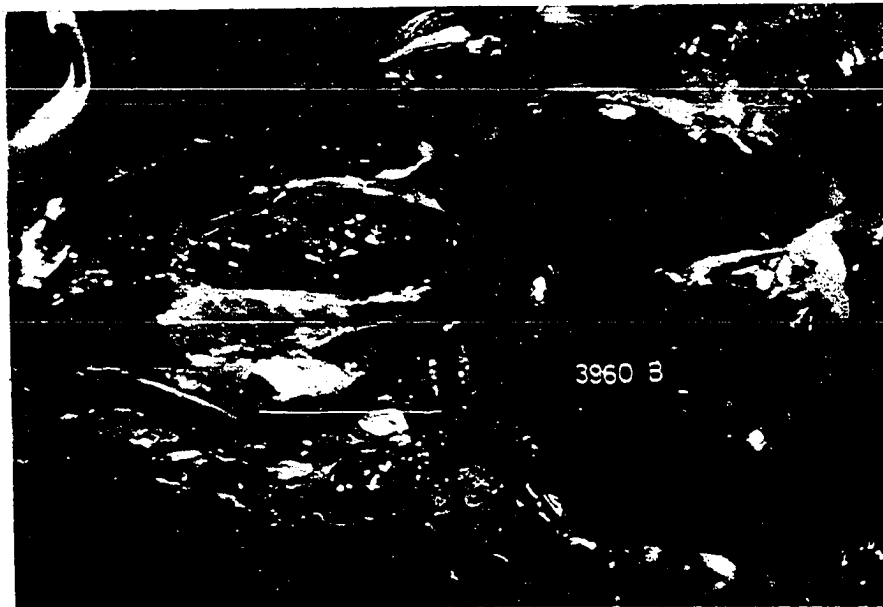
The first signs of illness were observed 2 or 3 days after inoculation with M. hyorhinis. Slight depression, shivering and a roughened hair coat were the earliest signs, followed by more pronounced depression with rapid respiration. The first signs of lameness were usually observed 5 or 6 days after inoculation. Lameness varying in severity from the "shifting weight" type to impaired limb movement were usually first observed at this time. Stretching movements and kicking at the abdomen were occasionally observed.

Depression and lameness persisted for the remainder of the experiment. Loss in condition and retarded growth were obvious in the principals at the termination of the experiment.

At necropsy, the animals had a serofibrinous peritonitis and pleuritis. Frequently a fibrinous pericarditis was also observed, so severe in some cases that the pericardium could not be removed intact from the heart (Figure 1). Numerous fibrin and fibrous tags were on the visceral pleura of the

Figure 1. Mycoplasma hyorhinis infection in the pig.
Fibrinous pericarditis 14 days after
intraperitoneal inoculation with M. hyorhinis,
strain SK-76

Figure 2. Mycoplasma hyorhinis infection in the pig.
Fibrous pleuritis 14 days after intraperitoneal
inoculation with M. hyorhinis, strain SK-76



lungs (Figure 2). Increased fluid was often noted in all the body cavities. Fibrin collections were frequently found in association with serous surfaces of the testicles (Figure 3).

The articulations involved were characterized by lesions varying in severity from synovial edema and congestion (Figure 4) to severe villous synovitis. Synovial fluid of these articulations was increased in amounts and varied in appearance from clear and colorless to blood-tinged and turbid. Cartilage changes were rarely observed.

Clinical Chemistry

Total lactic dehydrogenase (LDH)

Total plasma and synovial fluid LDH activity (Table 1) of the three groups were compared. Slaughter (85-100 kg) group plasma activity was significantly greater than that of the Control (14-28 kg) group. Control plasma activity on the other hand, was not significantly different from that observed in the Infected group. It appears as though weight variation had a greater effect than infection with M. hyorhinis on total plasma LDH activity.

The highest level of synovial fluid LDH activity was found in M. hyorhinis infected animals. This activity was significantly higher than that observed in the Control group. Slaughter animals also had significantly higher levels of activity than the Control group. Infection with M. hyorhinis apparently had a greater influence on total synovial fluid

Figure 3. Mycoplasma hyorhinis infection in the pig.
Fibrin collection on testicular serous
surfaces 14 days after intraperitoneal inocu-
lation with M. hyorhinis, strain SK-76

Figure 4. Mycoplasma hyorhinis infection in the pig.
Synovitis involving synovial membrane of
femorotibial articulation 14 days after
intraperitoneal inoculation with M. hyorhinis,
strain SK-76

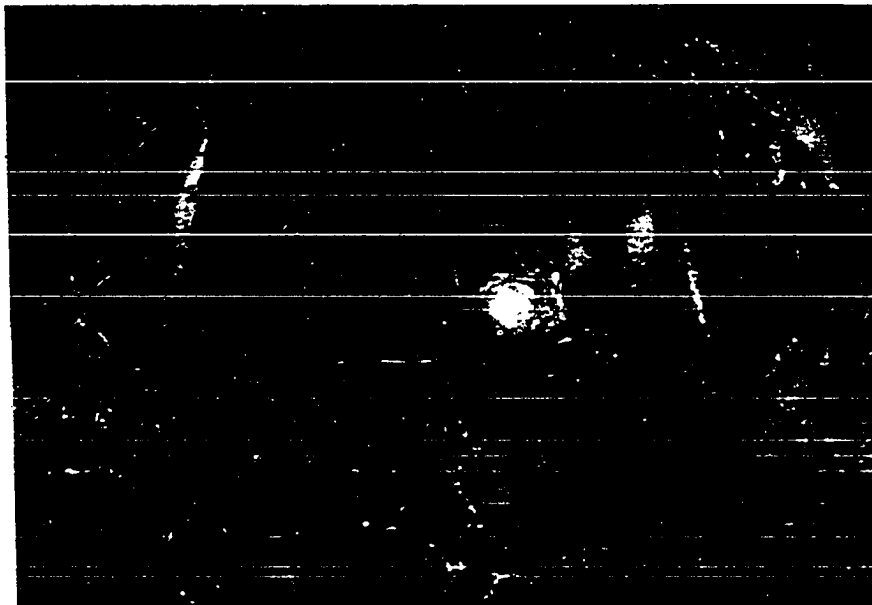
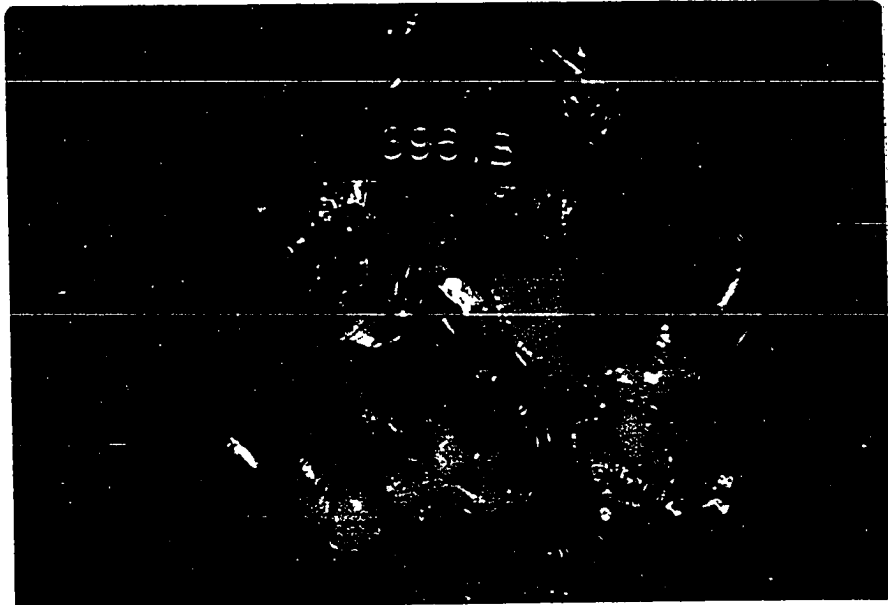


Table 1. Total plasma and synovial fluid LDH activity
(mean) levels in swine

	Animal group	Animal weight (kg)	No. animals	No. observa- tions
Plasma				
Code no. ^a				
1	Slaughter	85-100	50	50
2	Control	14-28	10	10
3	Infected	14-28	18	18
Synovial fluid				
4	Slaughter	85-100	50	50
5	Control	14-28	10	5
6	Infected	14-28	18	17

^aCode no. used for "Coded Statistical Comparisons"
(Student's "t" test).

^bBerger-Broida (B.B.) units/ml (Sigma Chemical Co.)
1 B.B. unit will reduce 4.8×10^{-4} μ moles of Pyruvate/min
at 25° C.

*Not significant at 5% level.

Mean activity ^b	Standard deviation	Range ^b	Coded ^a statistical comparisons	Result of comparisons
1634	± 318.3	800-2100	1 vs 2	P < .005
1285	± 419.5	700-1950	2 vs 3	n.s.*
1587	± 492.5	700-2025		t = 1.62
245	± 71.4	83-341	4 vs 5	P < .025
181	± 67.1	108-250	5 vs 6	P < .010
324	± 20.7	250-350		

LDH activity than did the weight of the animal.

LDH isoenzyme distribution

Electrophoretic isoenzyme distribution patterns such as those reproduced in Figures 5 through 10 were obtained. From these patterns, isoenzyme percentages were determined and group means established (Table 2).

A comparison of Slaughter (85-100 kg) and Control (14-28 kg) plasma means (Figure 11) was made to study the effect of weight on plasma LDH isoenzyme distribution. Mean activity levels of isoenzyme numbers 1, 2 and 5 were higher in the Control group than in the Slaughter group. Isoenzyme numbers 3 and 4 were higher in the Slaughter group. Mean differences between Slaughter and Control isoenzymes 1 and 4 were not statistically significant even though these differences were the largest of the entire weight group comparison. Plasma LDH isoenzyme distribution in the two weight groups was apparently quite similar.

When plasma and synovial fluid isoenzyme distribution were compared within the Slaughter group (Figure 12), plasma and synovial fluid were quite similar in isoenzymes 1 and 2 and plasma was only slightly higher in isoenzymes 3 and 5. Isoenzyme 4 was significantly higher than that in plasma. Synovial fluid appears to differ from plasma principally in the presence of higher levels of isoenzyme 4.

Isoenzyme distribution in Control group plasma was then

Figure 5. LDH isoenzymes of normal (85-100 kg) swine plasma (%). Typical isoenzyme distribution in plasma of Slaughter group swine

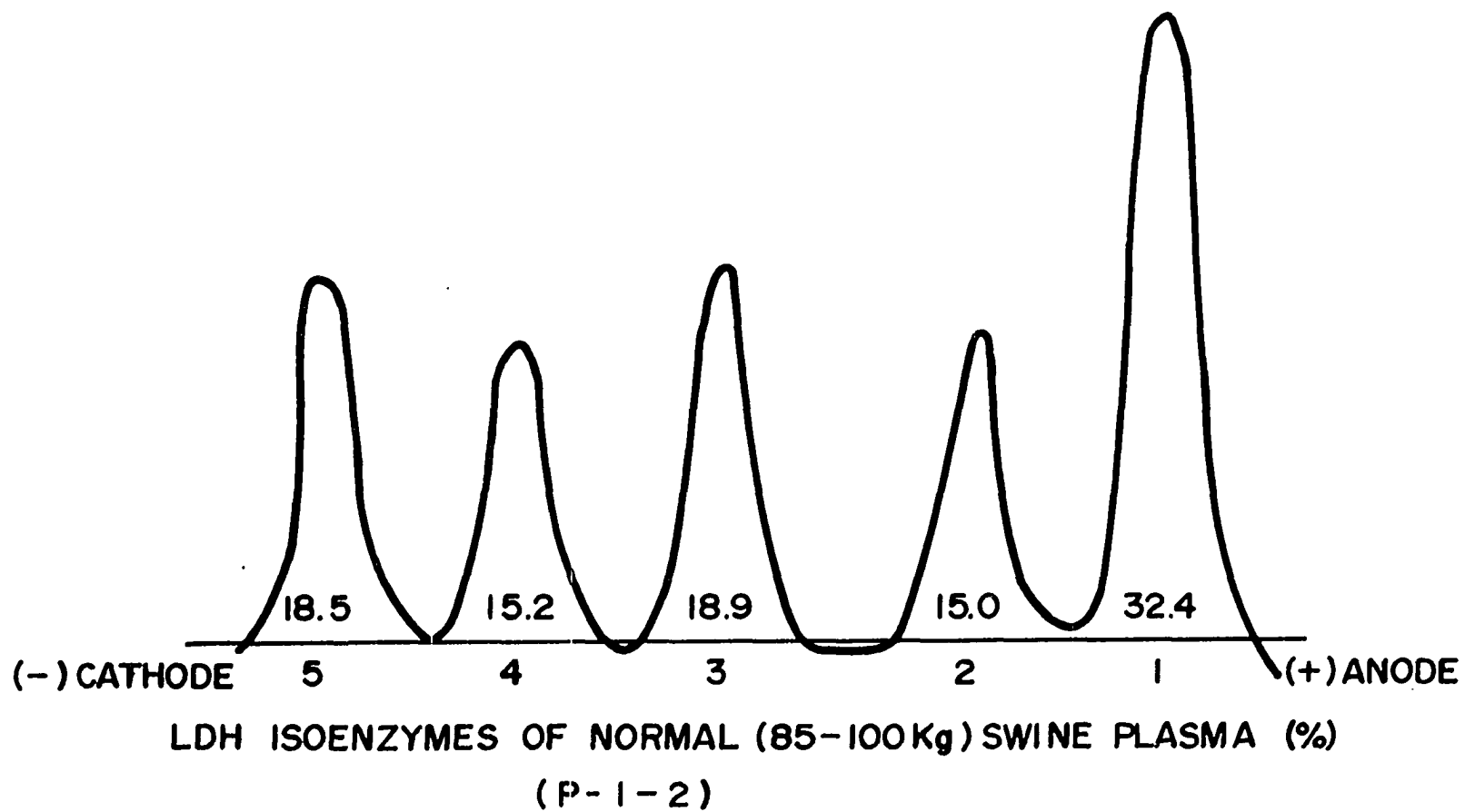
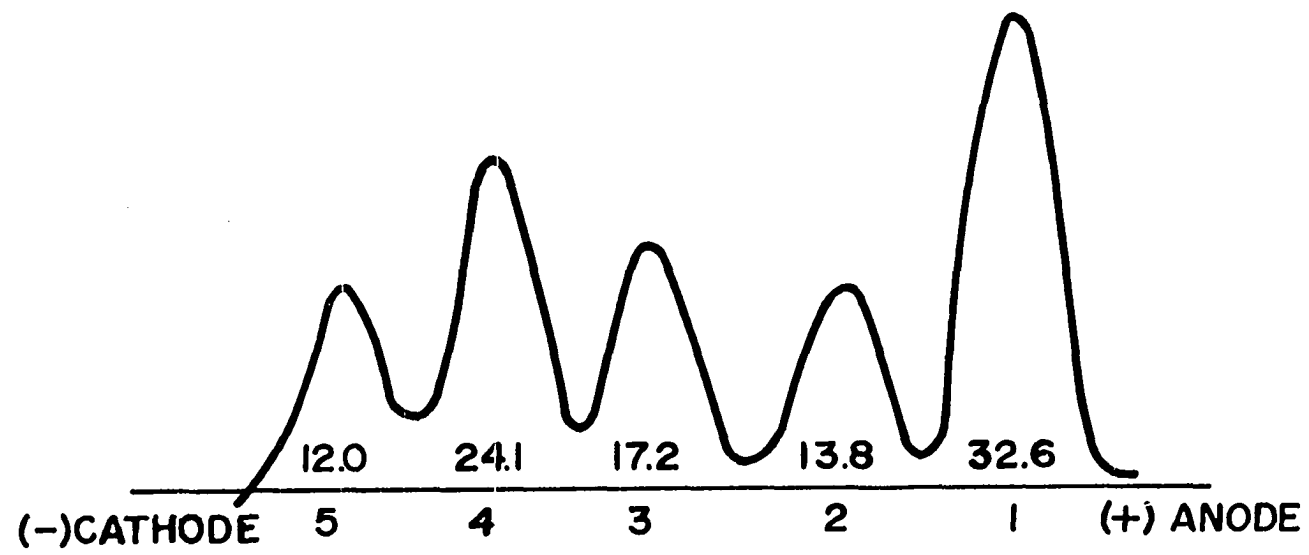


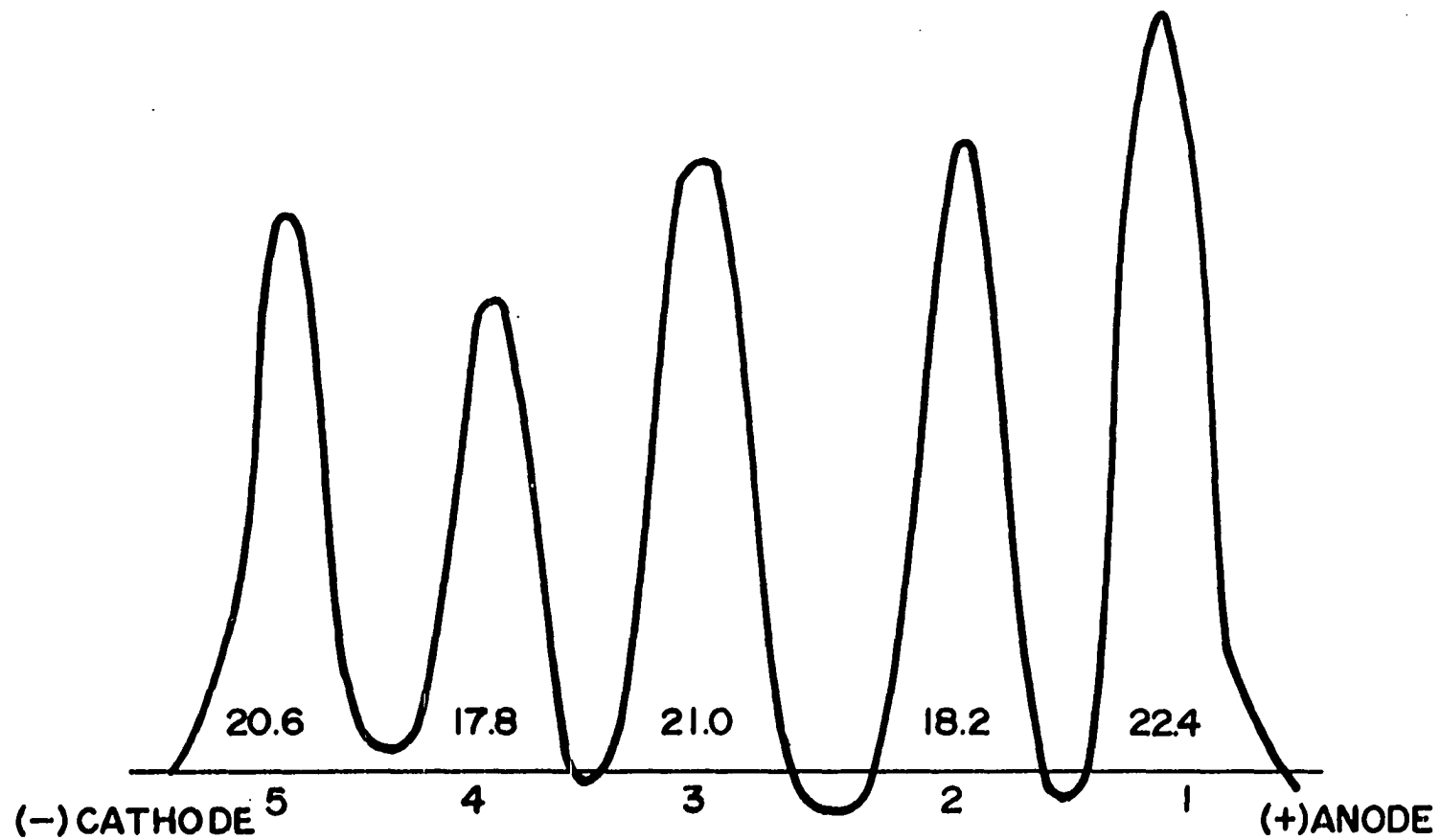
Figure 6. LDH isoenzymes of normal (85-100 kg) swine synovial fluid (%).
Typical isoenzyme distribution in synovial fluid of Slaughter group
swine



LDH ISOENZYMES OF NORMAL (85-100Kg) SWINE SYNOVIAL
FLUID (%)

(P-6-19)

Figure 7. LDH isoenzymes of normal (14-28 kg) swine plasma (%). Typical isoenzyme distribution in plasma of Control group swine



LDH ISOENZYMES OF NORMAL (14-28 Kg) SWINE PLASMA (%)
(5070 G)

Figure 8. LDH isoenzymes of normal (14-28 kg) swine synovial fluid (%). Typical isoenzyme distribution in synovial fluid of Control group swine

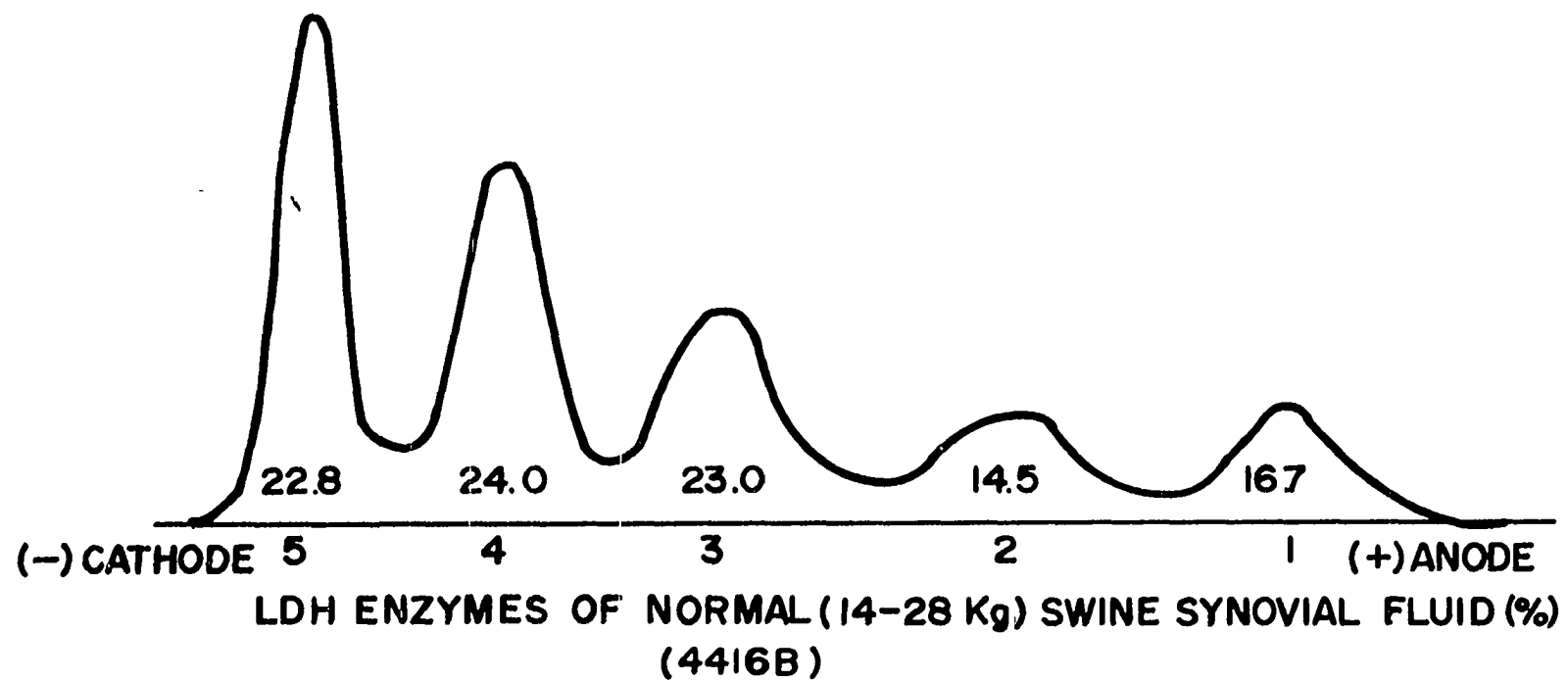


Figure 9. LDH isoenzymes of Infected swine plasma (%). Typical isoenzyme distribution in plasma of M. hyorhinis infected swine

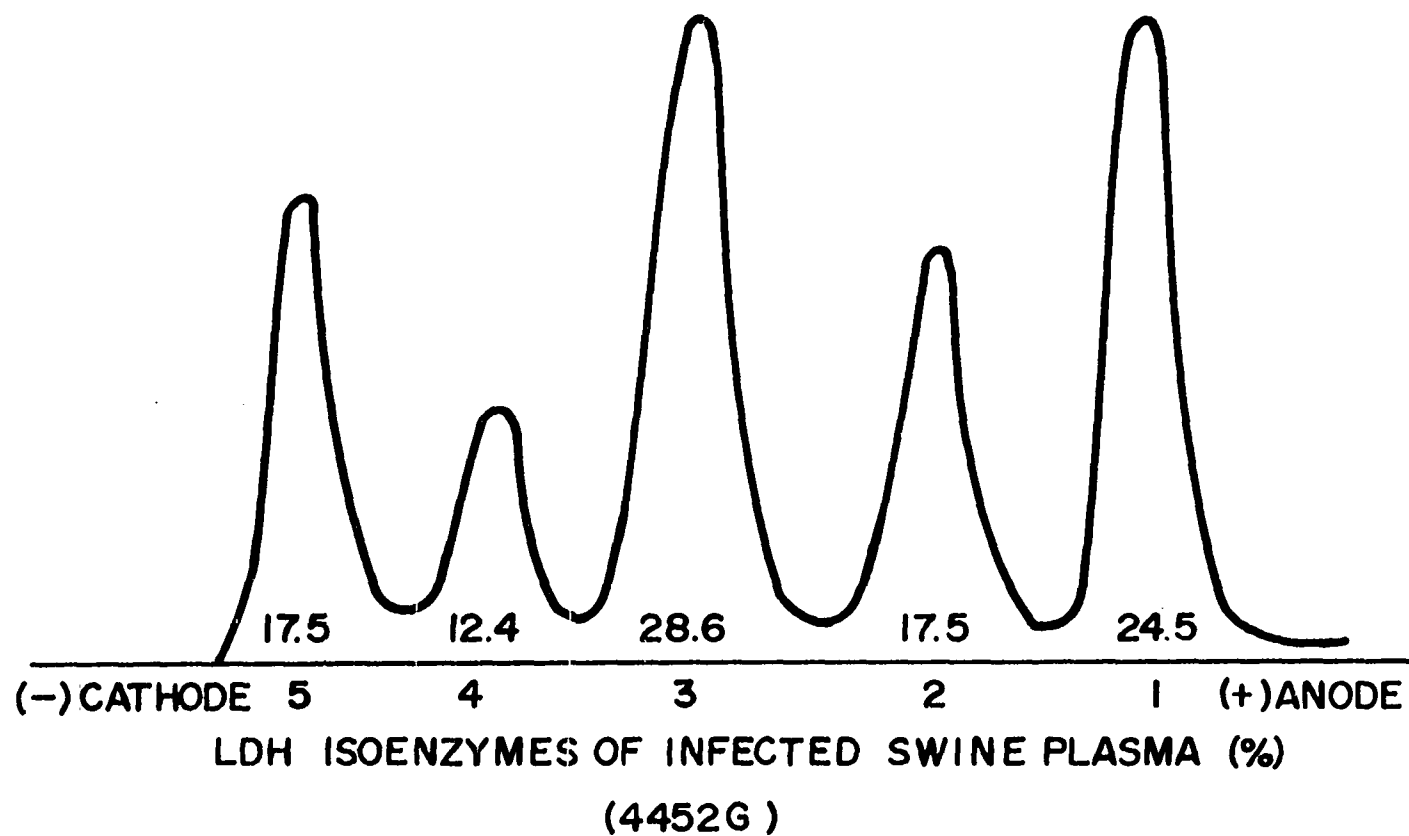


Figure 10. LDH isoenzymes of Infected swine synovial fluid (%). Typical isoenzyme distribution in synovial fluid of M. hyorhinis infected swine

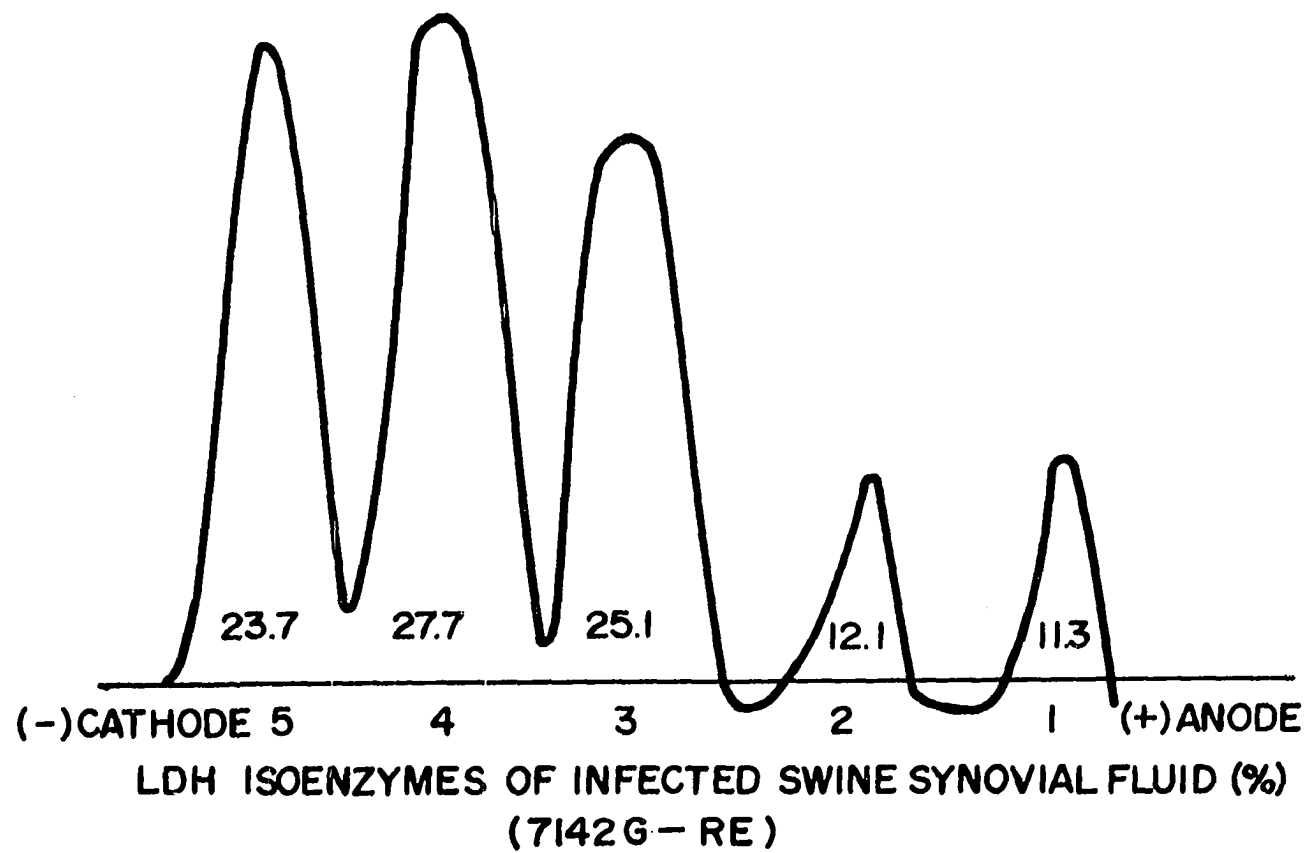


Table 2. Mean distribution (%) of swine plasma and synovial fluid LDH isoenzymes

	Animal group	Animal weight (kg)	Isoenzymes	
			1	2
Plasma				
Code no. ^a				
1	Slaughter	85-100	26.2	15.4
2	Control	14-28	30.6	17.5
3	Infected	14-28	22.4	17.5
Synovial fluid				
4	Slaughter	85-100	26.3	14.6
5	Control	14-28	19.6	15.9
6	Infected	14-28	10.8	13.4

^aCode no. used for "Coded Statistical Comparisons" (Student's "t" test).

Isoenzymes			Standard error	Coded ^a statistical comparisons	Result of comparisons
3	4	5			
19.7	18.7	19.9	± 1.11	1 vs 2 1 vs 4	See Fig. 7 See Fig. 8
16.9	14.3	20.7	± 2.36	2 vs 3 2 vs 5	See Fig. 9 See Fig. 10
25.6	16.6	17.9	± 1.28	3 vs 6	See Fig. 11
17.7	22.8	18.5	± 1.11	4 vs 5	See Fig. 12
20.2	24.6	19.6	± 2.36	5 vs 6	See Fig. 13
25.8	26.9	22.7	± 1.28		

Figure 11. Intergroup plasma mean interactions. The effect of weight on plasma LDH isoenzyme distribution

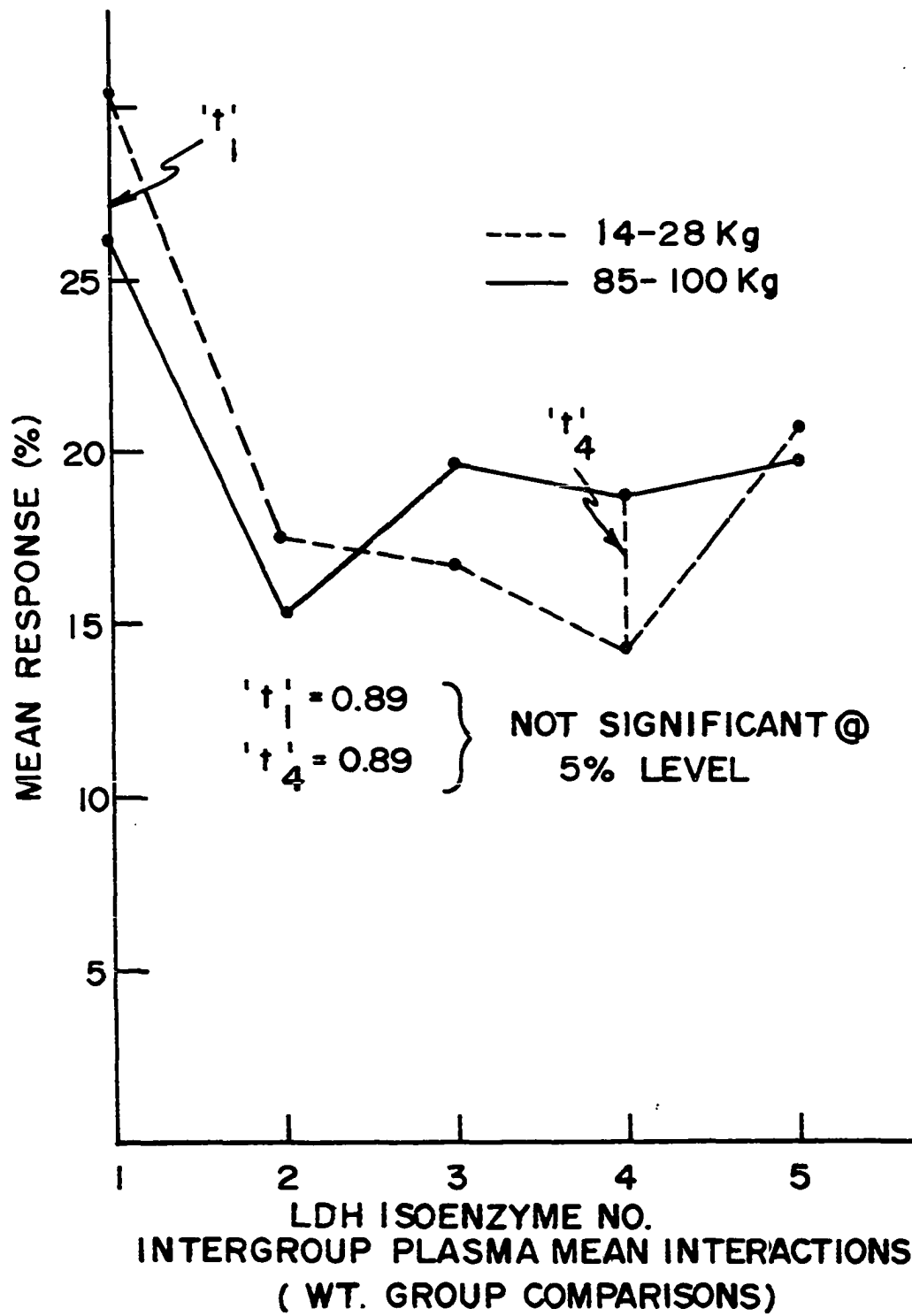
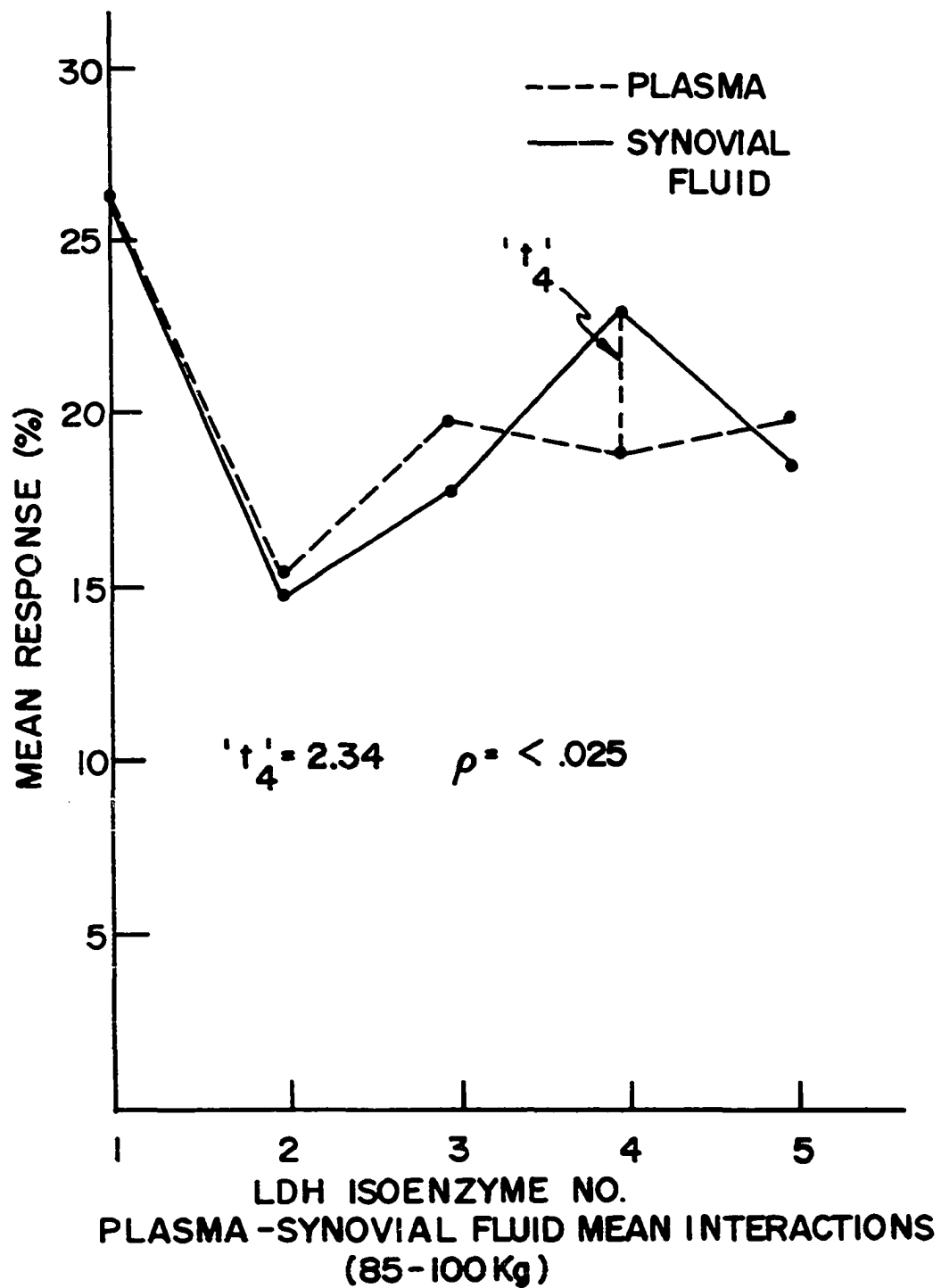


Figure 12. Plasma-synovial fluid mean interactions.
Comparison of fluids from Slaughter group
swine



compared with that of the M. hyorhinis infected (14-28 kg) group (Figure 13). Isoenzymes 1 and 5 in the Infected group were present in lower levels than those of the Control group, whereas isoenzymes 3 and 4 levels were higher. Isoenzyme 3 of the Infected group was greatly increased over that observed in the Control group. Infection appears to have produced a significant drop in isoenzyme 1 and a relatively pronounced elevation of number 3.

Comparisons of plasma and synovial fluid of the Control group (Figure 14), revealed that isoenzymes 1, 2 and 5 were in higher levels in plasma than in synovial fluid. Synovial fluid on the other hand, contained higher levels of isoenzymes 3 and 4. Statistically significant mean differences were obtained only with isoenzymes 1 and 4. Isoenzyme 4 of synovial fluid again as in the previously described Slaughter group, exceeded plasma levels.

The next comparison made was Infected group plasma versus synovial fluid (Figure 15). Isoenzyme distribution in the individual fluids of this group as in the Control group, were dissimilar. Isoenzymes 1 and 2 were considerably higher in plasma than in synovial fluid and levels of isoenzymes 4 and 5 were much greater in synovial fluid than they were in plasma. The plasma-synovial fluid relationship however, was generally similar to that observed in the Control group (Figure 14) with the exception of isoenzymes 2 and 5.

Figure 13. Control versus Infected plasma mean interactions.
The effect of M. hyorhinis infection on plasma
LDH isoenzyme distribution

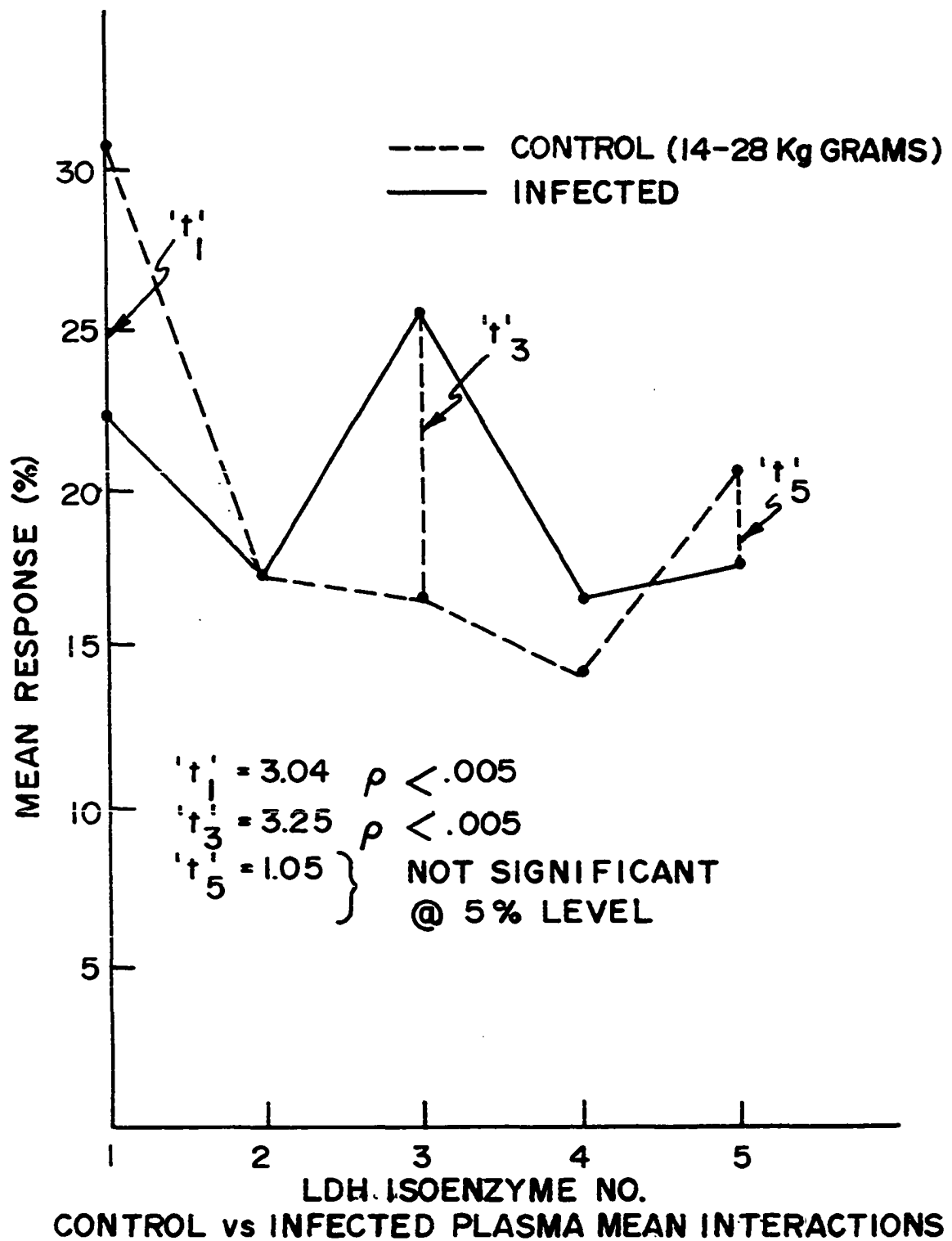


Figure 14. Plasma-synovial fluid mean interactions.
Comparison of fluids in Control group swine

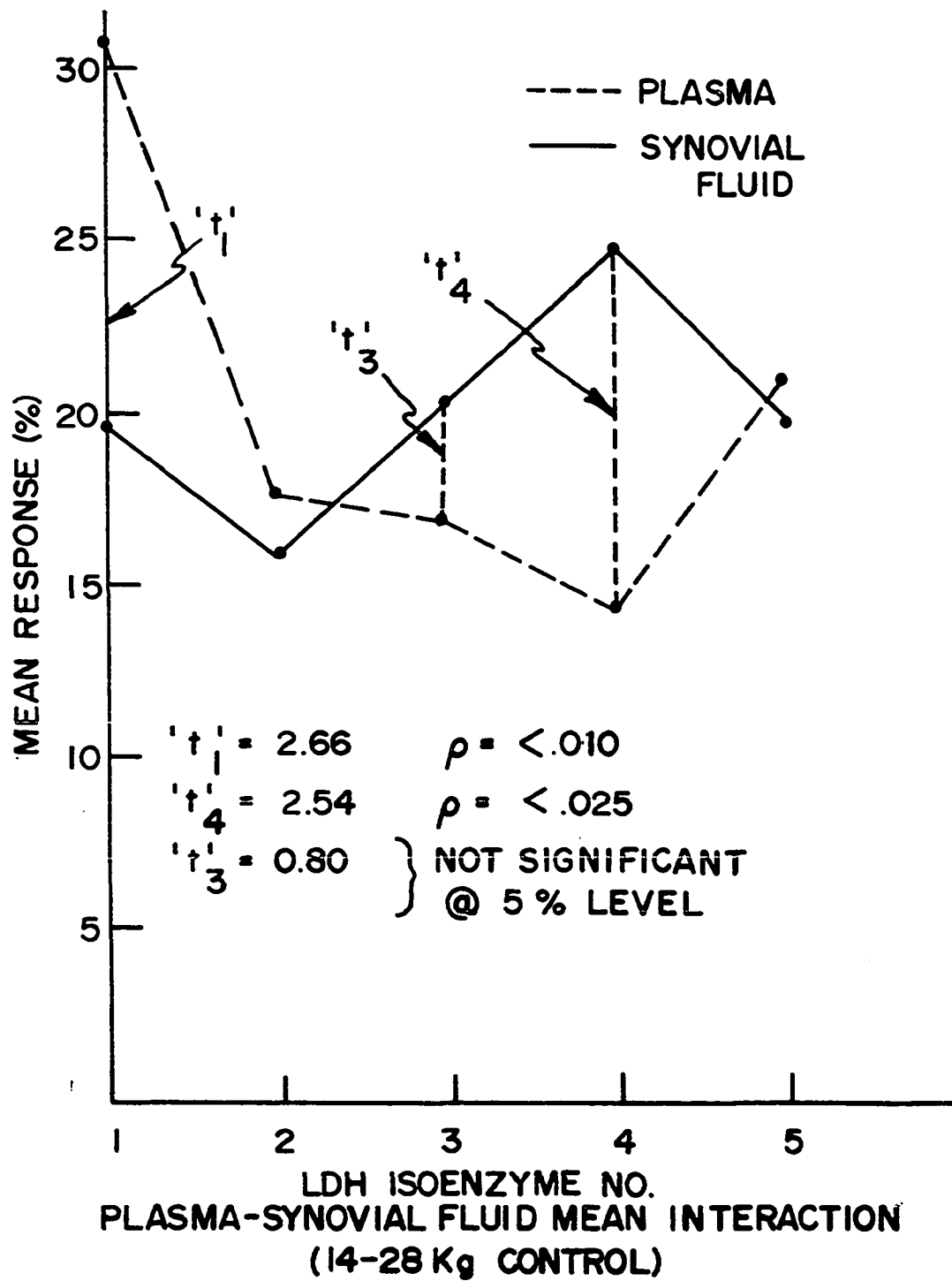
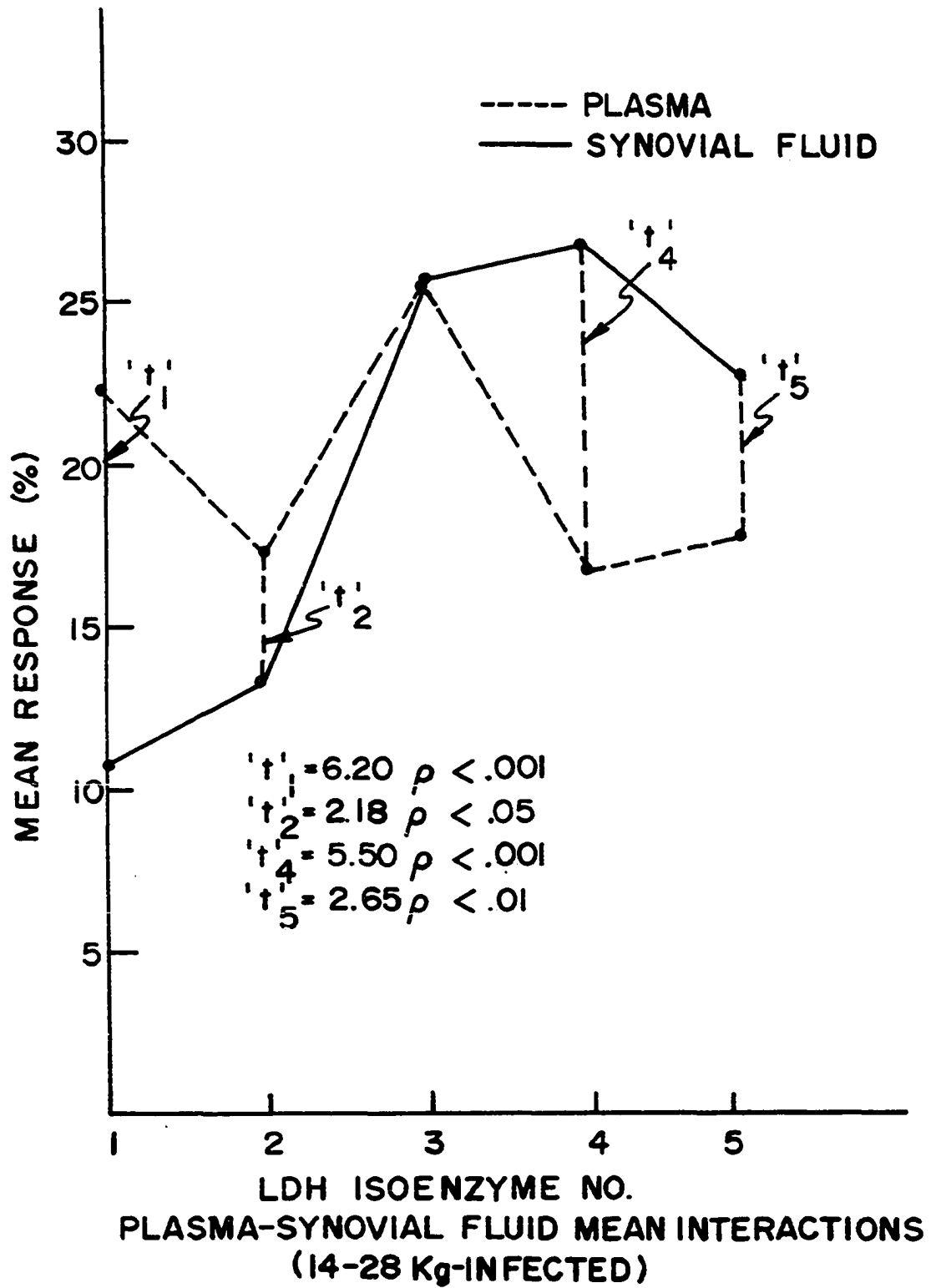


Figure 15. Plasma-synovial fluid mean interactions.
Comparison of fluids in M. hyorhinis infected
swine



In the Infected group, the mean differences for enzymes 2 and 5 of the respective body fluids were considerably larger than the mean differences observed in the Control group. During infection, isoenzyme distribution favored a decrease in synovial fluid isoenzyme 2 and an increase in isoenzyme 5 relative to plasma levels.

Synovial fluid comparisons between the various groups were also made with respect to isoenzyme distribution. The Slaughter-Control comparison (Figure 16) for the effect of weight on isoenzyme distribution showed Control fluid to be higher in all isoenzymes except number 1. Although differences between the two groups were observed, these differences were not supported by statistical significance. From this it appears there was no appreciable weight effect on isoenzyme distribution in the synovial fluid of these two groups.

When Control and Infected synovial fluids were compared (Figure 17), levels of isoenzymes 1 and 2 of Infected synovial fluid were found to be lower than those of Control fluid. Levels of isoenzymes 3, 4 and 5 in infected synovial fluid were higher than in control fluid. Group mean differences were statistically significant only in isoenzymes 1 and 3. The effect of infection was to lower levels of isoenzyme 1 and elevate isoenzyme 3 in synovial fluid.

Figure 16. Intergroup synovial fluid mean interactions.
The effect of weight on synovial fluid LDH
isoenzyme distribution

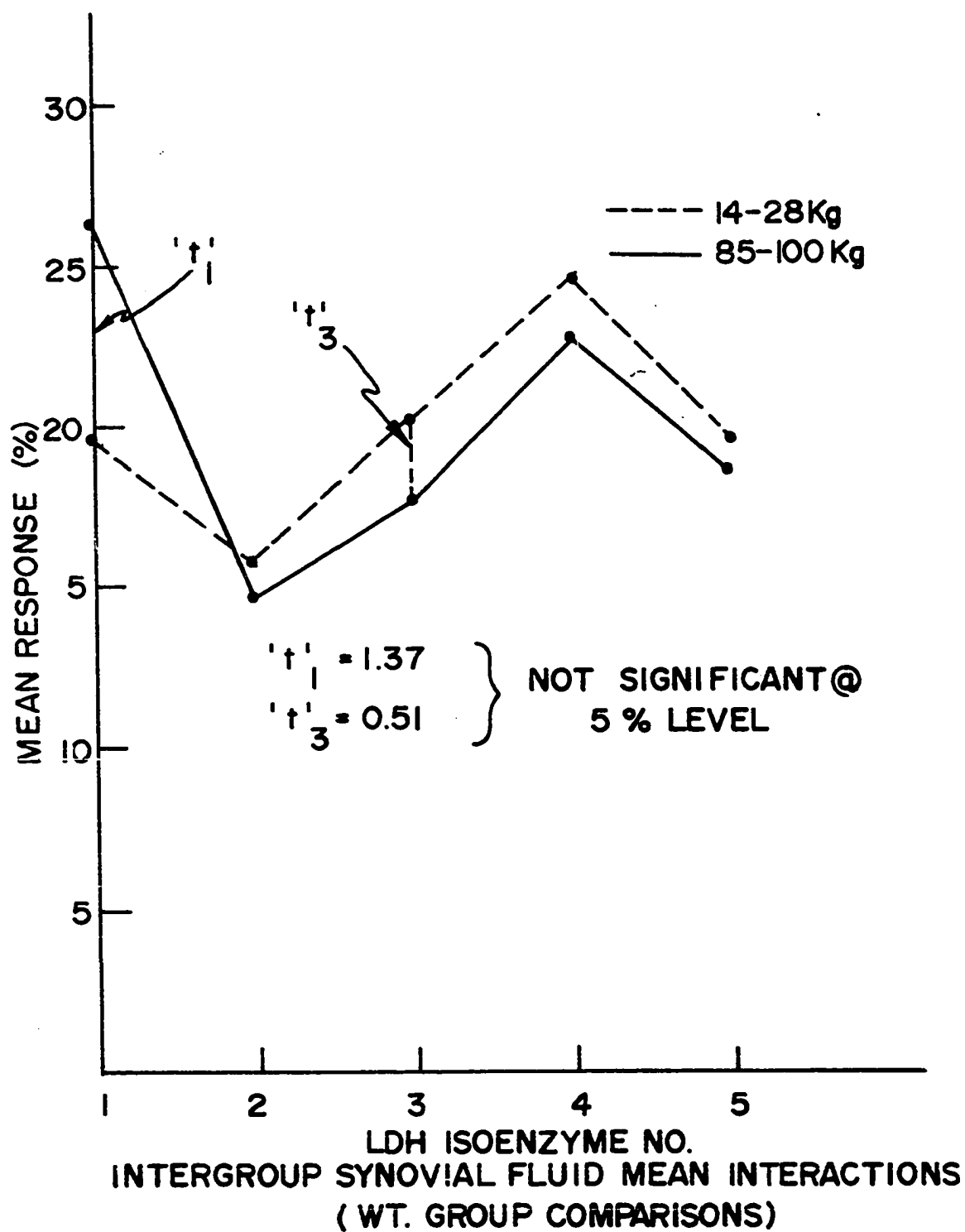
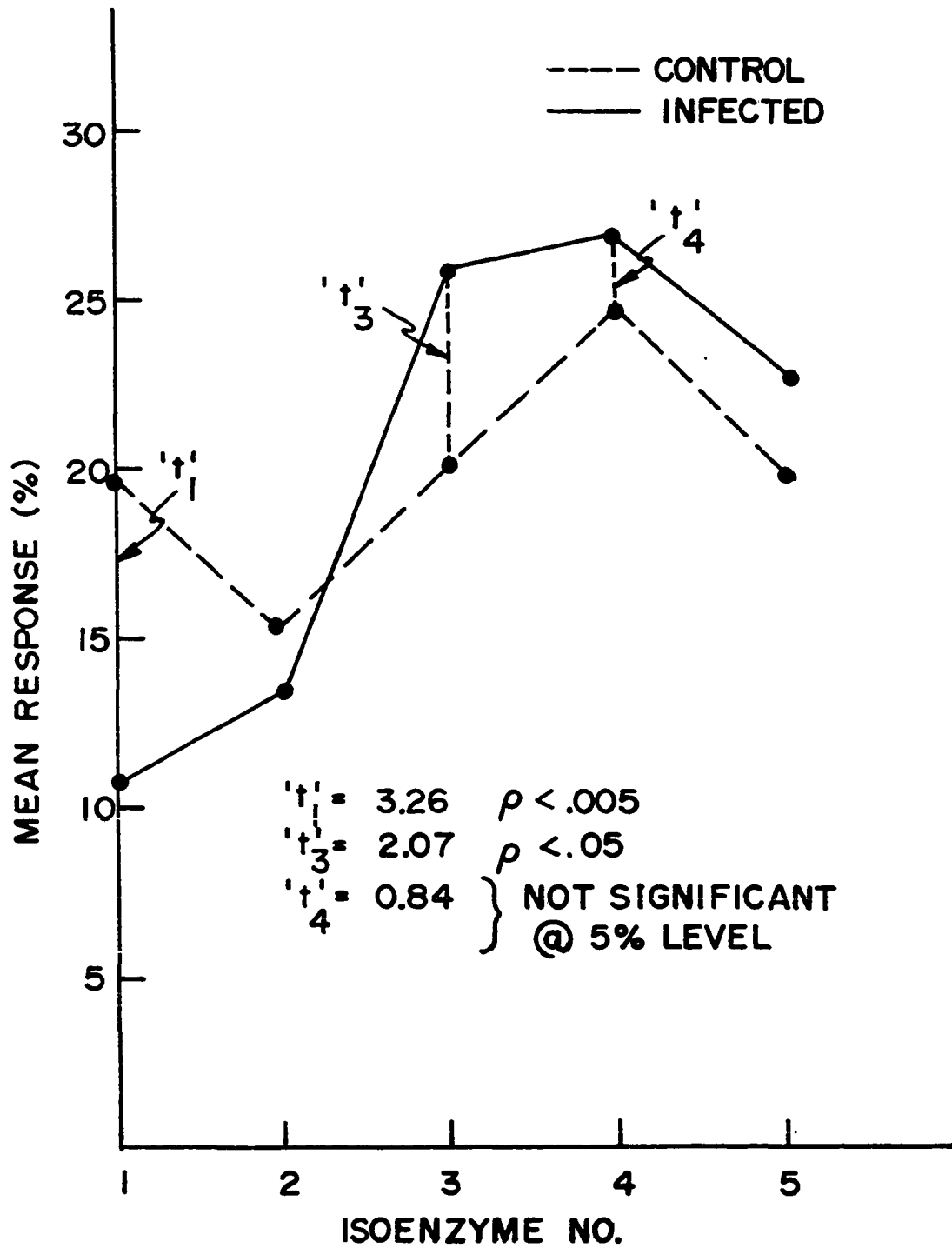


Figure 17. Control versus Infected synovial fluid mean interaction. The effect of M. hyorhinis infection on LDH isoenzyme distribution in synovial fluid



CONTROL vs. INFECTED SYNOVIAL FLUID MEAN INTERACTION

Total protein

Total serum and synovial fluid protein content of the three groups were compared (Table 3). Slaughter serum had the highest protein content followed closely by infected serum. The Slaughter-Control mean comparison revealed a statistically significant difference between the serum means of these two groups. Weight did have an influence on serum protein content. When control sera were compared to infected sera, again a significant difference was found to exist.

Total protein content of synovial fluid was found to be highest in infected synovial fluid, and lowest in control synovial fluid. A statistically significant difference existed between the synovial fluids of these two groups. Weight also had an influence as evidenced by the existence of a statistically significant difference between the synovial fluids of the Slaughter and Control groups.

Protein fraction distribution

Electrophoretic protein fraction patterns such as those reproduced in Figures 18 through 23 were obtained and from them fraction percentages and group means established (Table 4).

Slaughter-Control serum comparisons (Figure 24) for studying weight effects were made. In the Control animals, albumen, α_1 and β globulins were slightly higher than in Slaughter animals. Sera from the Slaughter animals on the

Table 3. Total serum and synovial fluid protein levels
(mean) in swine

	Animal group	Animal weight (kg)	No. Animals	No. observa- tions
Serum				
Code no.				
1	Slaughter	85-100	50	50
2	Control	14-28	10	10
3	Infected	14-28	15	15
Synovial fluid				
4	Slaughter	85-100	50	49
5	Control	14-28	10	5
6	Infected	14-28	15	13

^aCode no. used for "Coded Statistical Comparisons"
(Student's "t" test).

Mean (gm/100 ml)	Standard deviation	Range	Coded ^a statistical comparisons	Result of comparisons
6.6	± 0.54	5.4 - 7.8	1 vs 2	P < .001
4.1	± 0.99	3.0 - 6.4	2 vs 3	P < .05
5.7	± 0.63	4.7 - 6.9		
1.3	± 0.51	0.6 - 3.2	4 vs 5	P < .001
0.5	± 0.23	0.2 - 0.8	5 vs 6	P < .005
2.6	± 1.28	0.8 - 5.0		

Figure 18. Protein fractions of normal (85-100 kg) swine serum (%). Typical protein fraction distribution in serum of Slaughter group swine

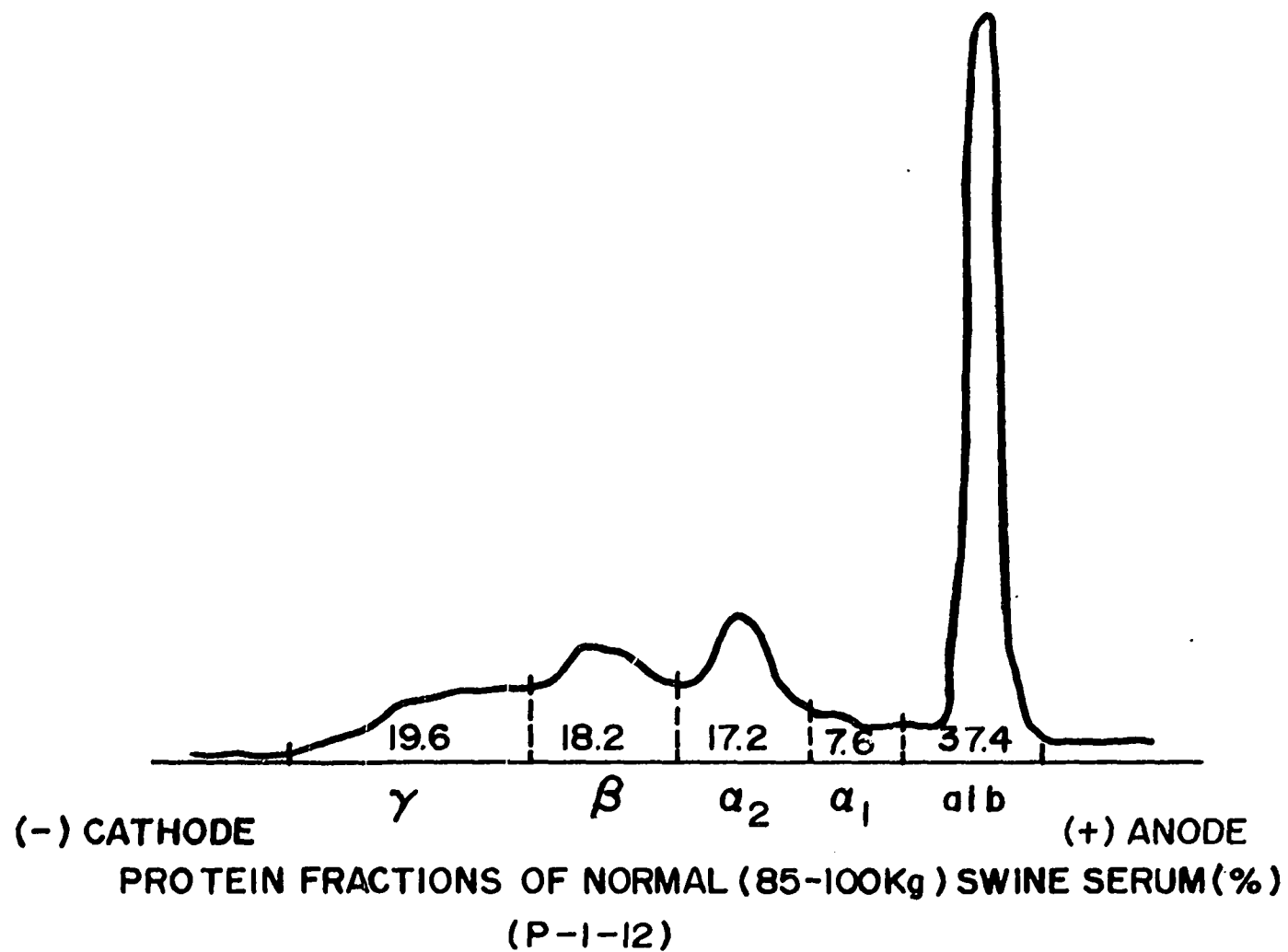
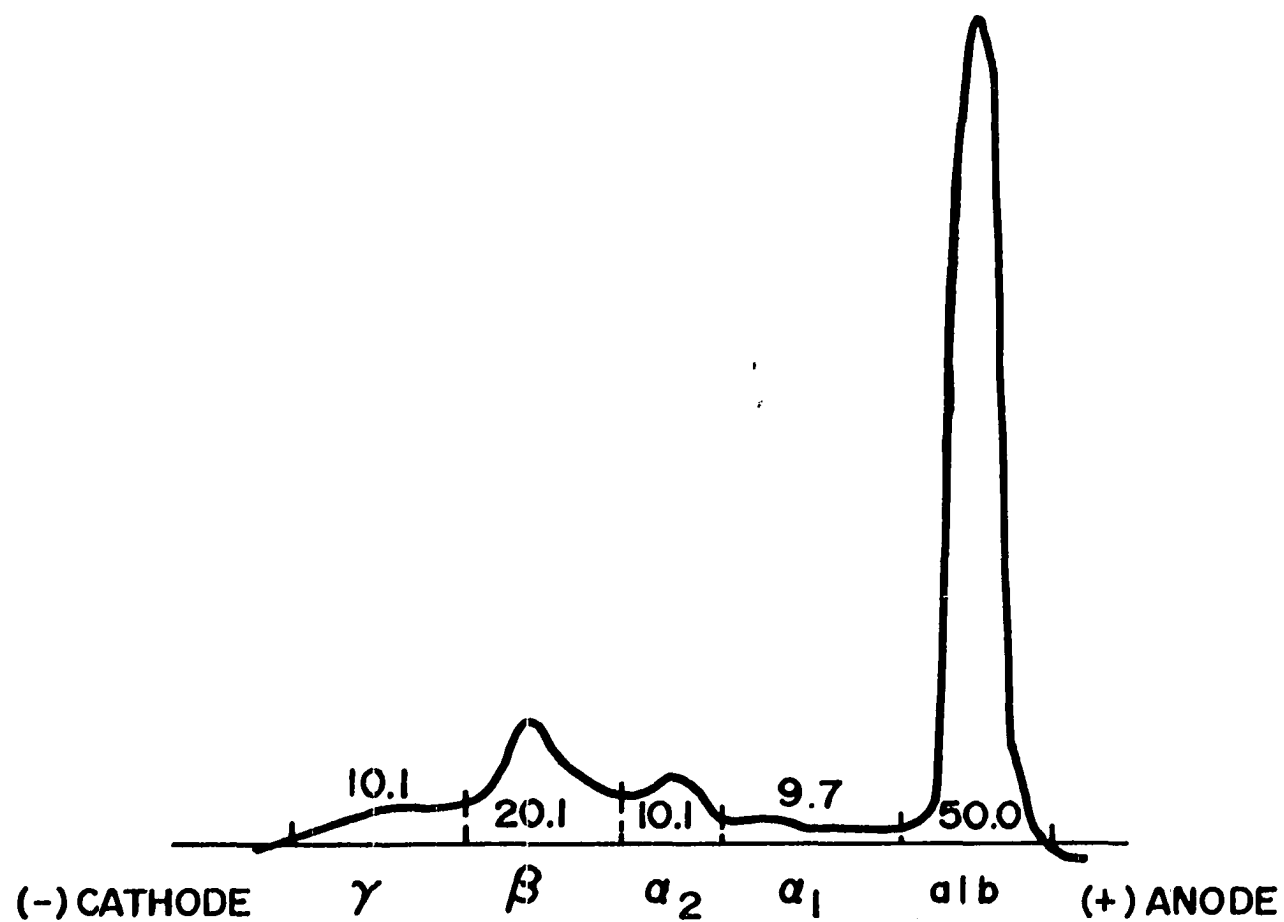


Figure 19. Protein fractions of normal (85-100 kg) swine synovial fluid (%).
Typical protein fraction distribution in synovial fluid of
Slaughter group swine



PROTEIN FRACTIONS OF NORMAL (85-100 Kg) SWINE SYNOVIAL
FLUID (%)

(P-1-4)

Figure 20. Protein fractions of normal (14-28 kg) swine serum (%). Typical protein fraction distribution in serum of Control group swine

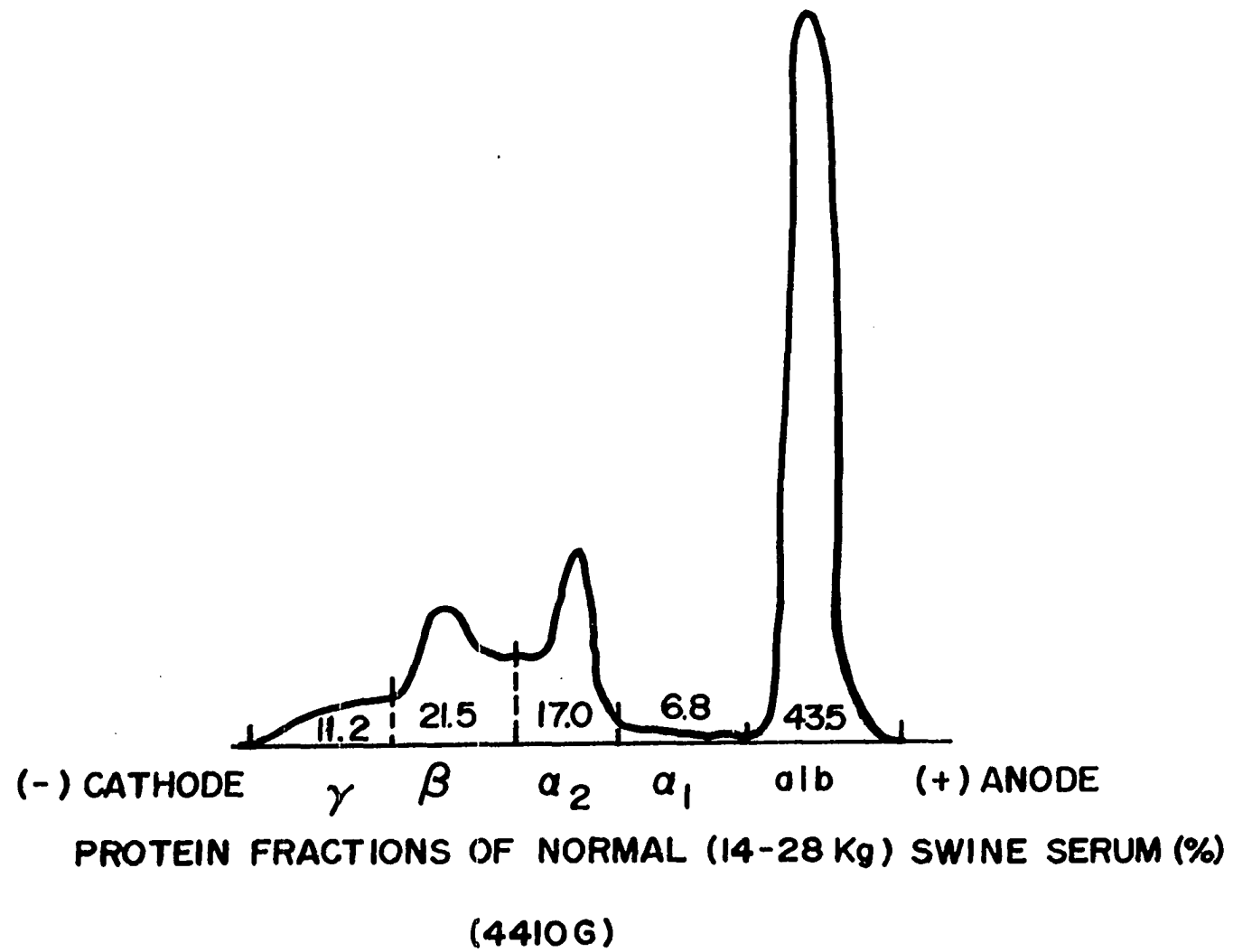
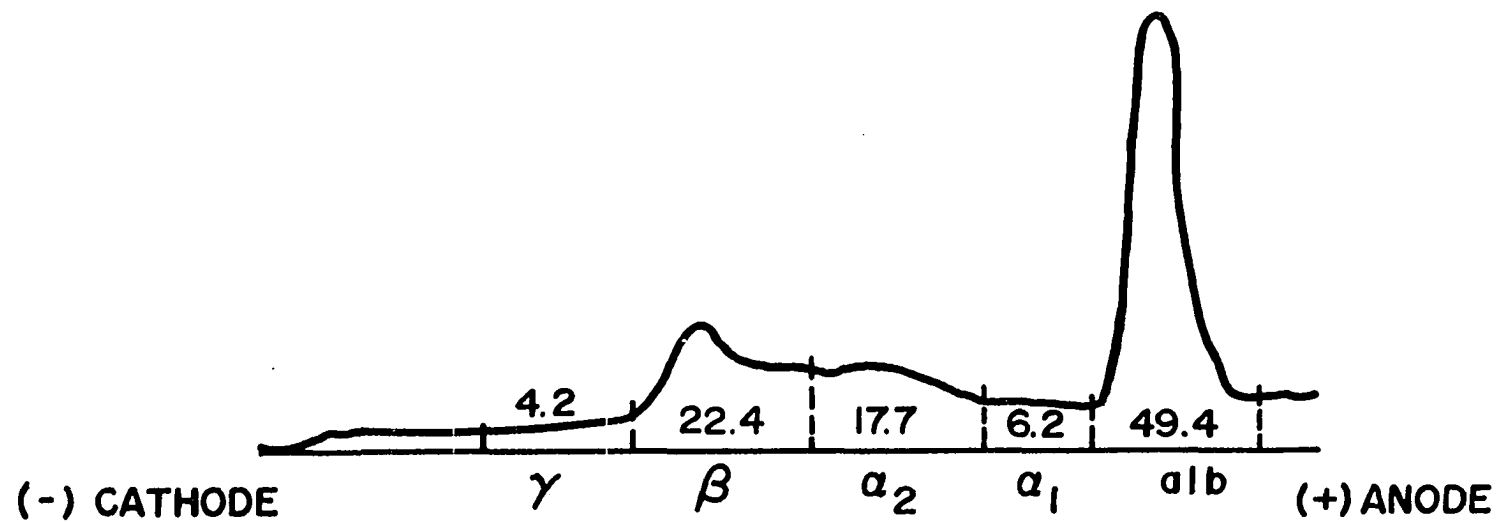


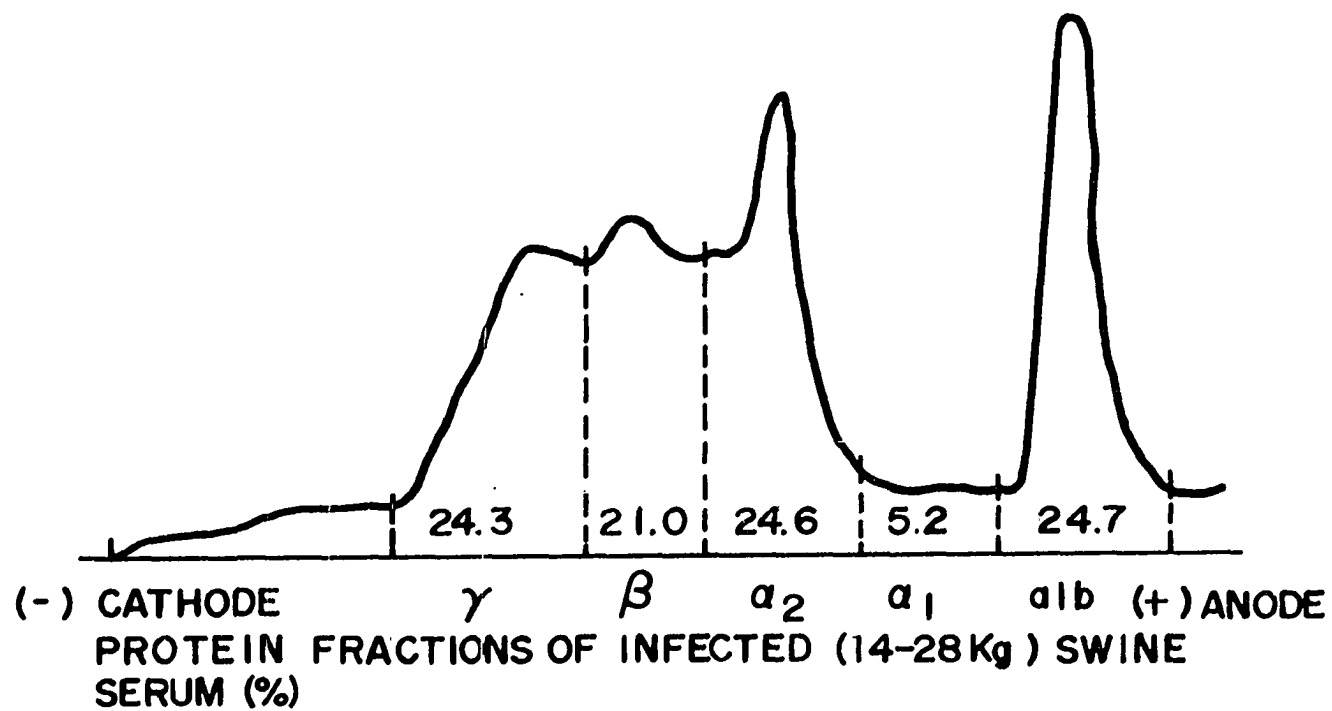
Figure 21. Protein fractions of normal (14-28 kg) swine synovial fluid (%).
Typical protein fraction distribution in synovial fluid of Control
group swine



PROTEIN FRACTIONS OF NORMAL (14-28 Kg) SWINE SYNOVIAL
FLUID (%)

(7140G-LE)

Figure 22. Protein fractions of Infected (14-28 kg) swine serum (%). Typical protein fraction distribution in serum of M. hyorhinis infected swine



(7144A)

Figure 23. Protein fractions of Infected (14-28 kg) swine synovial fluid.
Typical protein fraction distribution in synovial fluid of M.
hyorhinis infected swine

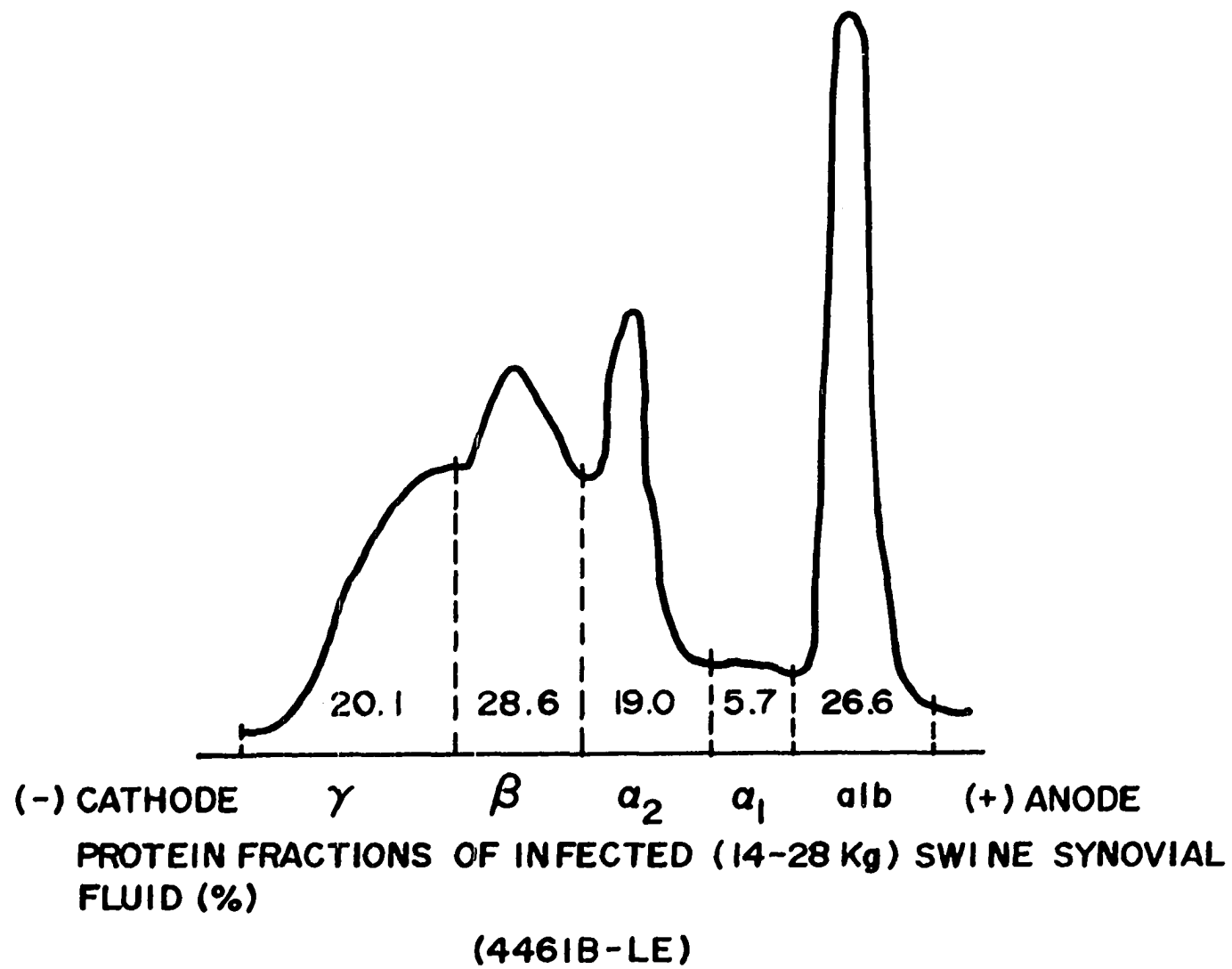


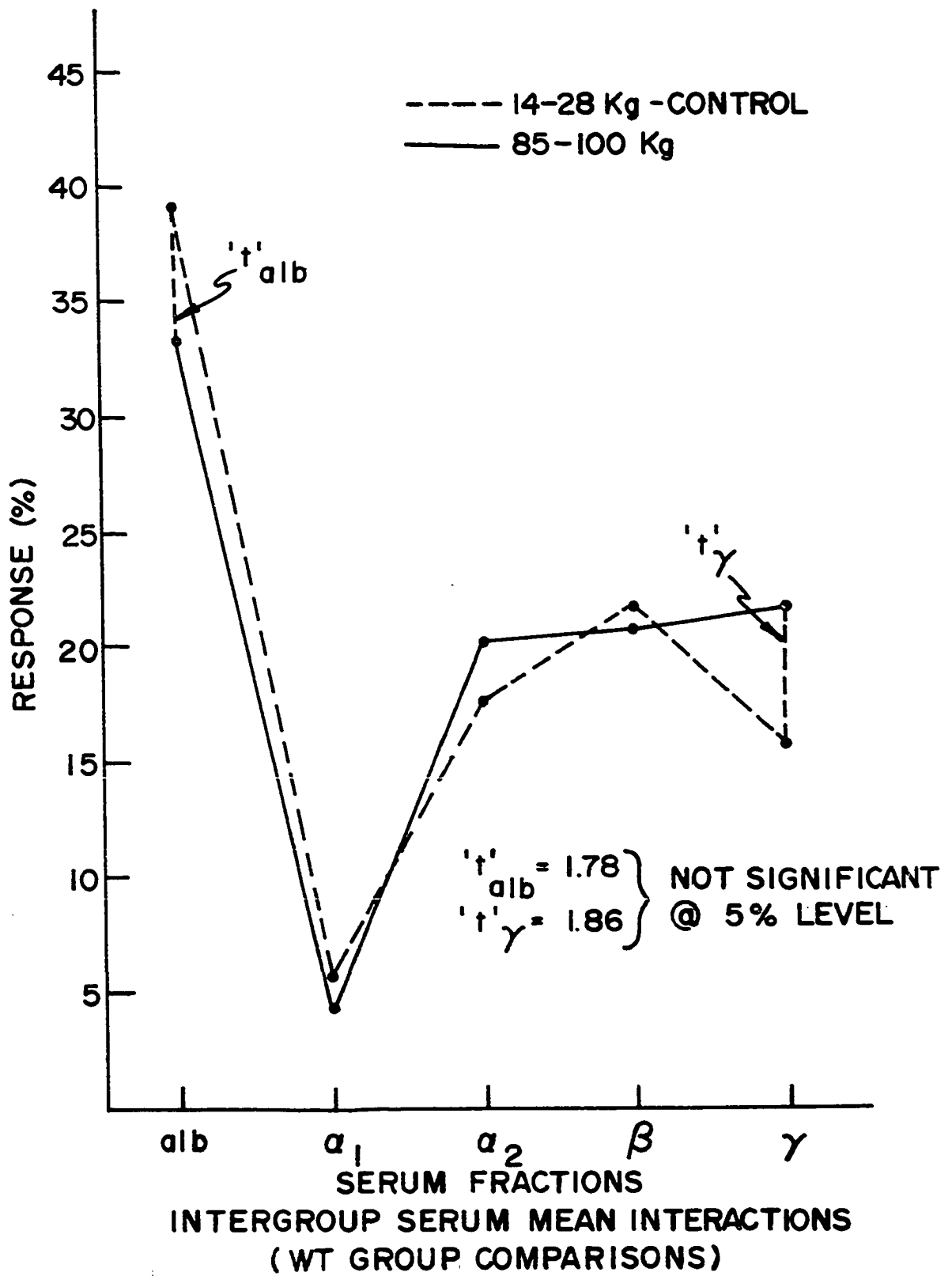
Table 4. Mean distribution (%) of swine serum and synovial fluid protein fractions

	Animal group	Animal weight (kg)	Albumin	Fractions Globulins α_1	α_2
Serum					
Code no.					
1	Slaughter	85-100	33.3	4.1	20.3
2	Control	14-28	39.0	5.7	17.7
3	Infected	14-28	29.4	5.3	21.7
Synovial fluid					
4	Slaughter	85-100	44.2	6.4	13.5
5	Control	14-28	41.6	6.9	19.0
6	Infected	14-28	30.6	4.7	18.1

^aCode no. used for "Coded Statistical Comparisons" (Student's "t" test).

B	γ	Standard error	A/G ratio	Coded ^a statistical comparisons	Result of comparisons
20.7	21.7	± 1.00	.50	1 vs 2 1 vs 4	See Fig. 20 See Fig. 21
21.9	15.6	± 1.82	.67	2 vs 3 2 vs 5	See Fig. 22 See Fig. 23
21.7	22.1	± 3.25	.41	3 vs 6	See Fig. 24
20.3	15.7	± 1.0	.70	4 vs 5	See Fig. 25
20.9	11.4	± 1.82	.72	5 vs 6	See Fig. 26
27.9	18.4	± 3.25	.44		

Figure 24. Intergroup serum mean interactions. The effect of weight on serum protein fraction distribution



other hand, were higher in α_2 and γ globulins. Although statistical significance was not achieved in comparing the group means, 't' values for albumen and γ globulin approached the level of significance. It appears that the principal weight effects were higher levels of albumen in the Slaughter group.

When serum was compared to synovial fluid in the Slaughter group (Figure 25) synovial fluid was higher in albumen and α_1 globulin than serum but lower in α_2 and γ globulins. Beta globulin levels appeared to be the same in both fluids. The mean differences of these fractions attained statistical significance. In characterizing the serum-synovial fluid relationship in the Slaughter group it was found that synovial fluid differs from serum in its higher albumen content and lower levels of α_2 and γ globulins.

The effect of infection on serum protein was observed by comparing control and infected sera (Figure 26). Albumen levels decreased and α_2 and γ globulin levels increased as a result of the infection. Levels of α_1 and β globulins did not change.

Serum-synovial fluid comparisons in the Control animals (Figure 27) had a greater fluid similarity than did the Slaughter group. Albumen α_1 and α_2 globulin serum means were only slightly lower than those of synovial fluid. Synovial fluid β and γ globulin means were slightly lower

Figure 25. Serum-synovial fluid protein mean interactions.
Comparison of fluids in Slaughter group swine

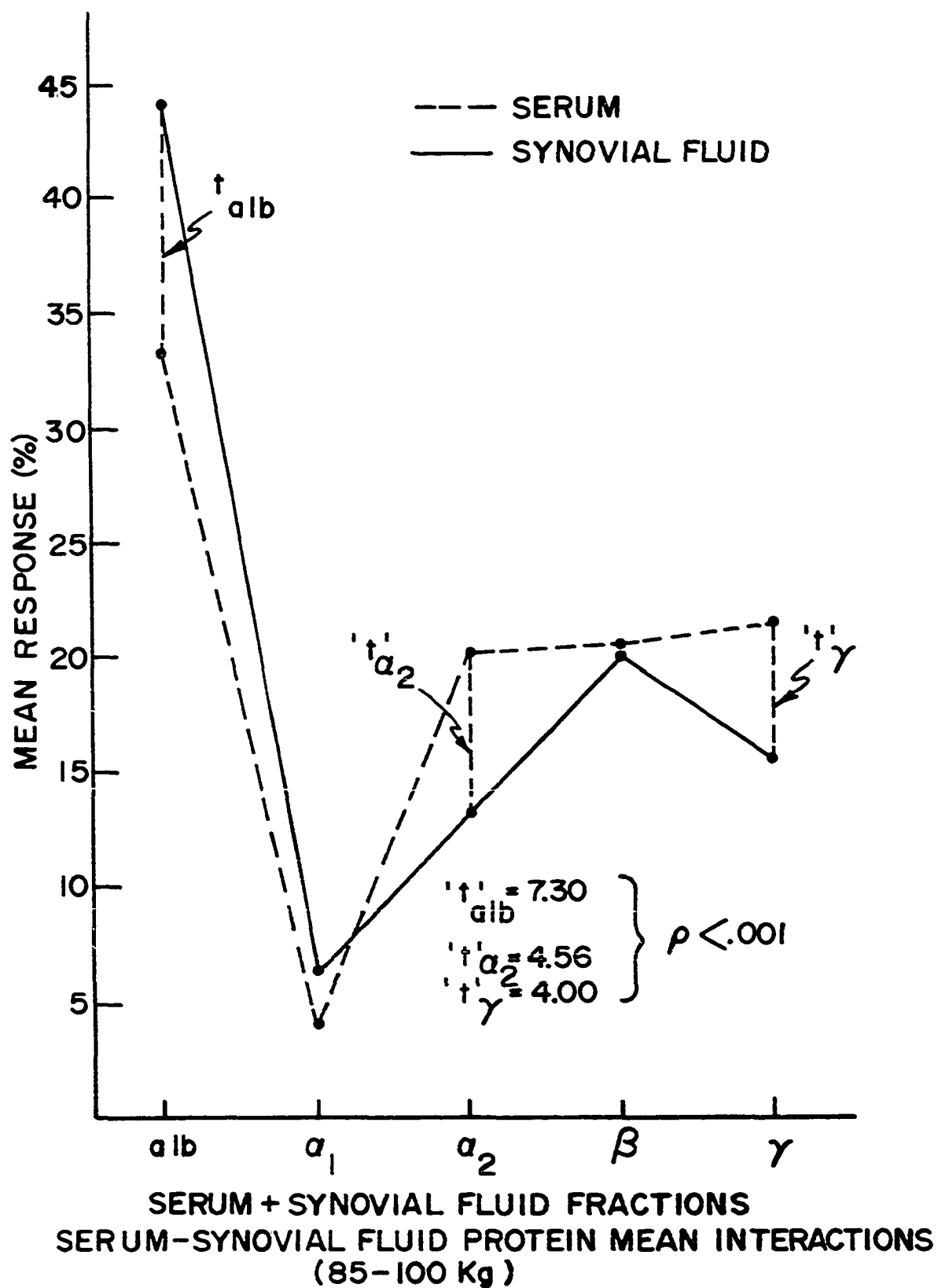


Figure 26. Control versus Infected serum protein mean interactions. The effect of M. hyorhinis on serum protein fraction distribution

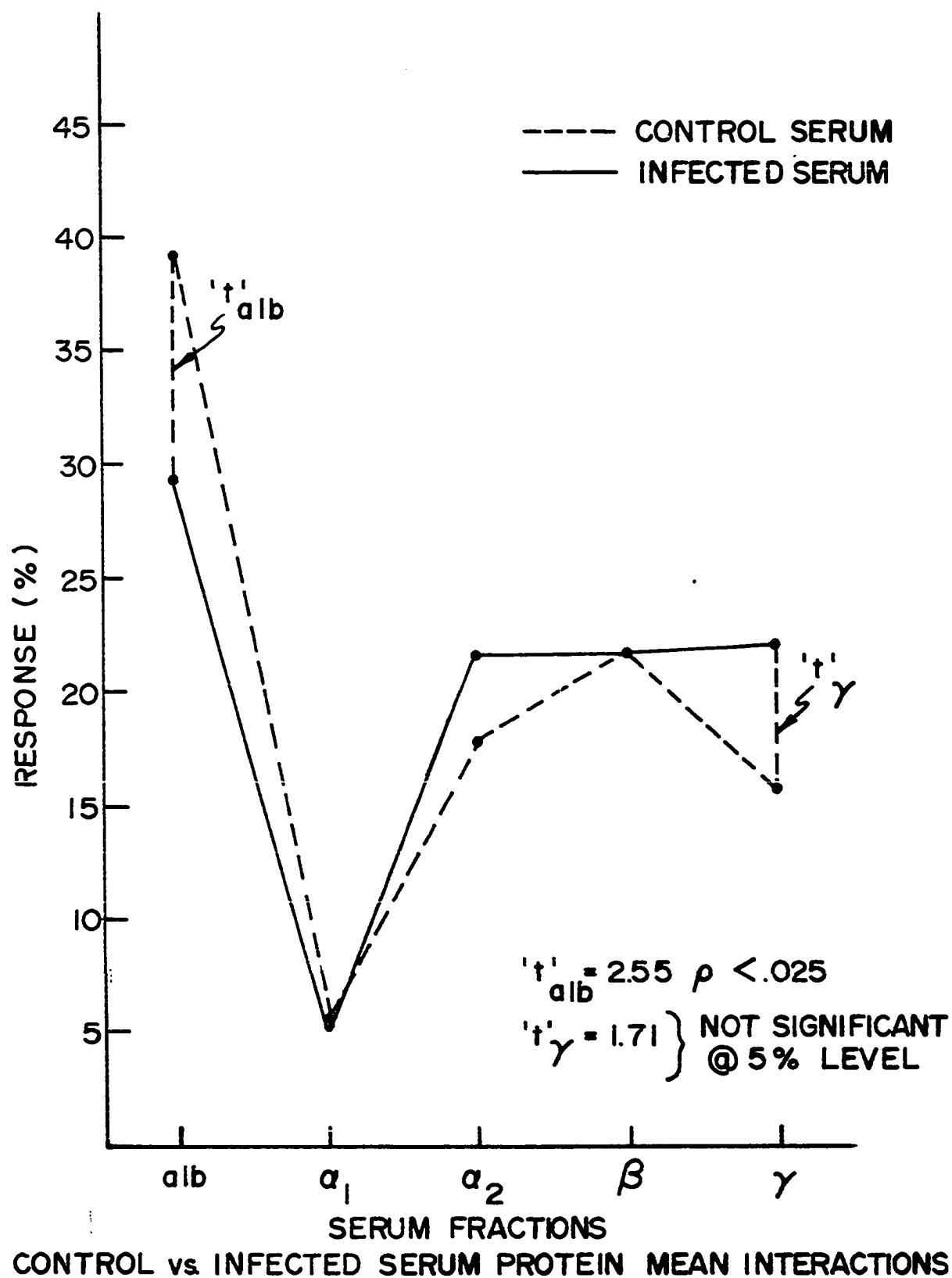
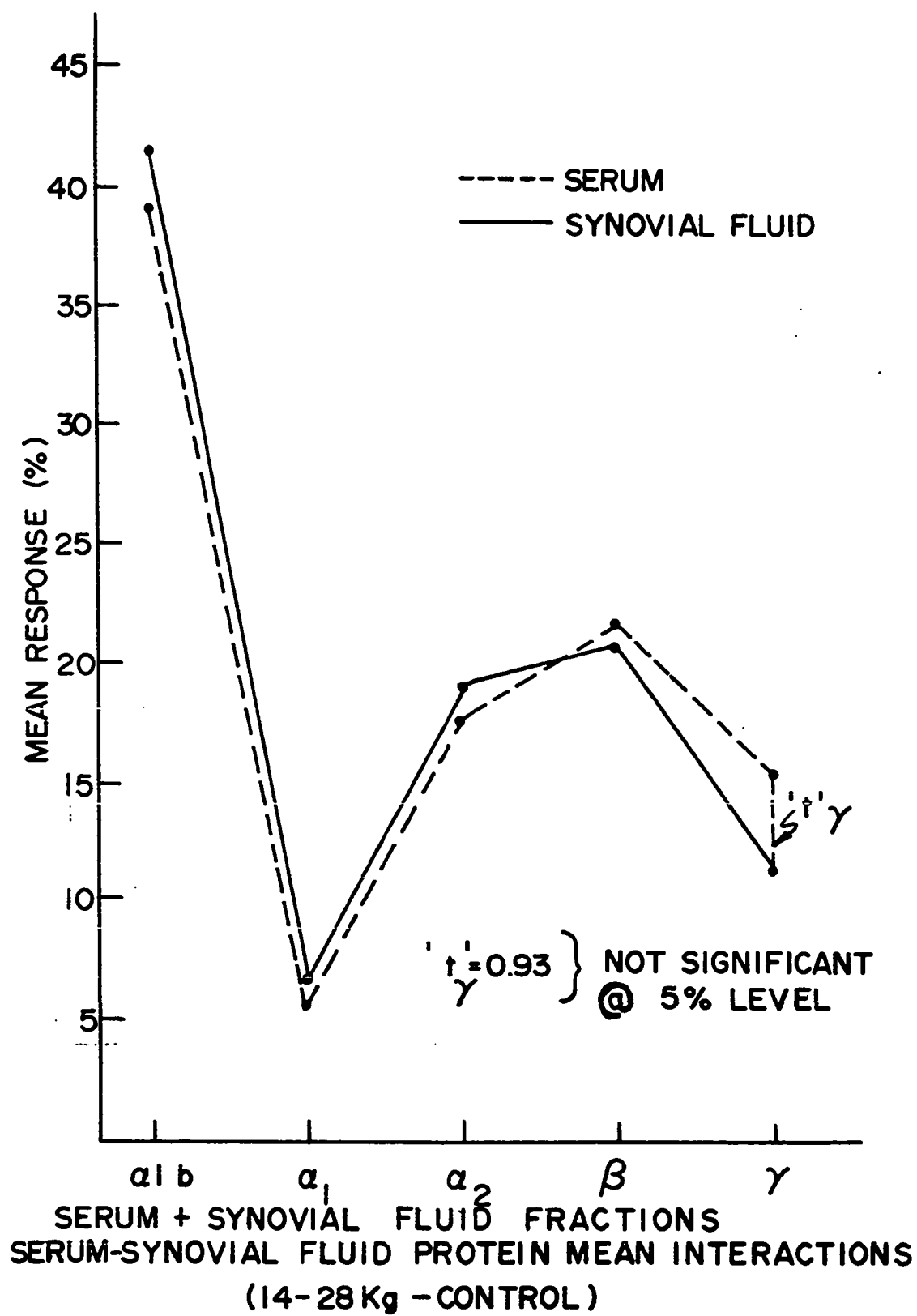


Figure 27. Serum-synovial fluid protein mean interactions.
Comparison of fluids in Control group swine



than those of serum. Statistical significance of these observed mean differences was not achieved. It appears that major fraction differences between the two fluids of this group did not exist.

A comparison of serum and synovial fluid from the Infected group (Figure 28) yielded results slightly different from those obtained with the Control group. Albumen and α_1 globulin of the infected fluids were essentially the same. Alpha-two globulin of serum was higher than that of synovial fluid. Beta globulin on the other hand was considerably higher in synovial fluid than in serum. Gamma globulin levels of serum were higher than those found in synovial fluid. Infection appears to have altered β and γ globulin distribution in the fluids. Synovial fluid β globulin and serum γ globulin levels were elevated as a result of infection.

Weight effects on synovial fluid were studied by comparing Slaughter and Control synovial fluids (Figure 29). Alpha-two globulin was higher in Control synovial fluid than in Slaughter fluid. Albumen and γ globulin of Slaughter synovial fluid however, was higher than that observed in the Control fluid. No apparent difference existed in β globulin levels. Statistical significance of mean differences was not achieved. The principal weight effects on synovial fluid were a reduction in α_2 globulin with increased weight and an increase in γ globulins.

Figure 28. Serum-synovial fluid protein mean interactions.
Comparison of fluids in M. hyorhinis infected
swine

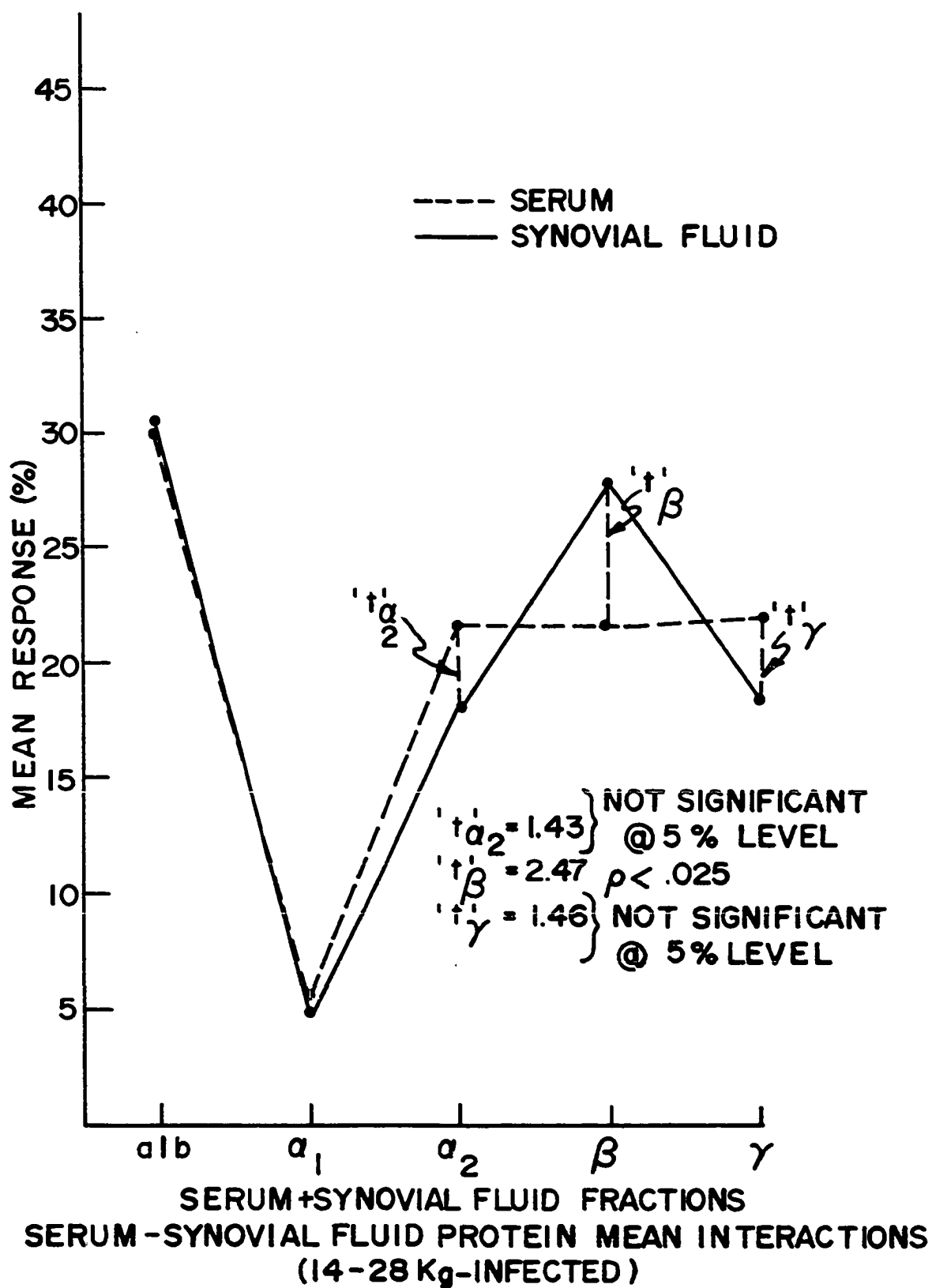
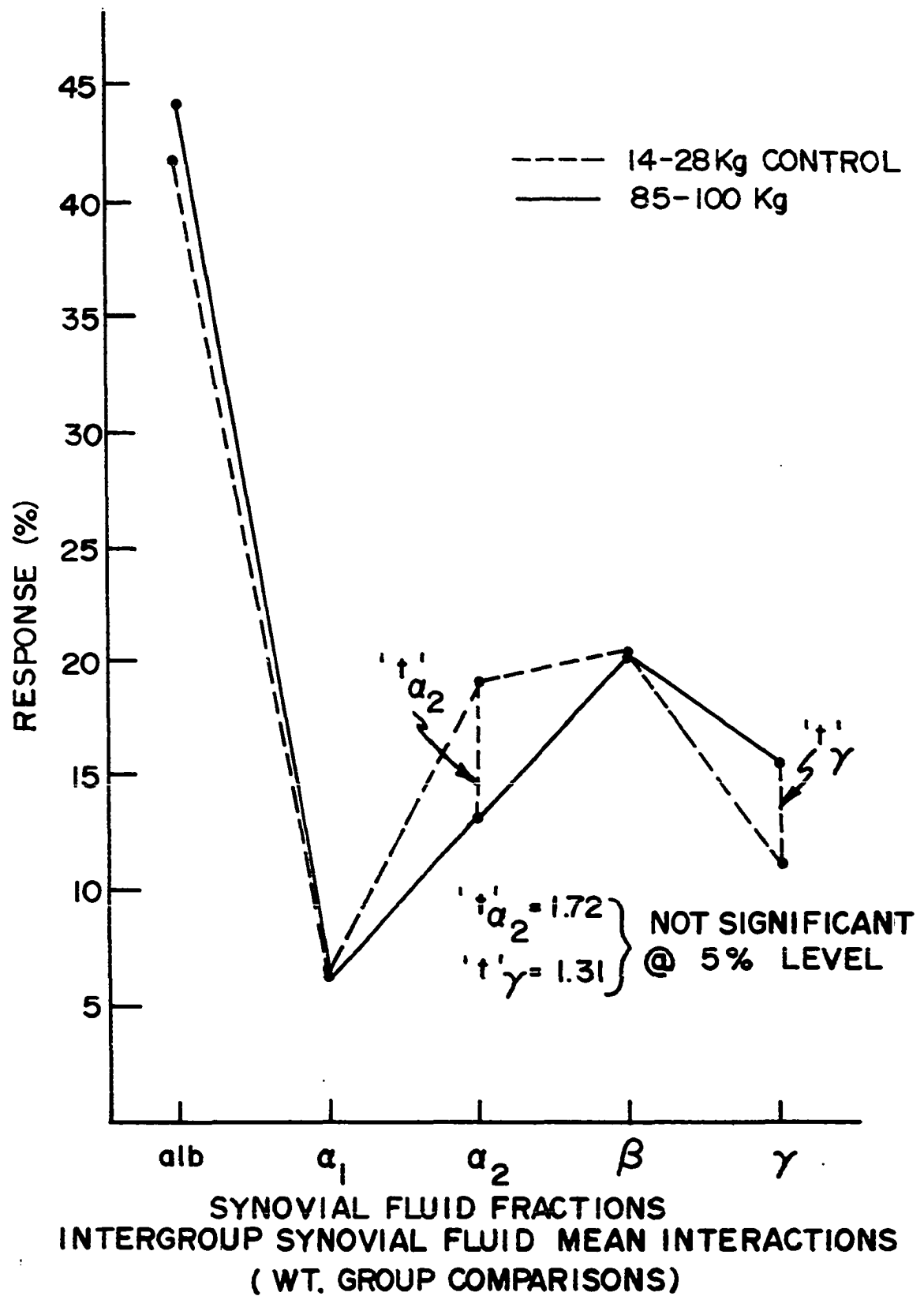


Figure 29. Intergroup synovial fluid mean interactions.
The effect of weight on synovial fluid
protein fraction distribution



The effect of infection on synovial fluid was then examined (Figure 30). With infection, albumen and α_1 globulin means were lowered and β and γ globulin means were elevated. Although statistical levels of significance were not achieved except with albumen means, β and γ globulin 't' values approached levels of significance. The effect of infection then, on synovial fluid was to produce a decreased albumen and elevated β and γ globulin levels.

Albumen/Globulin (A/G) ratios were calculated from the values in Table 4 and compared. Weight effect on the A/G ratio took the form of a reduced ratio with increasing weight as evidenced in the comparison of sera from the two different normal groups. Slaughter sera yielded a smaller A/G ratio than that of the Control sera. Infection tended to reduce the A/G ratio as evidenced in the comparison of Control and Infected sera.

Synovial fluid A/G ratios were for the most part larger than their respective serum ratios. Again the heavier of the two weight groups had the smallest ratio. Infection also reduced the synovial fluid A/G ratio to a level where it was almost the same ratio observed in infected serum.

Total glucose

Glucose levels of plasma and synovial fluid are summarized in Table 5. Plasma/Synovial ratios were calculated for each of the groups from the data in the table. The effect

Figure 30. Control versus Infected synovial fluid mean interactions. The effect of M. hyorhinis infection on synovial fluid protein fraction distribution

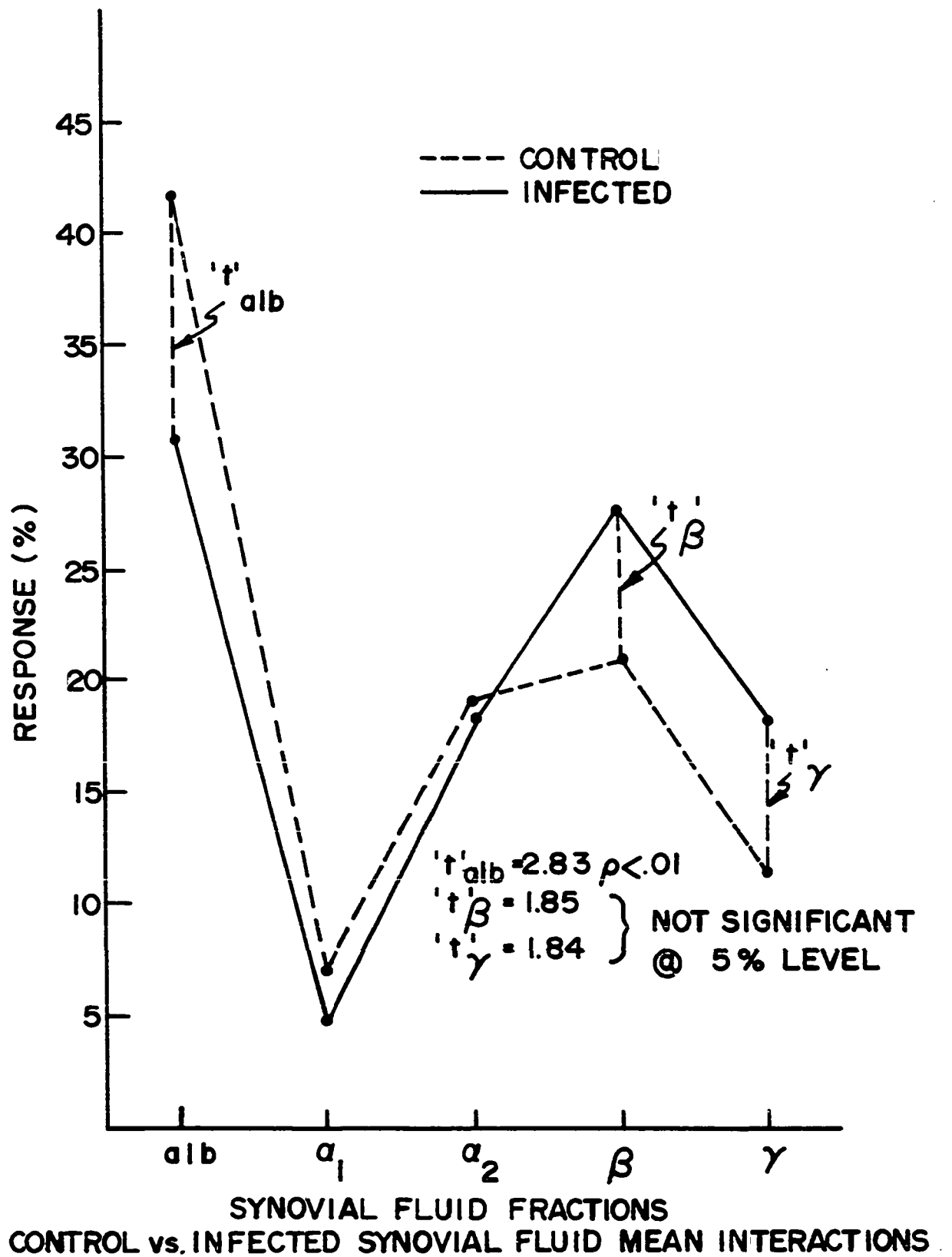


Table 5. Total plasma and synovial fluid glucose levels
(mean) in swine

	Animal group	Animal weight (kg)	No. animals	No. observa- tions
Plasma				
Code no.				
1	Slaughter	85-100	50	50
2	Control	14-28	10	10
3	Infected	14-28	18	18
Synovial fluid				
4	Slaughter	85-100	50	50
5	Control	14-28	10	5
6	Infected	14-28	18	14

^aCode no. used for "Coded Comparisons".

Mean (mg/100 ml)	Standard deviation	Coded ^a comparisons	Plasma/synovial fluid ratio
95.6	± 26.4	1 vs 4	1.76
101.0	± 26.4	2 vs 5	1.62
11.7	± 26.4	3 vs 6	0.58
54.3	± 26.4		
62.4	± 26.4		
20.2	± 26.4		

of weight upon glucose took the form of an increased plasma/synovial fluid ratio in the heavier Slaughter group when compared to the Control group. Infection produced a reduction in this ratio to a level approximately one-third of the Control group.

Hyaluronic acid content of synovial fluid

Levels within the three groups were not statistically different from one another (Table 6).

Normal Synovial Membrane

Morphology

Synovial membranes from each of three articulations sampled were similar morphologically. This similarity was observed between joints as well as between animals of differing weights. Each membrane was composed of several connective tissue cell types, blood vessels, and depending in part on the location within the particular joint and the specific joint examined, varying amounts of collagen and adipose tissue.

The most prominent connective tissue cells were the endothelial-like synovial lining (intimal) cells. These cells were relatively uniform in size and appearance, but occasionally cuboidal lining cells containing cytoplasmic vacuoles were observed. The thickness of the synovial lining varied with the nature of the subjacent supporting

Table 6. Hyaluronic acid levels (mean) of swine synovial fluid

Code no.	Animal group	Animal weight (kg)	No. animals	No. observations
1	Slaughter	85-100	50	32
2	Control	14-28	14	14
3	Infected	14-28	18	7

^aCode no. used for "Coded Statistical Comparisons" (Student's "t" test).

*Not significant at 5% level.

Mean (mg/100 ml)	Standard deviation	Coded ^a statistical comparisons	Results of comparisons
30.2	± 12.9	1 vs 2	n.s.* t = 0.68
26.6	± 12.9	2 vs 3	n.s. t = 0.47
24.2	± 12.9		

connective tissue. The lining was usually continuous and only one cell layer thick in regions subtended by relatively avascular adipose tissue (adipose synovial membrane, Figure 31). A continuous lining several layers thick was present on villi subtended by highly vascular loose areolar connective tissue (areolar synovial membrane, Figure 32). The areolar type could be found in conjunction with either fibrous or adipose connective tissues (fibroareolar and areoloadipose synovial membranes respectively (Figure 33). Fibrous synovial membranes exhibited a single layered discontinuous arrangement of its lining cells with spaces between adjacent cells.

The subjacent lamina propria, as alluded to above, was composed of numerous connective tissue cell types. Depending upon the region of the joint sampled, this tissue contained varying amounts of loose areolar connective tissue, adipose tissue or fibrous connective tissue. The vasculature was generally of a capillary type rather than of an arteriolar or venular type. Capillary content varied considerably with the nature of the supporting connective tissue. The most vascular synovial tissue was the villous areolar membrane and the least vascular was the smooth surfaced adipose membrane. All other classes of synovial membrane had levels of vascularization somewhere in between. The perivascular connective tissue of these capillaries was usually very

delicate and contained occasional mast cells.

The next layer, the inner aspect of the stratum fibrosum, consisted of a mixture of dense fibrous and loose areolar connective tissue. The fibrous type was permeated by arterioles, venules and lymphatics. The looser areolar tissue contained the same arrangement of vasculature and in addition, a few nerve bundles.

Normal Synovial Membrane-Acid Mucopolysaccharides

Alcian blue-alcian yellow

The synovial membrane from each articulation was a highly sulfated tissue as evidenced by a predominance of alcian blue positive tissue components. Dense fibrous connective tissue and all basement membranes were highly sulfated. Mast cells present in the perivascular connective tissues were invariably highly sulfated. A great deal of variability was seen however, in the lamina propria beneath the synovial lining cells.

The loose areolar lamina propria was a moderately sulfated tissue, but the delicate perivascular tissue of loose areolar or areoloadipose membranes frequently stained positively for carboxylated tissue with alcian yellow. In some regions alcian yellow uniformly stained the commonly alcian blue positive loose areolar tissue as well as the perivascular tissues. Thin bands of carboxylated tissue were also present at the interface of adipose and fibrous connective tissues. Yellow staining carboxylated tissue was not

present in the lamina propria of either the fibrous or adipose type synovial membranes. Major differences in localization of these two radicals in lamina propria of different weight groups were not observed.

Synovial lining cells from each articulation stained weakly positive for sulfate groups in some areas and negative for both radicals in other areas of the membrane. No major differences were noted in localization of these two radicals within the synovial lining cells of various weight groups.

Muscle and nerve tissue, other than their supporting connective tissues, were negative for both sulfate and carboxyl radicals in all articulations of the various weight groups.

Safranin O

The overall staining reaction of all synovial membranes was that of fast-green. Most tissue components were fast-green positive with the exception of the cytoplasm of smooth muscle and synovial lining cells which were Safranin positive. All cell nuclei were Safranin positive. Articulation and weight variations in acid mucopolysaccharide localization within the various synovial membranes were not observed.

Normal Synovial Membrane-Enzyme Histochemistry

In each of the following histochemical preparations, enzyme activity was confined to the cytoplasm of cells

within the tissues described (Table 7).

Enzyme localization within each tissue component was readily observed but no major differences in intensity of activity between the various articulations or between the various weight groups could be determined. Differences in intensity were most readily determined in the various components of a single tissue section where comparisons were greatly facilitated. The following descriptions are an attempt to describe the most prominent activity differences observed between the components of a tissue, between the various articulations of an animal and between homologous articulations of the various weight groups.

DPNH-diaphorase

The synovial lining (intimal) cells contained relatively large amounts of enzyme activity, evidenced by large accumulations of blue formazan granules imparting a dark blue or even black color to the cells (Figure 34).

Synovial vasculature was the next most prominent site of enzyme localization. Endothelium contained the greatest concentration of vascular activity followed by the muscular tunics of arterioles and venules. In addition to endothelium, lymphatics contained subintimal cellular activity. Adventitia of the synovial vasculature contained only very slight enzyme activity.

Perineurium of nerve bundles innervating the synovium

Table 7. Relative enzyme staining reactions of normal swine articular tissues

Structure	Tissue	Tissue component
Synovial membrane	Synovial intima	Synovial lining cells
		Vascular endothelium
	Blood vessel	Tunica media
		Tunica adventitia
		Perineurium
	Nerve	Endoneurium
	Connective tissue	Adipose tissue cells
	Leukocytes	Tissue neutrophils
	Muscle	Skeletal
Articular cartilage	Nonchondrogenic cells (Zones I & II)	Chondrocytes
		Matrix
	Chondrogenic region (Zones III & IV)	Chondroblasts
		Matrix
<hr/>		
- Negative		++ Moderate
+ Positive		+++ Intense

^aVariable - occasionally negative.

Diaphorases		Dehydrogenases			Phosphatases	
DPNH	TPNH	LDH	SDH	IDH	Alkaline	Acid
+++	+++	+++	+	+	-	+++
++	++	++	+	+	+++	++
+	+	+	++	++	-	+
+	+	+	-	-	-	++
++	+	+	-	-	-	+
+	+	+	-	++	-	+
+	+	+	++	++	-	-
-	-	-	-	-	+	-
+	+	+	++	+	-	+
+	+	+	² -	² -	+	+
-	-	-	-	-	-	-
+++	+++	+++	+	+	+++	+++
-	-	-	-	-	++	+

Table 7. (Continued)

Structure	Tissue	Tissue component
Articular cartilage cont.	Endochondral vasculature	Perivascular chondrocytes Vascular endothelium Perivascular tissue

^b++ Reaction in lighter animals with intact vessels;
- Reaction in heavier animals with vascular degeneration.

^c+ Reaction in lighter animals with intact vessels;
++ Reaction in heavier animals with vascular degeneration.

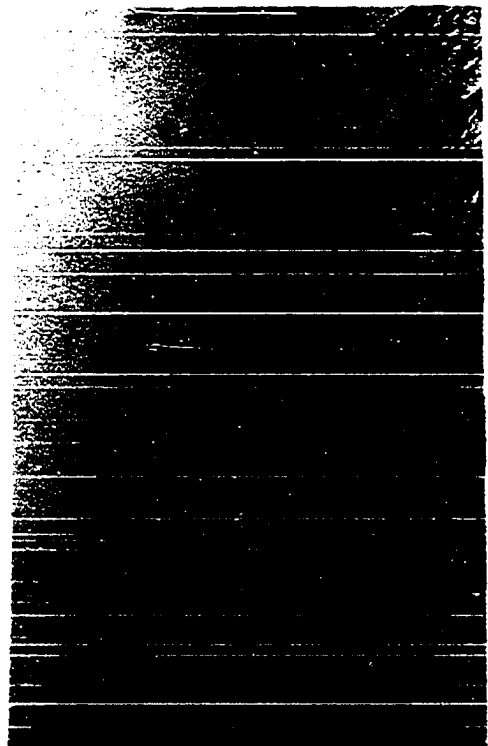
Diaphorases		Dehydrogenases			Phosphatases	
DPNH	TPNH	LDH	SDH	IDH	Alkaline	Acid
++(-) ^b	++(-) ^b	++(-) ^b	-	-	++(-) ^b	+
+(+) ^c	+(+) ^c	+(+) ^c	^{+a} -	^{+a} -	++	+
+(+) ^c	+(+) ^c	+(+) ^c	-	^{+a} -	-	+++

Figure 31. Upper left. Normal adipose synovial membrane. Hematoxylin and eosin. X 77

Figure 32. Upper right. Normal areolar synovial membrane. Hematoxylin and eosin. X 77

Figure 33. Lower left. Normal fibroareolar synovial membrane. Hematoxylin and eosin. X 120

Figure 34. Lower right. Normal synovial membrane showing enzyme localization in synovial lining cells. DPNH-diaphorase. X 77



exhibited considerable activity while endoneurium had less. Small vascular channels within nerve bundles and occasional Schwann cells were readily identified by their relatively concentrated activity.

Other mesenchymal tissues demonstrated varying levels of enzyme localization. Fat cells were often quite high in activity as were some individual muscle fibers and fibrocytes. It was interesting to note that individual fibers of a muscle bundle would often exhibit considerable enzyme compared to adjacent fibers, which would often contain only very slight activity. Major differences in enzyme localization or intensity resulting from the nature of the articulation or weight of the animal were not observed.

TPNH-diaphorase

Localization of this enzyme in synovial membrane was the same as that observed in DPNH-diaphorase preparations (Figure 35). Overall intensity of enzyme activity was only slightly less, however. Neither articulation nor weight influenced intensity or localization of enzyme activity.

Lactic dehydrogenase

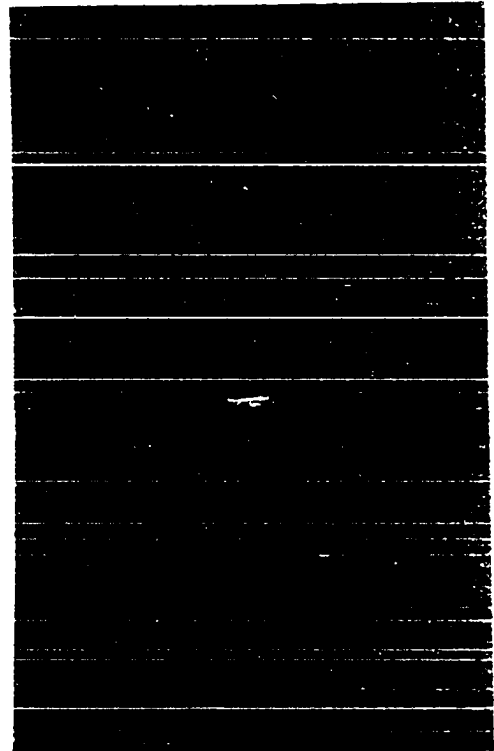
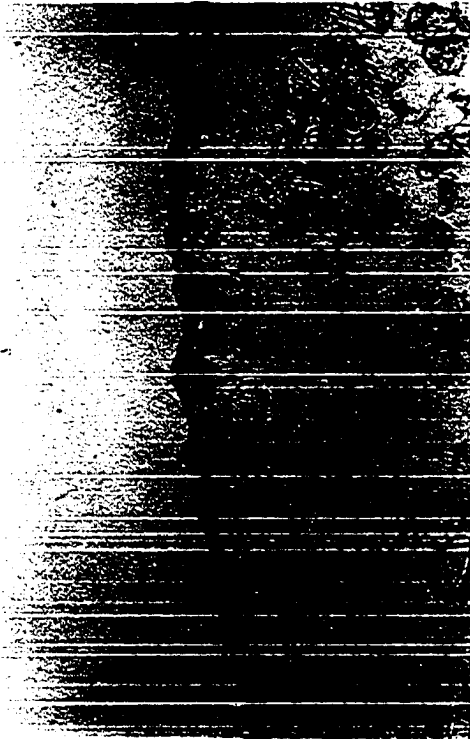
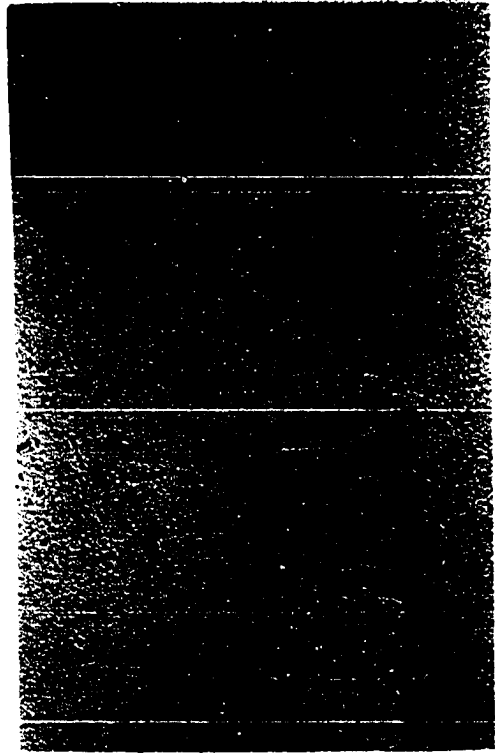
Enzyme localization observed in these preparations was the same as that observed in both diaphorase preparations (Figure 36). Enzyme intensity was slightly less, how-

Figure 35. Upper left. Normal synovial membrane showing predominant enzyme localization in synovial lining cells. TPNH-diaphorase. X 77

Figure 36. Upper right. Normal synovial lining cells showing intracellular localization of enzyme. Lactic dehydrogenase. X 390

Figure 37. Lower left. Normal synovial membrane showing absence of synovial lining activity and presence of vascular activity (arrows). Alkaline phosphatase. X 77

Figure 38. Lower right. Normal synovial membrane showing enzyme activity in synovial lining. Acid phosphatase. X 77



ever, than that observed in the TPNH-diaphorase preparations. Differences due to articulation or weight were not observed.

Alkaline phosphatase

The fine capillary network beneath the synovial lining stained heavily for enzyme activity as evidenced by large accumulations of blue diazo salt precipitate in endothelial cytoplasm (Figure 37). These otherwise "invisible" vessels were readily observed and easily traced via their dichotomously arranged branches. Occasionally, minute venular valves were outlined by the blue precipitate. An occasional neutrophil which was present in subsynovial connective tissue was clearly delineated by the heavy concentration of enzyme activity within its cytoplasm, and readily identified by the "ghost" of its "negatively stained" polymorphic nucleus. No other structures were alkaline phosphatase positive. Articulation or weight differences were not observed.

Acid phosphatase

Synovial lining cells contained high levels of enzyme activity as evidenced by numerous large granules of blue diazo salt (Figure 38).

Synovial membrane vasculature was the next most prominent area of localization. Endothelium and adventitia had considerable activity, the medial musculature somewhat less. Slight enzyme activity was also observed in nerve bundles

and muscle fibers. Articular or weight differences were not observed.

Succinic dehydrogenase

Succinic dehydrogenase localization in synovial membrane was inconsistent from preparation to preparation. In tissues that contained enzyme activity fat cells and the tunica media of arterioles and venules exhibited moderate activity. Synovial lining cells and endothelial cells of blood vessels contained only a slight amount while skeletal musculature contained moderate activity. Intensity and localization varied more from preparation to preparation than between articulations or weight groups.

Isocitric dehydrogenase

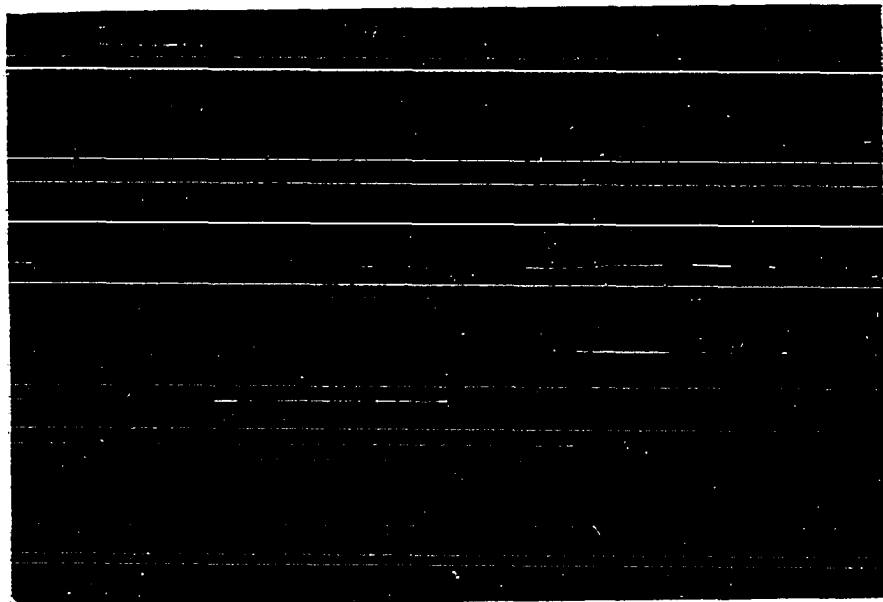
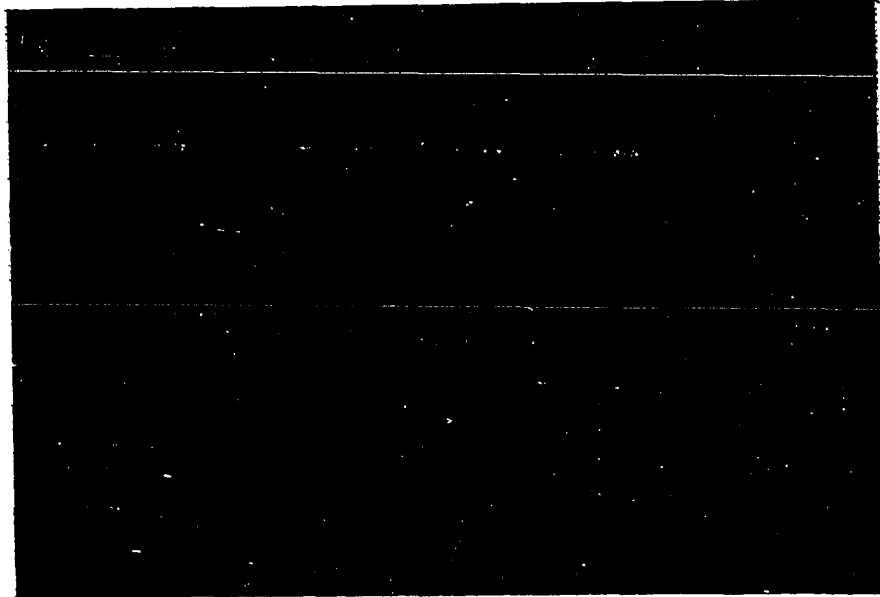
Enzyme localization and intensity in the various synovial membrane components closely paralleled those of succinic dehydrogenase. In addition nerve fibers exhibited moderate enzyme activity and skeletal muscle exhibited only a slight amount. Variations in intensity and localization were greater between preparations than between articulations or weight groups.

Normal Articular Cartilage-Morphology

Articular cartilage morphology varied considerably with the articulation thus necessitating individual discussion (Figure 39).

Figure 39. Articular cartilage from a normal 40-45 kg pig. Hematoxylin and eosin. X 77

Figure 40. Articular cartilage from a normal 20-25 kg pig showing intact endochondral vasculature. Hematoxylin and eosin. X 77



Cubital articulation

The proximal articulation of the radius, the glenoid cavity, was covered by a thin, relatively uniform sheet of weight bearing articular cartilage. The relatively uniform thickness of this cartilage permitted easy distinction of four morphologic zones (Barnett, 1961). The transitional zone (Zone II) showed the greatest variability in staining with eosin. All other zones stained homogeneously. In 8-10 kg and 20-25 kg swine endochondral vessels were observed in various stages of degeneration. These degenerative changes will be described as observed in the cartilages of the femorotibial articulation. The heavier weight groups exhibited no functional endochondral vasculature.

Femorotibial articulation

Two cartilage samples were selected from this joint, weight bearing (proximal articulation of the tibia) and non-weight bearing (distal trochlear articulation of the femur) surfaces. The thickness of the weight bearing cartilage was generally similar to that of the cubital articulation. Thickness was observed to vary however, with the weight group studied. The lighter animals (8-10 and 20-25 kg) had a slightly thicker cartilage than the heavier groups. All four zones were readily distinguishable. The most prominent difference between light and heavy groups was in the amount of endochondral vasculature. The vascular content of the

cartilage of the lightest groups was greater than that of any of the other weight bearing cartilages. The 8-10 kg group contained the greatest number of intact endochondral vessels (Figure 40), the 20-25 kg and 40-45 kg groups contained varying degrees of endochondral vascular degeneration. The heaviest (90-100 kg) group contained no intact endochondral vasculature.

Degenerative changes observed in cartilages of the younger groups ranged from vascular obstruction by thrombosis to vascular hemorrhage and necrosis (Figures 41 and 42). Vascular necrosis was followed by formation of a "cartilage scar" (Figure 43). These changes most often originated at the level of Zone IV (calcified zone) or within Hunter's circle.

The nonweight bearing cartilage was in general, thicker but less uniform due to its trochlear morphology. Zonal arrangement of the various cartilagenous layers was not as apparent as in other cartilages, especially in the thicker regions of the trochlear prominences. Endochondral vasculature was more pronounced in this cartilage than in any other. Zones III and IV of the heaviest group had a greater tendency toward basophilia than in the lightest ones.

Figure 41. Articular cartilage from a normal 20-25 kg pig. Endochondral vascular degeneration characterized by perivascular hemorrhage. Hematoxylin and eosin. X 120

Figure 42. Articular cartilage from a normal 20-25 kg pig. Endochondral vascular degeneration characterized by perivascular hemorrhage and vascular necrosis. Hematoxylin and eosin. X 120

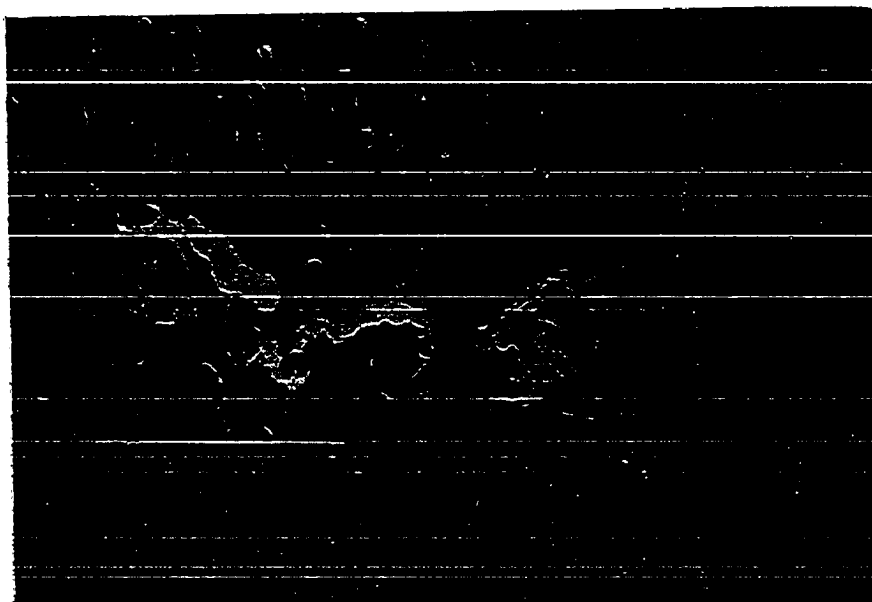
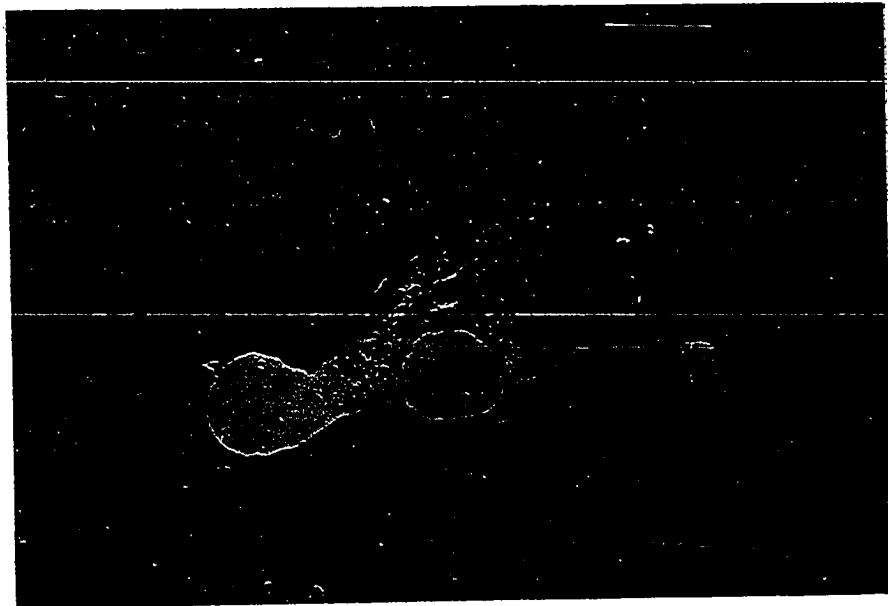


Figure 43. Articular cartilage from a normal 90-100 kg pig. Endochondral vessel replaced by chondrocytes and matrix with resultant "cartilage scar" formation. Hematoxylin and eosin. X 192

Figure 44. Articular cartilage from a normal 40-45 kg pig. Variability in anion group localization is quite marked in Zone II. Alcian blue-Alcian yellow. X 77

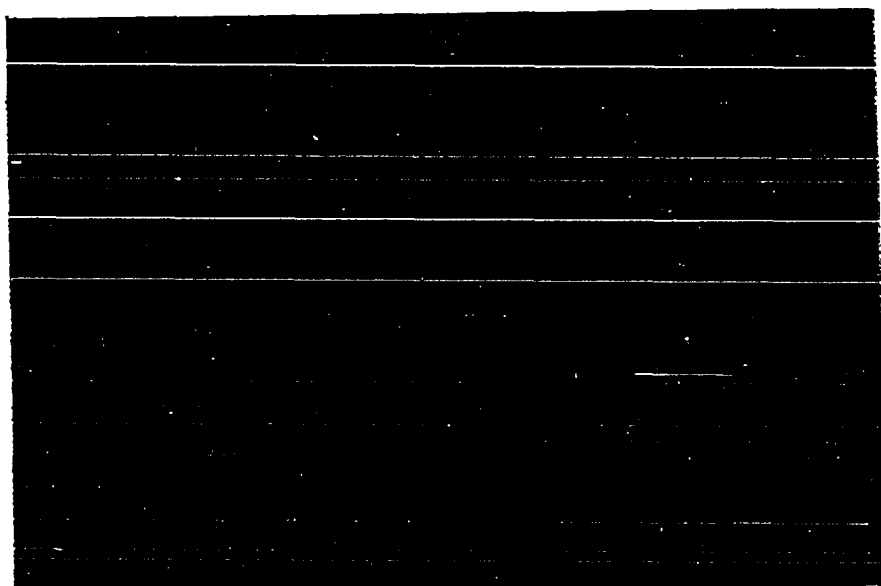
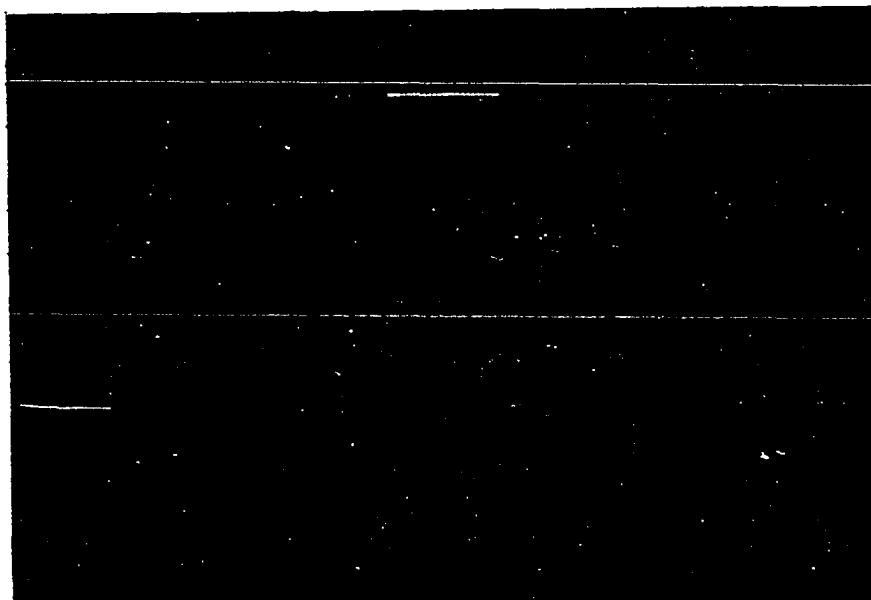
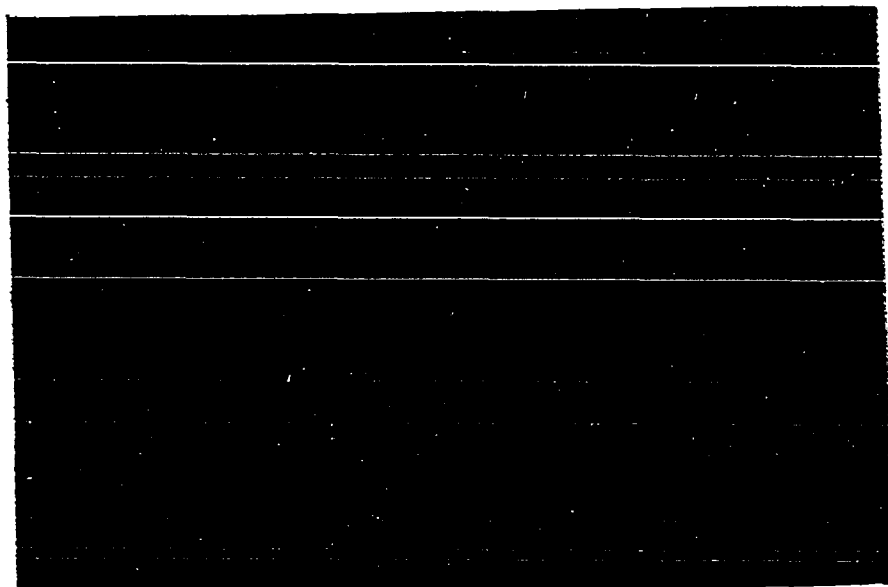
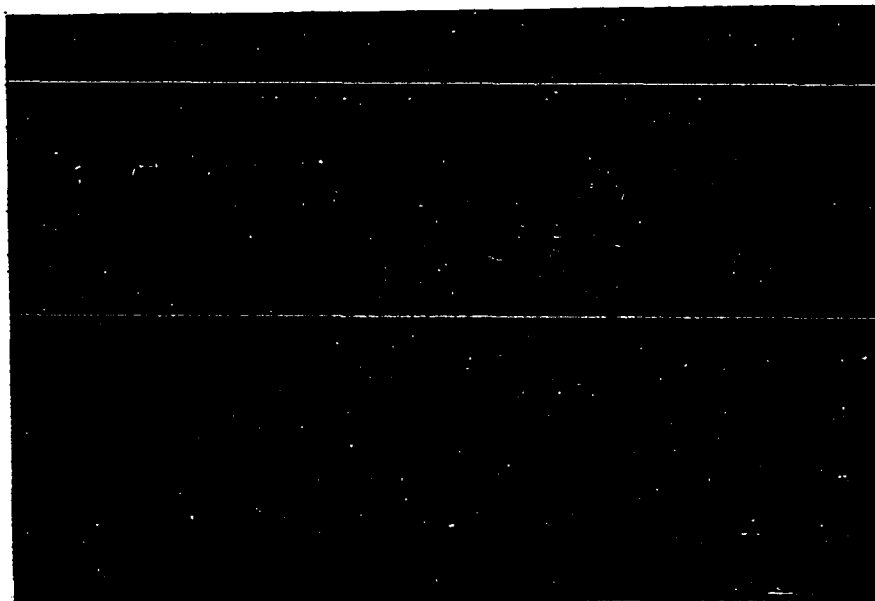


Figure 43. Articular cartilage from a normal 90-100 kg pig. Endochondral vessel replaced by chondrocytes and matrix with resultant "cartilage scar" formation. Hematoxylin and eosin. X 192

Figure 44. Articular cartilage from a normal 40-45 kg pig. Variability in anion group localization is quite marked in Zone II. Alcian blue-Alcian yellow. X 77



Tibiotarsal articulation

The weight bearing articular cartilage covering the proximal articulation of the tibiotarsal bone was less uniform in thickness than the cubital articular cartilage, and more uniform than the nonweight bearing cartilage of the femorotibial articulation. The cartilage overlying the trochlear prominences was for the most part thicker than that overlying the trochlear groove. Identification of definite zones was difficult in the thick regions, but easier in thinner areas. Zone II of the thin regions of the trochlea was quite narrow. Trochlear prominences contained a number of endochrondial vessels. These vessels too were observed to undergo degenerative changes paralleling an increase in weight. Vessels were replaced by cartilage matrix in the heaviest groups.

Normal Articular Cartilage-Acid Mucopolysaccharides

Alcian blue-alcian yellow-Cubital articulation

Anion group localization in each of the four morphologic zones was not always coincident with zone morphology (Figure 44). Some of the zones varied considerably with regard to the nature of the anionic groups they contained (Table 8). The staining character of the zones will be described individually in the order from most superficial (Zone I) to the deepest (Zone IV).

Table 8. Acid mucopolysaccharide (AMPS) staining reaction of normal swine articular cartilages

Weight group	Articulation	Morphologic zone	Alcian blue-alcian yellow	
			SO ₄ (blue)	COO ⁻ (yellow)
8-10 kg	Cubital	I	++	<u>±</u>
		II	++(+)	<u>±</u> (+)
		III & IV	+++	-
	Femorotibial	I	++	<u>±</u>
		II	++(+)	<u>±</u>
		III & IV	+++	-
	Tibiotasal	I	++	<u>±</u>
		II	++	<u>±</u>
		III & IV	+++	<u>±</u>
20-25 kg	Cubital	I	++	<u>±</u>
		II	++(+)	<u>±</u> (+)
		III & IV	+++	-
<hr/>				
- Negative	++	Moderate		
<u>±</u> Negative to light	++(+)	Moderate to heavy		
+ Light	+++	Heavy		
	<u>±</u> (+)	Negative to moderate		

^aFunctional-perivascular matrix predominately sulfated.

Safranin O		Endochondral vasculature
Safranin (red)	Fast green (green)	
<hr/>		
-	++	
++(+)	<u>+</u> (+)	
+++	<u>+</u>	
-	++	
+	++	-a
+++	<u>+</u>	
-	++	
++(+)	<u>+</u> (+)	
+++	<u>+</u>	
-	++	
++(+)	<u>+</u> (+)	
+++	<u>+</u>	

Table 8. (Continued)

Weight group	Articulation	Morphologic zone	Alcian blue-alcian yellow	
			SO ₄ (blue)	COO ⁻ (yellow)
20-25 kg	Femorotibial	I	++	±
		II	++(+)	±
		III & IV	+++	-
	Tibiotarsal	I	++	±
		II	++(+)	++(+)
		III & IV	+++	++(+)
	Cubital	I	++	±
		II	++(+)	±(+)
		III & IV	+++	-
45-50 kg	Femorotibial	I	++	++(+)
		II	++	++(+)
		III & IV	+++	++(+)

++(+) Light to moderate

^bEarly degenerative changes. Perivascular matrix shows reduced sulfation.

^cObliterated by encroaching matrix. Perivascular matrix weakly sulfated with carboxylated or reduced sulfation only.

Safranin O		Endochondral vasculature
Safranin (red)	Fast green (green)	
-	++	_b
+	++	
+++	<u>+</u>	
-	++	
++(+)	<u>+</u> (+)	
+++	<u>+</u>	
-	++	
++(<u>+</u>)	<u>+</u> (+)	
+++	<u>+</u>	
-	++	_c
+	++	
+++	<u>+</u>	

Table 8. (Continued)

Weight group	Articulation	Morphologic zone	Alcian blue-alcian yellow	
			SO_4 (blue)	COO^- (yellow)
45-50 kg	Tibiotarsal	I	++	\pm
		II	+++	+(+)
		III & IV	+(+)	+(+)
90-95 kg	Cubital	I	++	\pm
		II	++(+)	\pm +(+)
		III & IV	+++	-
	Femorotibial	I	++	+(+)
		II	++	+(+)
		III & IV	+++	+(+)
	Tibiotarsal	I	++	\pm
		II	+(+)	+(+)
		III & IV	+++	+(+)

^dAbsent-matrix "scar" stains similar to surrounding matrix becoming almost indistinguishable from it.

Safranin O		Endochondral vasculature
Safranin (red)	Fast green (green)	

-	++	
++(+)	<u>+</u> (+)	
+++	<u>+</u>	
-	++	
++(+)	<u>+</u> (+)	
+++	<u>+</u>	
-	++	
+	++	_d
+++	<u>+</u>	
-	++	
++(+)	<u>+</u> (+)	
+++	<u>+</u>	

Zone I (Gliding or Flattened Zone) The gliding zone was primarily a thin sheet of flattened chondrocytes surrounded by homogeneous alcian blue staining sulfated matrix. The site of greatest variability in anion localization was the cartilage territorial matrix (cartilage capsules), which was normally sulfated. On occasion this matrix contained both sulfate and carboxyl groups. The major difference between weight groups was the "apparent" greater overall thickness of Zone I of the lightest weight groups. The appearance of increased thickness was due in fact to the extension of homogeneously stained sulfate positive matrix from the outer limits of Zone II into the inner region of Zone I. In the heavier animals, sulfate localization was confined primarily to Zone I and a line of demarcation clearly separated Zones I and II.

Zone II (Transitional zone or zone of ovoid cell clumps) This was the widest and most heterogeneously stained of the four zones. All possible permutations of sulfated and carboxylated territorial and inter-territorial (matrix between cartilage capsules) matrix were observed. Staining varied from carboxylated only, to mixtures of carboxyl and sulfate groups to sulfated only. The region of greatest homogeneity was the sulfated outer limit bordering on Zone I described above.

Zones III and IV (Radial and Calcified zones respectively) These two zones are included together because they usually stained identically. The territorial matrix invariably contained only sulfate groups. Inter-territorial matrix was more variable and often contained both sulfate and carboxyl groups. Weight variations in anion group predominance or localization were not observed.

Safranin O-cubital articulation

The localization of this stain more closely paralleled zone morphology (Figure 45).

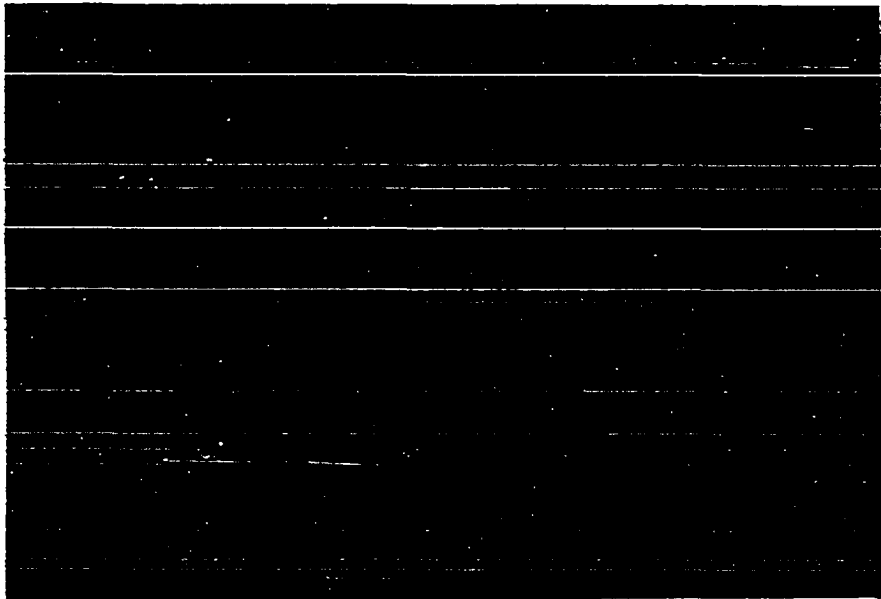
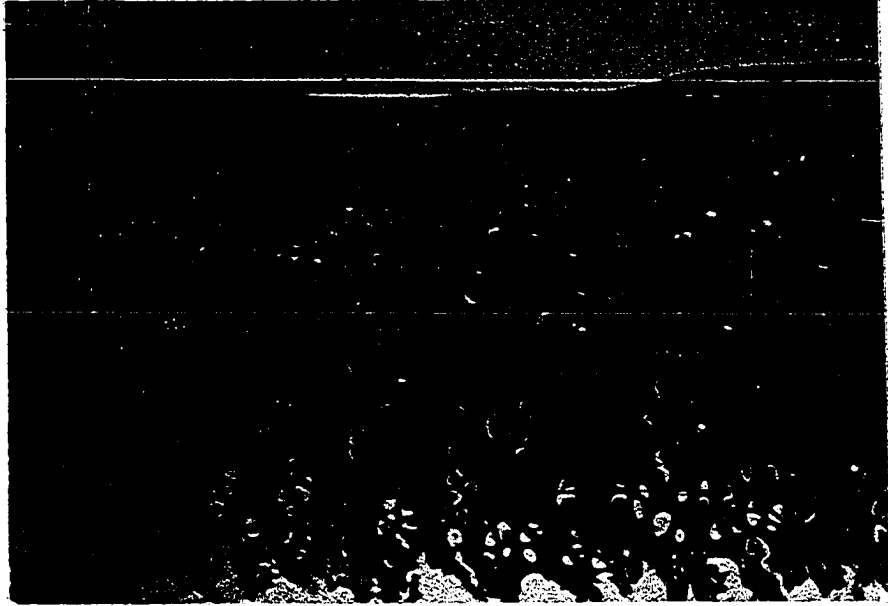
Zone I This zone was most commonly fast-green positive and safranin negative. Again weight variation was noted as a difference in "apparent" thickness of Zone I.

Zone II Again this zone had the greatest variability in staining. Territorial matrix was safranin positive. Inter-territorial matrix was quite variable with regard to its safranin intensity, often staining a blue-red indicative of uptake of both safranin and fast-green. The outer region of Zone II was often weakly safranin positive and stained heavily with fast-green, especially in the lighter weight groups. The cytoplasm of chondrocytes within this zone was safranin positive and occasionally contained large fast-green positive cytoplasmic inclusions.

Zones III and IV These zones were generally deeply safranin positive. Territorial matrix was deeply safranin

Figure 45. Articular cartilage from a normal 40-45 kg pig.
Safranin O. X 77

Figure 46. Articular cartilage from a normal 20-25 kg
pig. Intact endochondral vasculature (dark
spot is an artifact). Alcian blue-alcian
yellow. X 77



positive and inter-territorial matrix was more variable in intensity. Inter-territorial matrix often stained with both safranin and fast-green but some variation in stain intensity was noted. Chondrocytic cytoplasm stained either safranin positive or weakly with both safranin and fast-green. Occasionally, fine granules of fast green material were observed within the cytoplasm.

Alcian blue-alcian yellow (femorotibial articulation)

Localization of anionic groups in femorotibial weight bearing and nonweight bearing articular cartilages was generally similar to that described under cubital articulation (Alcian blue-alcian yellow) with the following exceptions.

Zone I The presence of both carboxyl and sulfate groups in cartilage matrix imparted a green color to it in contrast with the blue staining character of this zone in other articular cartilages of the lighter weight swine. The outer limits of Zone II (bordering on Zone I) were primarily sulfated in the lightest weight group but contained both sulfate and carboxyl groups in the heavier animals.

Zones II, III and IV These three zones in nonweight bearing cartilages of the lightest weight group contained intact, functional endochondral vessels surrounded by blue sulfated territorial matrix (Figure 46). In the heavier groups it was observed that as vascular degeneration

(Figures 47 and 48) progressed, the sulfated territorial matrix would gradually fade and almost disappear. The weakly sulfated "cuff" would either remain pale blue, become carboxylated giving the matrix a green color, or fade and become almost totally colorless. As chondrocytes and matrix gradually filled in the site, the area would become very weakly sulfated (Figure 49). As the old vessel site was completely replaced by matrix, it would take on the staining characteristics of the surrounding matrix and become almost indistinguishable from it.

Safranin O-femorotibial articulation

Localization of this stain closely paralleled morphologic zoning in the articular cartilage of the femorotibial articulation.

Zone I In both weight bearing and nonweight bearing cartilages this zone was safranin negative and fast-green positive. Cells of this zone in lighter animals were plump and contained vacuolated cytoplasm where as in heavier animals the zone was thin and cells were flattened.

Zone II This zone in both weight and nonweight bearing cartilages of the heaviest group shared a highly irregular interface with Zone I. Zone II was usually weakly safranin positive and highly fast-green positive. The appearance of irregularity was produced by extension of fast-green positive matrix from Zone I deep into Zone II. The

Figure 47. Articular cartilage from a normal 40-45 kg pig. Endochondral vascular thrombosis and early vascular degeneration. Alcian blue-alcian yellow. X 120

Figure 48. Articular cartilage from a normal 40-45 kg pig. Endochondral vascular necrosis and hemorrhage with reduced sulfation of matrix immediately adjacent to vessels. Alcian blue-alcian yellow. X 48

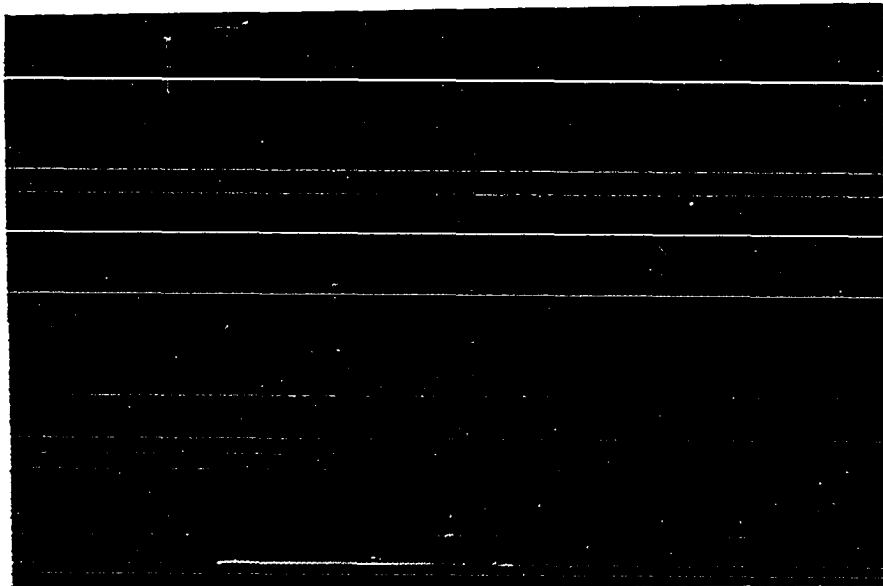
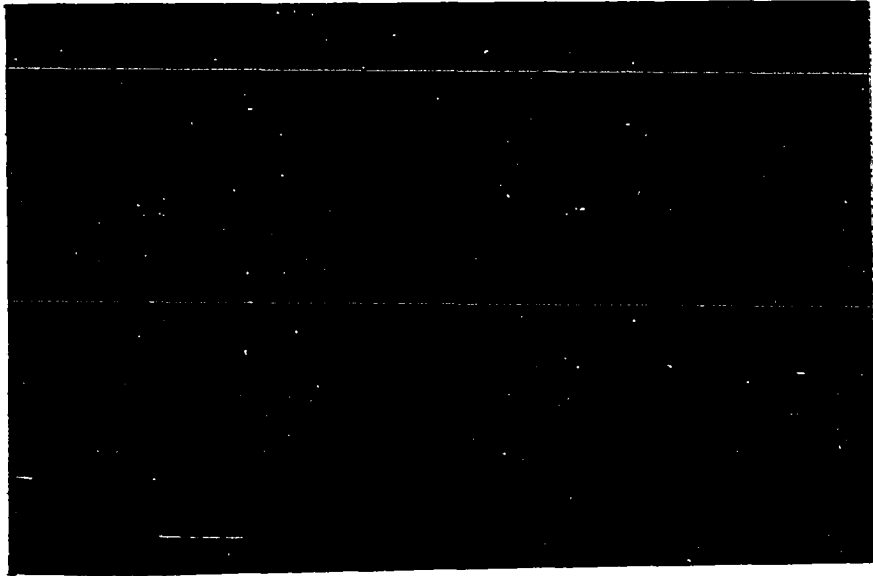
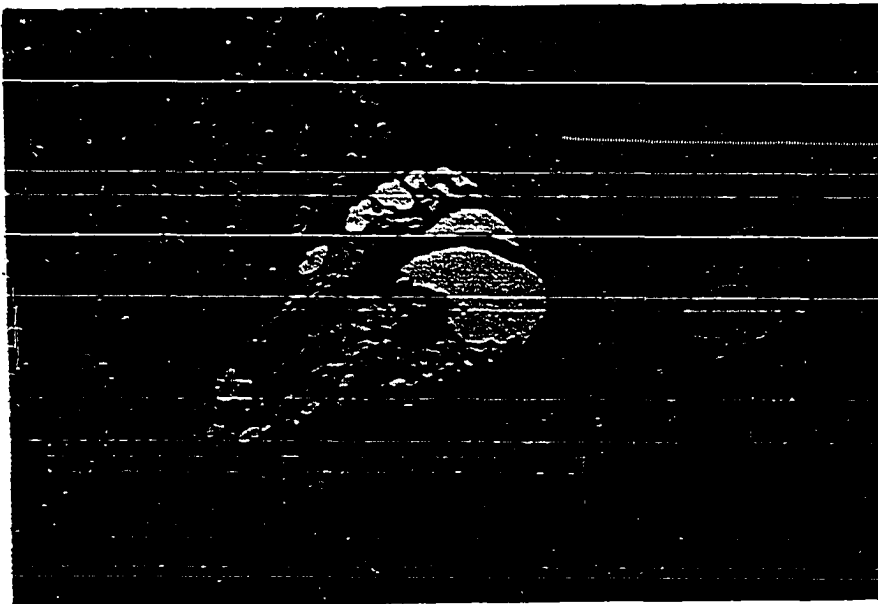
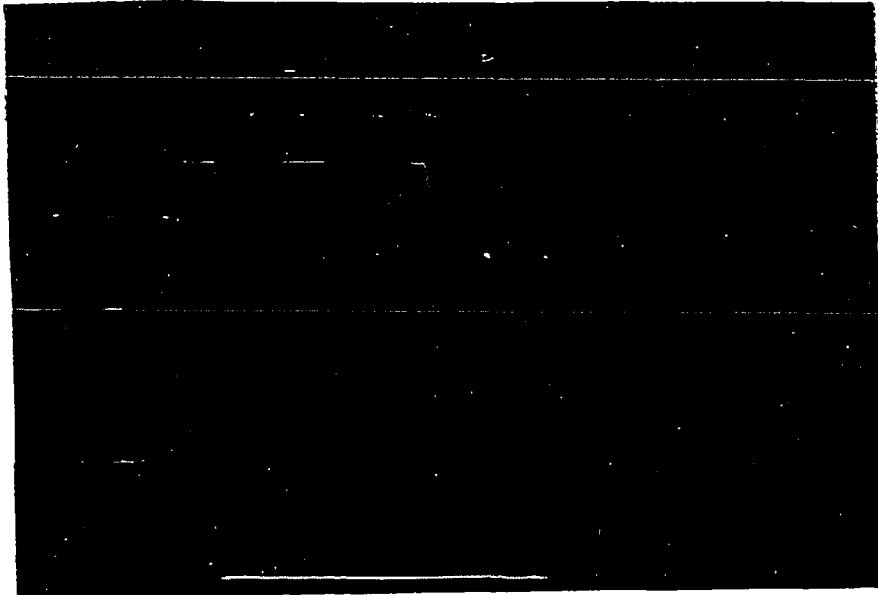


Figure 49. Articular cartilage from a normal 40-45 kg pig. Endochondral vasculature replaced by a weakly sulfated "cartilage scar". Alcian blue-alcian yellow. X 120

Figure 50. Articular cartilage from a normal 20-25 kg pig. Intact endochondral vessels surrounded by safranin negative matrix. Safranin O. X 77



inner region of Zone II bordering on Zone III was deeply safranin positive. Fast green positive cytoplasmic inclusions were observed in chondrocytes of this zone.

Zones III and IV These two zones were deeply safranin positive. Intact endochondral vessels were surrounded by a safranin negative, fast green positive cuff (Figure 50). In the areas of vascular degeneration, a gradual loss of fast-green staining of the perivascular matrix and an increase in safranin staining was observed. Areas where matrix entirely replaced vessels were weakly safranin positive and contained foci of fast-green staining material. The same staining reaction of chondrocytic cytoplasm observed in Zone II of the cubital articulation was noted in both types of articular cartilage of the femorotibial articulation.

Alcian blue-alcian yellow-tibiotarsal articulation

Staining characteristics of this articular cartilage were quite similar to those already described for the cubital articular cartilage.

Zone I Anion localization was similar to that observed in the cubital articulation.

Zone II The thin regions (between trochlear prominences) in the lightest group articular cartilage was predominantly sulfated, whereas the thin regions of the other weight groups contained varying mixtures of both

carboxyl and sulfate groups. Functional endochondral vasculature was present in the lightest weight group. These vessels were surrounded by sulfated matrix in the lightest group but were surrounded by matrix containing mixtures of both anions in the 20-25 kg group.

Zones III and IV The lightest group had a predominantly sulfated territorial and inter-territorial matrix, the other weight groups had sulfated territorial matrix but an inter-territorial matrix containing both anion groups.

Safranin O-tibiotarsal articulation

The staining reaction of cartilage from the tibiotarsal articulation was the same as that described in the other articulations.

Normal Articular Cartilage-Enzyme Histochemistry

Again, as in the case of synovial membrane, enzyme activity was confined to cell cytoplasm (Table 7). One exception, cartilage matrix, will be described later.

DPNH-diaphorase

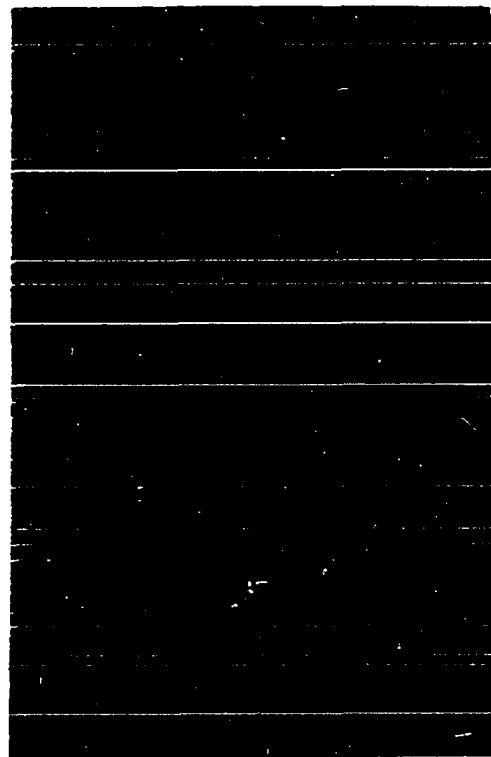
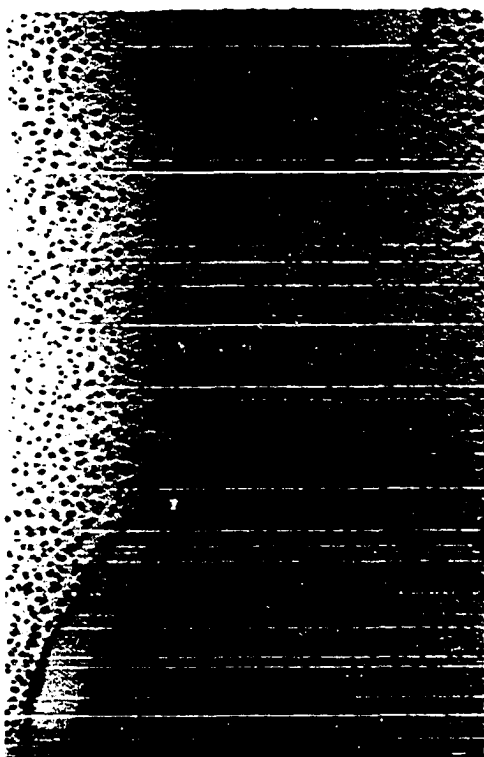
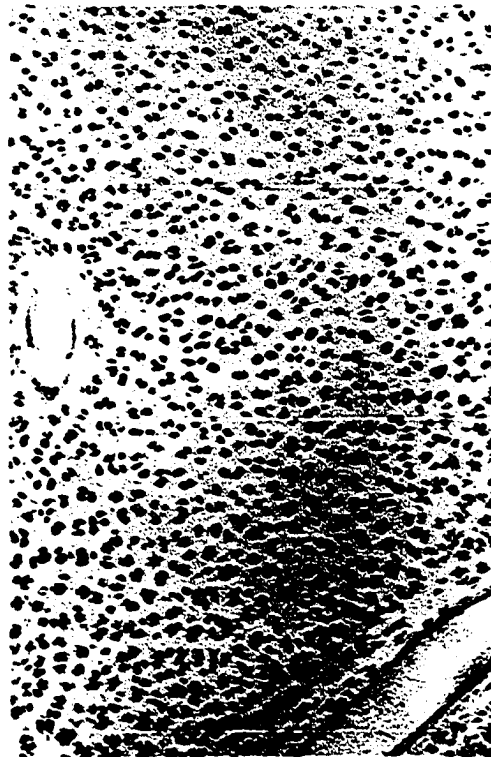
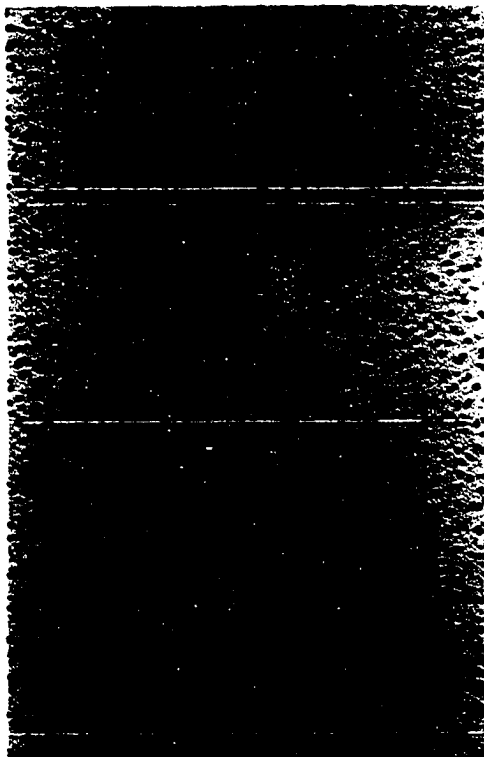
The area of most prominent enzyme localization was the region of chondrogenesis (Zones III and IV). Levels of activity gradually diminished with each successive layer of chondrocytes toward the articular surface, but never disappeared entirely (Figure 51).

Figure 51. Upper left. Articular cartilage from a normal 20-25 kg pig. Endochondral vessels are surrounded by chondrocytes containing intense enzyme activity. DPNH-diaphorase. X 48

Figure 52. Upper right. Articular cartilage from a normal 20-25 kg pig. Endochondral vessels are surrounded by chondrocytes containing intense enzyme activity. LDH. X 62

Figure 53. Lower left. Articular cartilage from a normal 40-45 kg pig. Intense enzyme activity is confined to Zones III and IV(A). Alkaline phosphatase. X 48

Figure 54. Lower right. Articular cartilage from a normal 20-25 kg pig. Moderate chondrocytic enzyme activity is present throughout the articular cartilage and in endochondral vasculature. Acid phosphatase. X 62



Endochondral vasculature of the lightest weight groups was surrounded by chondrocytes which had a higher level of activity than their counterparts at the same level within the matrix. Mesenchymal tissue supporting the endochondral vasculature also contained enzyme activity as did the endothelium of the vessels themselves.

The vasculature in the two heavier groups was undergoing degenerative changes and contained less activity than that observed in the two lighter groups. Perivascular chondrocytes adjacent to these vessels were practically devoid of enzyme activity.

TPNH-diaphorase

Localization of this enzyme paralleled exactly that of DPNH-diaphorase.

Lactic dehydrogenase

Localization of this enzyme also paralleled that of the diaphorases (Figure 52).

Alkaline phosphatase

Enzyme activity of Zones III and IV was the most prominent found anywhere in the cartilage (Figure 53). Cytoplasm of chondrocytes exhibited intense localization in this region. Relatively high levels of activity were also observed in the matrix surrounding chondrocytes and chondroblasts. Very slight activity was observed in Zones

I and II. Endochondral vasculature in the deeper regions of cartilage from the lightest weight group was surrounded by chondroblasts with higher levels of enzyme activity than their counterparts at the same morphologic level. Chondrocytes surrounding the more superficial endochondral vasculature did not contain as high a level of enzyme activity.

The intima of endochondral vessels was quite high in activity and clearly delineated vascular lumina, some of which contained neutrophils with intense enzyme localization.

Acid phosphatase

Acid phosphatase activity was localized in the cytoplasm of chondrocytes and cells of supportive tissues associated with articular cartilage (Figure 54). The highest levels of activity observed in these cartilage preparations were observed in Zones III and IV. The chondroblasts and chondrocytes of this region were higher in activity than any other areas of articular cartilage. Only slight activity was observed in cartilage matrix of this region.

Perivascular tissue of endochondral vasculature was the next most prominent site of enzyme activity and served to clearly delineate vascular channels in the otherwise uniform matrix. Vascular endothelium contained slight enzyme activity.

Succinic dehydrogenase

The demonstration of enzyme activity was inconsistent from preparation to preparation, independent of the weight group or articulation studied. When enzyme activity could be demonstrated, the predominant activity was localized in the chondrogenic (Zones III and IV) region. Chondrocytes were variable, some had slight enzyme activity and others none. Endochondral vasculature occasionally contained slight activity in the vascular endothelium and smooth muscle of the tunica media.

Isocitric dehydrogenase

Enzyme localization closely paralleled that observed in succinic dehydrogenase preparations but intensity was usually greater. In addition to the previously described areas of localization, perivascular connective tissue also exhibited slight enzyme activity.

Infected Synovial Membrane-Morphology

The following morphologic changes were observed in the synovial membranes of articulations from infected animals irrespective of the articulation examined. One of the earliest morphologic changes observed in synovial membranes 14 days after inoculation was an increased prominence of synovial lining cells characterized by cellular hypertrophy (Figure 55) and increased eosinophilia of lining cell

- Figure 55. Upper left. Synovial membrane from M. hyorhinis infected pig. Synovial lining cells of normal morphology (A) contrasted with hypertrophic lining cells (B) present as an early change in M. hyorhinis infection. Hematoxylin and eosin. X 48
- Figure 56. Upper right. Synovial membrane from M. hyorhinis infected pig. Synovial membrane showing vascular congestion (A), edema (arrows) and early mononuclear cell infiltration (B). Hematoxylin and eosin. X 120
- Figure 57. Lower left. Synovial membrane from M. hyorhinis infected pig. Synovial membrane showing more advanced inflammatory changes characterized by perivascular infiltration by mononuclear inflammatory cells (arrows). Hematoxylin and eosin. X 48
- Figure 58. Lower right. Synovial membrane from M. hyorhinis infected pig. Fibrinous synovitis characterized by fibrin exudate (A) and thickened synovial membrane containing numerous inflammatory cells (B). Hematoxylin and eosin. X 62



cytoplasm. This change occurred concurrently with congestion of synovial membrane vasculature and edema of the lamina propria (Figure 56).

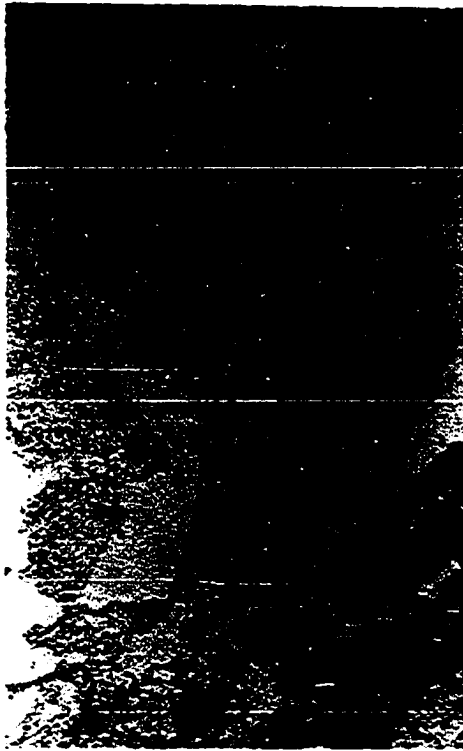
Following the earlier changes, perivascular accumulation of plasma cells, lymphocytes and other mononuclear inflammatory cells occurred (Figure 57). An occasional neutrophil would be observed in the tissue, but this cell type did not predominate until exudation of fibrin had occurred. An increased cellularity and thickening of the synovial membrane resulted from both perivascular accumulation and proliferation of subsynovial fibroblasts and synovial cell progenitors (Figure 58). Villous hypertrophy of the synovial membrane was the final result but villi remained short and not elongate.

The most severe changes observed were frank necrosis of the synovial lining (Figure 59) and fibrin exudation into the lamina propria and onto the synovial membrane surface. Neutrophils were present in large numbers both in the lamina propria and on the fibrin coated synovial lining.

Infected Synovial Membrane-Acid Mucopolysaccharides Alcian blue-alcian yellow

Alterations in either intensity or localization of sulfate or carboxyl groups were not observed as a result of the infectious process.

- Figure 59. Upper left. Synovial membrane from M. hyorhinis infected pig. Synovial lining cell and subsynovial tissue necrosis (arrow). Hematoxylin and eosin. X 120
- Figure 60. Upper right. Synovial membrane from M. hyorhinis infected pig. Synovial membrane showing high levels of enzyme activity in synovial lining cells and subsynovial connective tissue. DPNH-diaphorase. X 48
- Figure 61. Lower left. Synovial membrane from M. hyorhinis infected pig. Synovial membrane showing high levels of enzyme activity in synovial lining cells, subsynovial tissue (A) and capillaries. TPNP-diaphorase. X 48
- Figure 62. Lower right. Synovial membrane from M. hyorhinis infected pig. Synovial membrane showing high levels of enzyme activity in synovial lining cells and subsynovial tissue. Lactic dehydrogenase. X 48



Safranin O

The cytoplasm of synovial lining cells varied in staining characteristics. Lining cell cytoplasm was usually fast green positive. Hypertrophied lining cells of membranes which exhibited some of the earlier hyperplastic and inflammatory changes contained cytoplasm which was safranin positive. Numerous lining cells in these membranes would contain fast-green positive cytoplasmic inclusions.

In the later stages when fibrin and neutrophils became prominent, they stained fast-green positive. The overall appearance of the membrane in the most advanced inflammatory stages observed was that of a thickened highly cellular purple colored membrane. The purple color was due to the presence of an admixture of large numbers of safranin positive mesenchymal cells and fast-green positive neutrophils and fibrin.

Infected Synovial Membrane-Enzyme HistochemistryDPNH-diaphorase

The lining cells of synovial membrane were quite prominent with regard to enzyme activity (Figure 60). Intensity of staining was markedly increased over that observed in normal control animals. This intense staining reaction was carried into immediately subjacent subsynovial tissue also. Many of the proliferating mesenchymal cells and inflammatory cells had high levels of activity giving the

synovial membrane a "wider band" of enzyme activity than that observed in the normal controls. Synovial vasculature also exhibited a more intense staining reaction.

TPNH-diaphorase

Localization and intensity of this enzyme activity was quite similar to that observed in DPNH-diaphorase preparations (Figure 61).

Lactic dehydrogenase

Localization and intensity of this enzyme activity was also quite similar to that observed in DPNH-diaphorase preparations (Figure 62).

Alkaline phosphatase

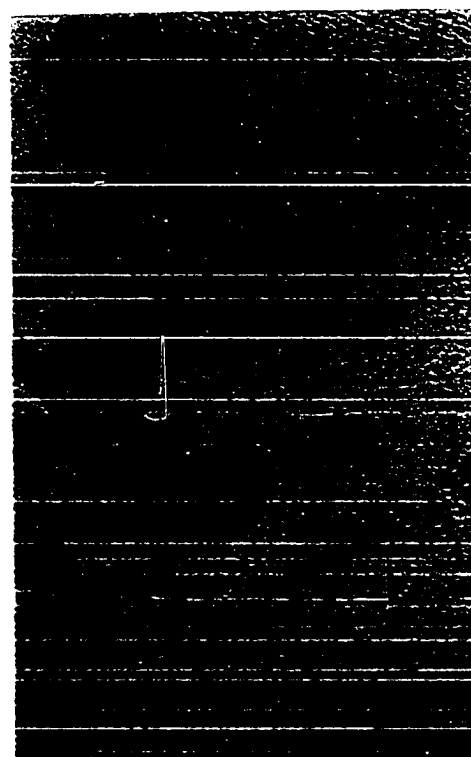
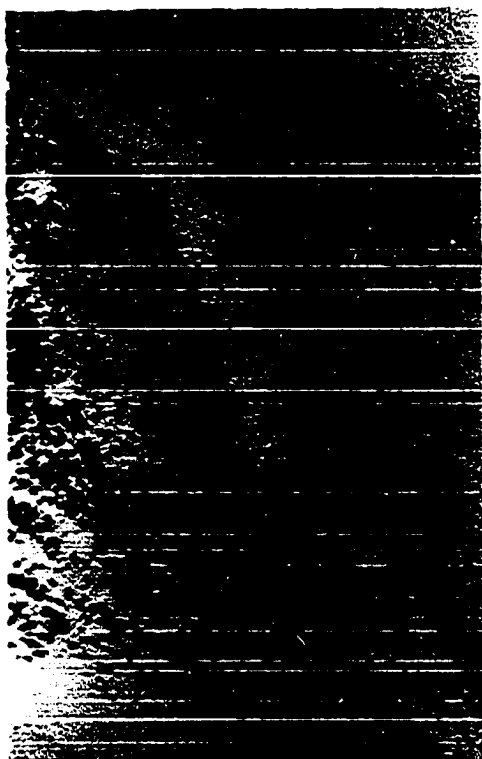
The difference between normal and inflammed tissues was quite striking (Figure 63). In addition to the numerous enzyme positive capillaries and vessels of the subsynovial tissue, neutrophils were quite high in enzyme activity.

The large numbers of enzyme positive neutrophils present in regions of advanced inflammation gave the predominantly enzyme negative tissue a "peppered" positive appearance.

Acid phosphatase

Synovial lining cells and proliferating subsynovial mesenchymal cells were quite high in enzyme activity even

- Figure 63. Upper left. Synovial membrane from M. hyorhinis infected pig. Synovial lining heavily infiltrated with enzyme positive neutrophils (B) some of which have escaped into the lumen of the joint cavity (A). Alkaline phosphatase. X 48
- Figure 64. Upper right. Synovial membrane from M. hyorhinis infected pig. Synovial lining cells containing high levels of enzyme activity. Acid phosphatase. X 130
- Figure 65. Lower left. Synovial membrane from M. hyorhinis infected pig. Thickened synovial lining containing moderate levels of enzyme activity. Succinic dehydrogenase. X 48
- Figure 66. Lower right. Synovial membrane from M. hyorhinis infected pig. Thickened synovial lining containing moderate levels of enzyme activity. Isocitric dehydrogenase. X 48



more so than that observed in normal control tissues (Figure 64).

Succinic dehydrogenase

When activity was observed, it was more intense than that observed in positive normal control tissues. Synovial lining cells were intensely stained in enzyme positive preparations from infected animals. The tunica media of blood vessels from these same tissues was the next most prominent site of enzyme activity.

Isocitric dehydrogenase

Enzyme intensity and localization was quite similar to that observed in succinic dehydrogenase positive preparations (Figure 66).

Infected Articular Cartilage-Morphology

The following changes, though inconsistent from one infected articulation to another, were observed. The nature of the specific articulation did not appear to have had an influence on the presence or absence of these changes.

Subarticular osteochondromyelitis characterized by the destruction and disappearance of chondroblasts from the chondrogenic region (Zone IV particularly) and the infiltration of bone marrow with large numbers of neutrophils was observed (Figures 67 through 69). Osteoclasts were present in moderate numbers along trabeculae of bone at the base of

Figure 67. Articular cartilage from M. hyorhina infected pig. Osteochondromyelitis extending outward into cartilage. Cartilage destruction is evident at top center and top right. Hematoxylin and eosin. X 48

Figure 68. Articular cartilage from M. hyorhina infected pig. Osteochondromyelitis and cartilage destruction resulting in reduced sulfation of degenerating cartilage at right. Alcian blue-alcian yellow. X 48

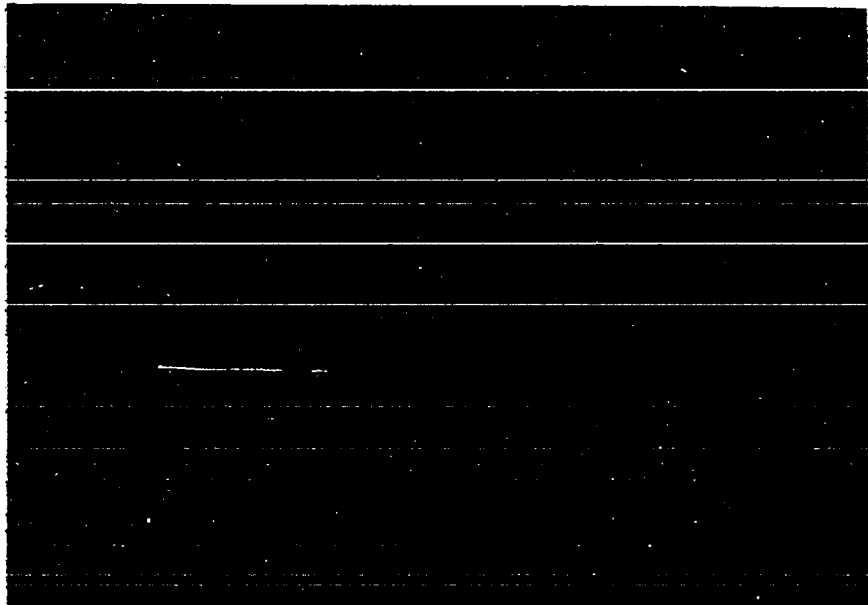
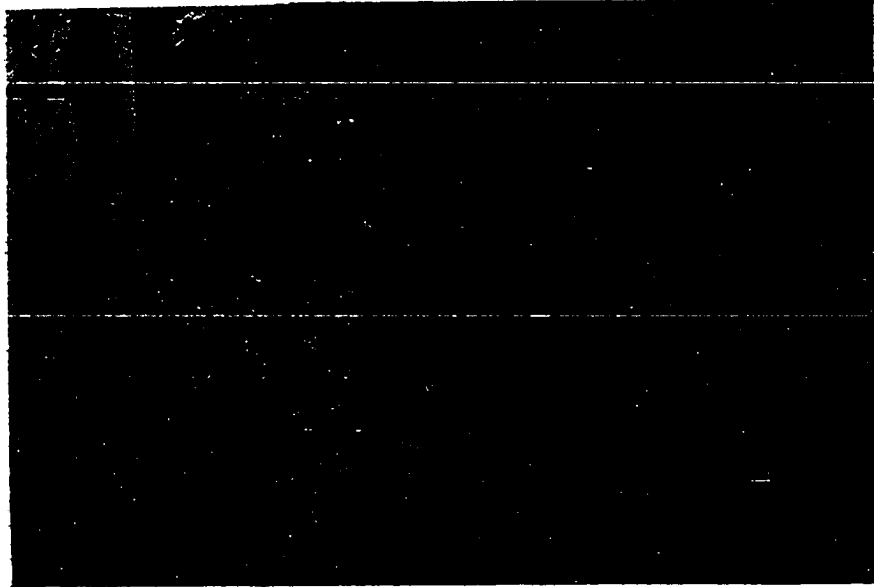
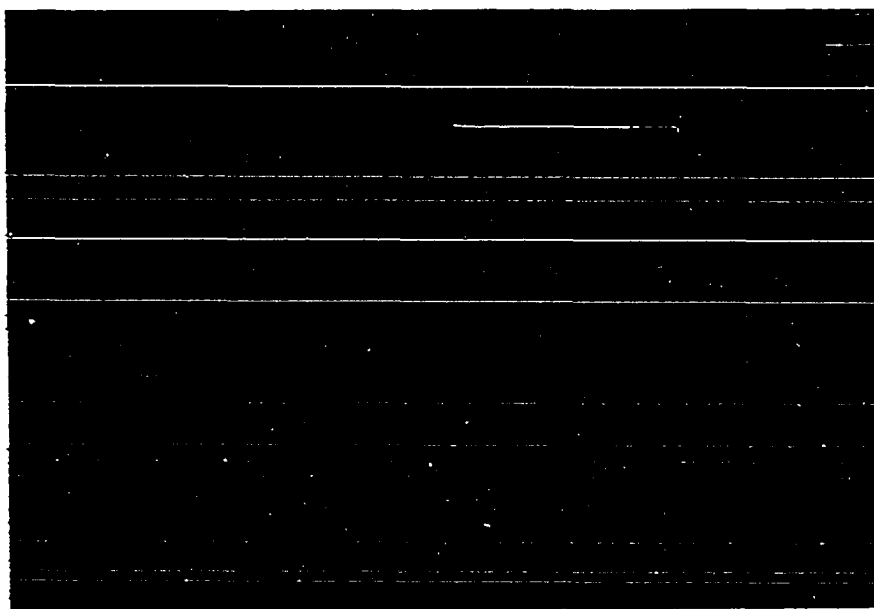
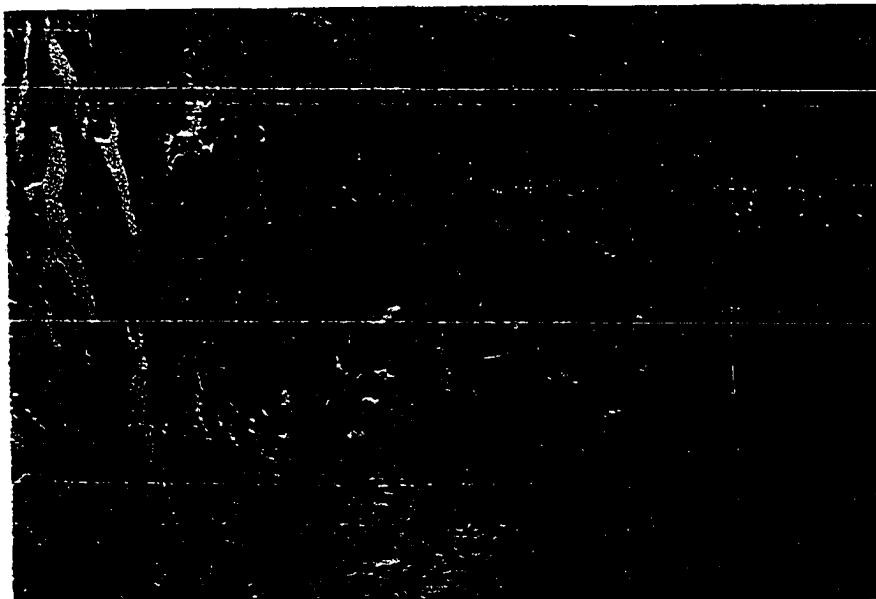


Figure 69. Articular cartilage from M. hyorhinis infected pig. Osteochondromyelitis and cartilage destruction resulting in reduced staining with safranin. Safranin O. X 48

Figure 70. Endochondral vasculitis and osteochondromyelitis of articular cartilage from M. hyorhinis infected pig. Vessels at left and right are infiltrated by inflammatory cells. Bone marrow and Zone IV are extensively infiltrated by neutrophils and mononuclear inflammatory cells. A few osteoclasts are evident. Hematoxylin and eosin. X 77



the chondrogenic region.

On occasion, cartilage destruction were observed along the route of the endochondral vasculature as it entered the articular cartilage from Hunter's circle or from the vascular network just below the chondrogenic region (Figures 70 through 72). This destruction was accompanied by replacement of matrix with fibrous connective tissue, neutrophils and other inflammatory cells. The superficial branches of some vessels seemed to "fill in" with matrix giving the overall impression that the cartilage came from a heavier weight group than it had in reality.

Infected Articular Cartilage-Acid Mucopolysaccharides

Alcian blue-alcian yellow

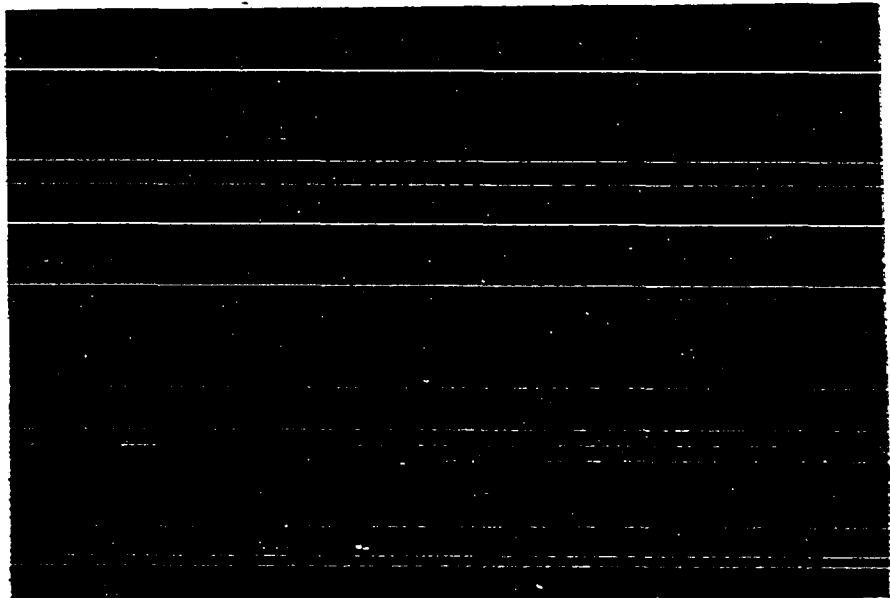
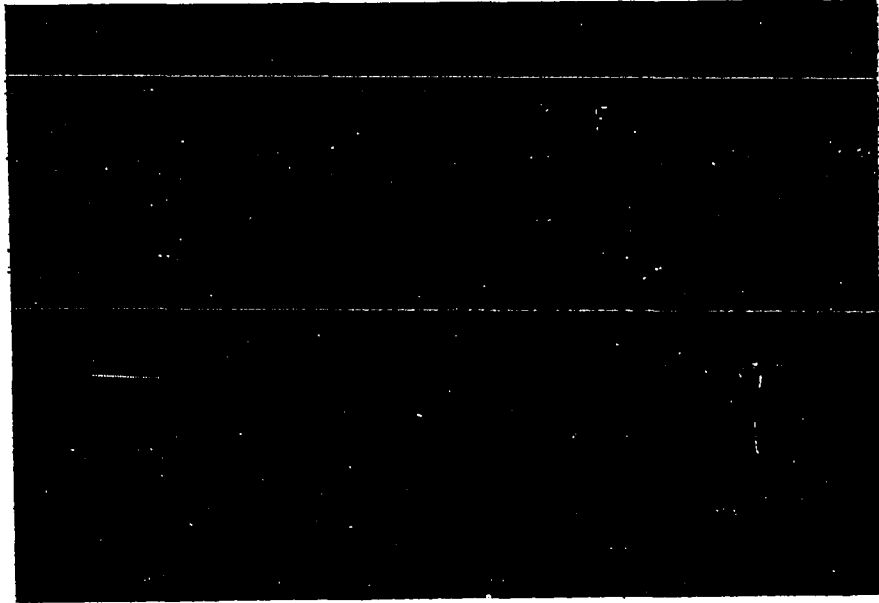
The only changes in localization of anionic groups were noted in areas of cartilage destruction. In areas where cartilage was replaced by connective tissue and inflammatory elements, sulfation of the tissue and matrix was considerably reduced (Figure 68). This reduction in sulfation was also observed in matrix surrounding endochondral vessels whose perivascular tissues were filled with inflammatory cells (Figure 71).

Safranin O

Sites of cartilage destruction and endochondral perivascular inflammation were manifested by reduced

Figure 71. Endochondral vasculitis and osteochondromyelitis of articular cartilage from M. hyorhina infected pig. Vessels at top center and bone marrow are infiltrated by inflammatory cells. Perivascular cartilage matrix shows reduced sulfation. Osteoclasts are evident in bone marrow and along bone trabeculae. Alcian blue-alcian yellow. X 77

Figure 72. Endochondral vasculitis and osteochondromyelitis of articular cartilage from M. hyorhina infected pig. Vessel at top center and bone marrow are infiltrated by inflammatory cells. Perivascular cartilage matrix shows reduced safranin staining. Osteoclasts are evident. Safranin O. X 77



safranin staining capability (Figures 69 through 72).

Infected Articular Cartilage-Enzyme Histochemistry

DPNH-diaphorase

The only variation in intensity or localization of enzyme activity in articular cartilage from infected animals was in the endochondral vasculature. Some endochondral vessels contained no enzyme activity whatsoever in contrast to that observed in control tissues (Figure 73).

TPNH-diaphorase

Observations on TPNH-diaphorase localization were similar to those described above under DPNH-diaphorase.

Lactic dehydrogenase

Observations on LDH localization were quite similar to those described above under DPNH-diaphorase (Figure 74).

Alkaline phosphatase

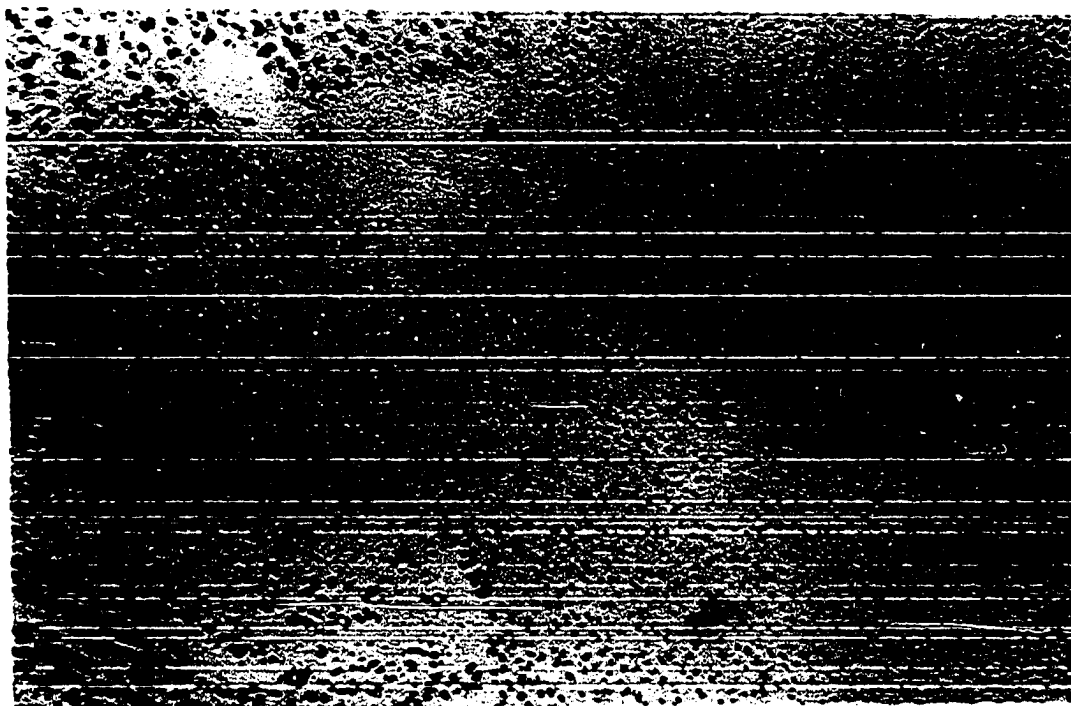
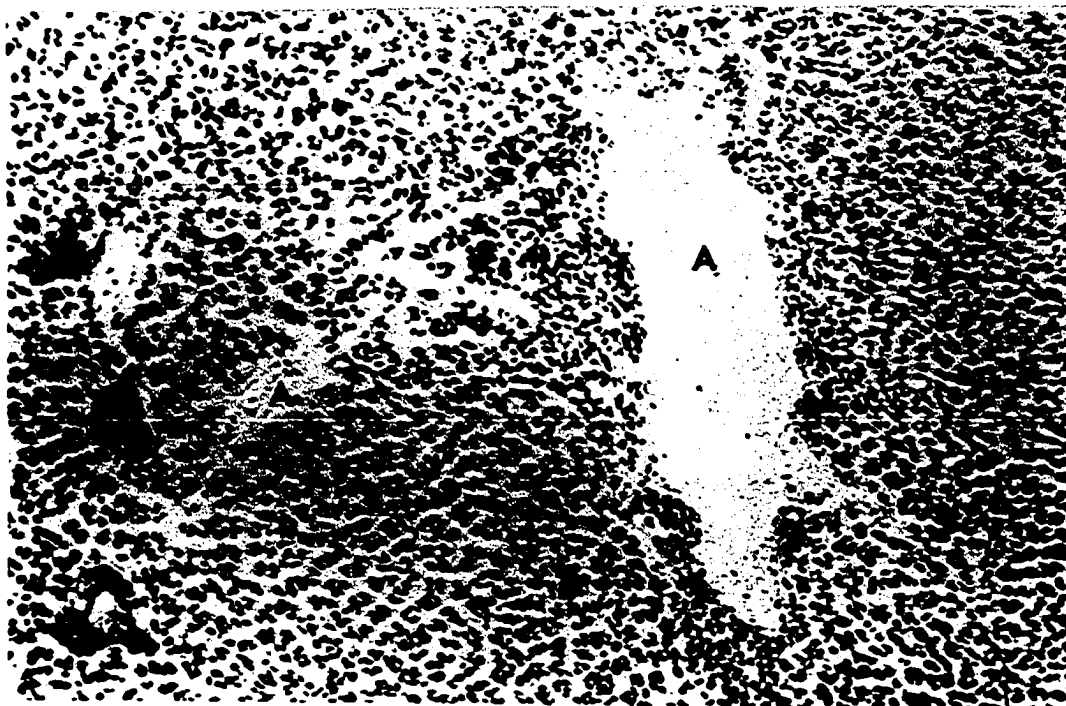
Differences in intensity or localization between infected and control tissues were not observed.

Acid phosphatase

Differences between control and infected tissues were not observed.

Figure 73. Articular cartilage from M. hyorhina infected pig. Endochondral vasculature (A) showing absence of enzyme activity in both vascular and perivascular tissue resulting in endochondral vessel "ghost". DPNH-diaphorase.
X 48

Figure 74. Articular cartilage from M. hyorhina infected pig. Endochondral vasculature (A) showing absence of enzyme activity in both vascular and perivascular tissue. Lactic dehydrogenase.
X 48



Succinic dehydrogenase

Differences between control and infected tissues were not observed.

Isocitric dehydrogenase

Differences between control and infected tissues were not observed.

DISCUSSION

Clinical and Gross Pathologic Observations
on Control and Infected Groups

Clinical and gross observations on M. hyorhinis infected swine were in agreement with those described by Roberts et al. (1963a, b) with the exception of the more chronic bone changes. Pannus and osteoporosis were not observed in this study probably because the pigs were not held on experiment for longer than 14 days.

Clinical Chemistry-LDH Activity

Intragroup comparisons

Total LDH In each of the three groups studied plasma was found to contain approximately 5-7 times the level of LDH activity observed in respective synovial fluids (Table 1). Cohen (1964) and Veys and Wieme (1968) in their studies of blood and synovial fluid in various human arthritides inferred that synovial fluid and blood LDH levels were approximately the same. This lack of agreement between human and swine data may be attributable to ontogenetic variformity (Hinks and Masters, 1966).

LDH isoenzyme distribution in plasma of the Slaughter, Control and Infected groups differed from that in respective synovial fluids (Figures 8, 10 and 11). Isoenzyme 4 (LDH₄) was higher in synovial fluid from Slaughter, Control and Infected groups than in plasma from these three groups.

Since each tissue of a species has a characteristic isoenzyme distribution (Plagemann et al., 1960), the presence of elevated LDH₄ levels may reflect the metabolic activity of the tissue of origin. Fine et al. (1963) and Agostini and Vergani (1966) describe specialized metabolic roles for the various LDH isoenzymes. Aerobic tissues such as heart muscle generally contain higher levels of anodal isoenzymes (LDH₁ and LDH₂) than anaerobic tissues such as skeletal muscle which is higher in the cathodal isoenzymes (LDH₄ and LDH₅). In the light of this information the presence of higher LDH₄ levels in synovial fluid may reflect the existence of predominantly anaerobic activity in normal articular tissues.

Plasma from Control and Infected swine were significantly higher in LDH₁ than were the respective synovial fluids (Figures 10 and 11) reflecting the greater role of aerobic metabolism in constituent sources of plasma LDH than in articular tissue.

Plasma and synovial fluid from Infected swine, in addition to the differences described above, differed in LDH₂ and LDH₅ content (Figure 11). Plasma was significantly higher in LDH₂, than was synovial fluid. Synovial fluid was higher in LDH₅ than was plasma. These shifts in distribution may reflect increased emphasis on aerobic metabolism in plasma and anaerobic metabolism in synovial fluid of Infected swine. The effect of infection on isoenzyme

distribution will be considered in greater detail later under Intergroup comparisons.

Intergroup comparisons

Total LDH There were differences in total plasma LDH activity among the three groups. Plasma from the heavier, more mature Slaughter animals had higher levels of total LDH activity than the lighter Control animals. As swine mature, total LDH levels in some tissues increase (Van den Hende et al., 1968). As tissue mass becomes greater, each tissue contributes a greater portion of activity to overall body LDH content. These increases in tissue levels of total LDH activity may be reflected in elevated plasma levels.

No significant differences in total plasma LDH levels were observed between Control and Infected groups, both of which were from the same weight class. Evidently weight had a greater effect on total plasma levels than infection with M. hyorhinis. Total blood levels of LDH are not altered in rheumatoid arthritis of man (Vesell et al., 1962).

A comparison of total synovial fluid LDH activity (Table 1) among the three groups indicated a considerable difference between Control and Infected animals. This difference was reflected as a significant increase in Infected synovial fluid LDH activity. Vesell et al. (1962), West et

al. (1963) and Veys and Wieme (1968) have reported increases in total LDH activity of synovial fluid from human patients with rheumatoid arthritis. Elevated levels in man have been attributed to increased production by synovial lining cells and/or contribution to total activity by leukocytes within synovial tissues and fluid (West et al., 1963; Jasani et al., 1967; Kerby and Taylor, 1967 and Veys and Wieme, 1968). Although pathologic swine synovial fluids were not routinely examined cytologically, gross turbidity of the majority of samples from infected animals and previous experience with abnormal fluids, suggests the presence of large numbers of leukocytes. On the basis of these observations the contribution of synovial lining cells and leukocytes to increased total activity in synovial fluid is quite probable.

Differences, between Slaughter and Control synovial fluid total LDH levels although significant, were not as great as between Control and Infected fluids. Higher synovial fluid levels in the heavier Slaughter group may be explained on a basis similar to that given for the higher plasma levels.

LDH isoenzyme distribution Comparisons of plasma isoenzyme distribution of the three groups revealed major differences between Control and Infected animals (Figure 9). Infected plasma LDH₁ was significantly lower than that of

plasma from Control animals. Infected plasma LDH₃ was significantly higher than that of plasma from Control animals. Although total plasma levels were unaffected by M. hyorhinis infection, there was a shift in isoenzyme distribution favoring anaerobic metabolic activity. This shift in response to M. hyorhinis infection may reflect altered cellular differentiation or metabolic events in LDH producing cells of pathologic serosal and articular tissues. Kjellberg and Karlsson (1966) observed altered isoenzyme patterns in milk of several species including swine during various periods of lactation in these animals. They attributed the shift in isoenzyme patterns to altered cellular differentiation and metabolic events in mammary tissues. Their findings are certainly applicable to this study in the light of morphologic and histochemical alterations observed in serosal and articular tissues of M. hyorhinis infected animals.

Comparison of plasma isoenzyme distribution in Slaughter and Control animals (Figure 7) revealed differences between the two groups. These differences did not reach statistical significance, however. Although statistical significance could not be achieved, discussion of these differences is warranted. With an increase in weight, plasma levels of LDH₁ dropped and LDH₄ increased. A shift in emphasis of plasma isoenzyme distribution from an anodal (LDH₁) to a

cathodal (LDH_4) type occurred. Similar findings are reported by Philip and Vesell (1962), Vesell and Philip (1963) and Fieldhouse and Masters (1966) in developing animal tissues and aging tissue cultures. From birth to maturity then, developmental changes in LDH isoenzyme distribution take the form of a shift in LDH from an aerobic type to an anaerobic one. The observed shift in swine plasma LDH isoenzyme distribution favoring anaerobic metabolism is believed to reflect normal developmental changes in swine tissues.

Comparison of synovial fluid isoenzyme distribution of Control and Slaughter groups (Figure 12) revealed differences which were not statistically significant. Although statistical significance was not achieved, the differences observed in LDH_1 and LDH_3 are worthy of comment. An elevation in LDH_1 of Slaughter synovial fluid appears to contradict the proposed maturation hypothesis; but upon closer investigation, this is not the case. The existence of an "embryonic" and "adult" form of LDH may explain the apparent contradiction. Vesell and Philip (1963) reported that frequently tissues containing predominantly anodal isoenzymes early in life contained predominantly cathodal isoenzymes later in life and vice versa. This led to the description of an "embryonic" and "adult" form of LDH. It is possible that the "adult form" of synovial fluid LDH does in fact contain

primarily anodal (LDH_1 and LDH_3) isoenzymes. In other words, "maturation" of swine synovial fluid LDH may take a direction opposite that observed in swine plasma.

The final comparison made was of synovial fluid isoenzyme distribution in Control and Infected animals (Figure 13). A significant drop in LDH_1 of Infected synovial fluid was coupled with a significant increase in LDH_3 . Increases in LDH_4 and LDH_5 of Infected synovial fluid, although not statistically significant, were observed. The overall picture obtained from these observations is a shift in anaerobic activity from one level to another of greater magnitude. Increased emphasis on anaerobic metabolic activity appeared to take place in synovial tissues as a result of M. hyorhinis infection. Similar, although not equivalent changes have been observed in human patients with various forms of arthritis. Vesell et al. (1962), Wieme (1963), Cohen (1964) and Veys and Wieme (1968) have all reported elevated levels of LDH_5 in various forms of arthritis including rheumatoid arthritis. The source of increased synovial fluid LDH activity has been attributed to synovial lining cells and/or leukocytes present in tissues and fluid. Another possible source must also be considered in the light of recent work by Tushan et al. (1969). Articular cartilage of several species has been found to contain high levels of LDH_4 and LDH_5 . These

isoenzymes may escape from articular cartilage in the early stages of joint inflammation and contribute to the observed shift in isoenzyme distribution.

A few additional points on LDH observations in this work should be discussed. During hemorrhagic shock and other stress situations, elevations in total LDH activity and isoenzyme levels have been reported by several workers. This condition has been noted to produce marked elevations in dogs after an initial lag phase of 1-3 hours (Vesell et al., 1959). Hemorrhagic shock was not produced in swine used in this study. Hessel-de-Heer (1968) reported the occurrence of elevated plasma LDH₅ levels in some breeds of pigs subjected to stress. She ascribed the elevation to a breed characteristic. She also reported that stunning had only a limited effect on plasma LDH₅ levels. Plasma LDH₅ levels of swine used in this study were not consistently elevated. Hyldgaard-Jensen (1967) reported a substantial increase in total plasma LDH activity of pigs during exsanguination. Although absolute values could have been affected in this study, comparative observations did not reflect an elevation. Hyldgaard-Jensen also reported elevated LDH₄ and LDH₅ levels in the plasma of exsanguinated pigs. Again, comparative studies did not reveal absolute LDH₄ and LDH₅ elevations attributable to exsanguination. The major point to be kept in mind is the use of standard,

uniform technical procedures throughout this study.

Clinical Chemistry-Protein

Intragroup comparisons

Total protein Total serum protein of the three groups was compared with that of respective synovial fluid (Table 3). Serum protein levels in the Slaughter and Control groups were from 5-8 times that observed in synovial fluid. Decker et al. (1959b) reported similar findings in studies on normal bovine serum and synovial fluid. Schur and Sandson (1963) in immunoelectrophoretic comparisons of normal human serum and synovial fluid reported marked reduction of large molecular weight proteins in synovial fluid. This probably accounts for the total protein gradient observed between normal serum and synovial fluid. Selective permeability of the synovial membrane prevents entrance of large molecular weight proteins into synovial fluid resulting in reduced total protein levels.

The total protein gradient between serum and synovial fluid of Infected animals was not as large as that observed in Slaughter and Control animals. Serum protein, although slightly elevated, was only about twice that observed in synovial fluid. Infection with M. hyorhinis apparently resulted in increased synovial membrane permeability reflected as an increase in total protein content of the synovial fluid. Elevations in synovial fluid protein have

also been described in erysipelatous arthritis (Crimmins and Sikes, 1965) and hydroarthrosis of swine (Bollwahn, 1967). The effect of infection on protein will be discussed in detail later under Intergroup comparisons.

Protein fraction distribution Serum protein fraction distribution was compared with that of synovial fluid in all three groups (Figures 21, 23 and 24). The migration patterns of synovial fluid proved to be identical to those of serum. Fraction distribution in Slaughter and Control serum was different from that observed in synovial fluid. In both groups synovial fluid albumen and α_1 globulin were higher and γ globulin lower than in serum. Significant differences in β globulin were not present. These findings are in agreement with human studies by Sandson and Hamerman (1958). They also describe lower α_2 globulin levels in normal human synovial fluid. Slaughter animals had lower levels of synovial fluid α_2 globulin, but Control synovial fluid levels were slightly higher. A possible weight effect may be present but this will be discussed in detail later under Intergroup comparisons.

Infected synovial fluid contained significantly higher levels of β globulin than serum. An increase in certain β globulin components has also been described in human inflammatory synovial fluids (Schur and Sandson, 1963). Albumen levels of serum and synovial fluid were the same.

A slight drop in synovial fluid albumen and elevation of serum albumen levels from the normal is possible in the light of a similar finding in human osteoarthritis (Sandson and Hamerman, 1958).

Although statistical significance was not achieved, differences between Infected serum and synovial fluid α_2 and γ globulin levels warrant brief mention. A downward shift in synovial fluid α_2 globulin levels resulted in Infected serum levels being higher than synovial fluid. The relationship of γ globulin in plasma and synovial fluid was maintained. The effect of M. hyorhinis infection on protein fraction distribution will be discussed in detail later under Inter-group comparisons.

Albumen/globulin (A/G) ratios of synovial fluid were generally higher than those of serum because of the higher albumen levels present in synovial fluid. Normal synovial membrane is more permeable to small molecular weight proteins such as albumen than to the larger globulins, therefore synovial fluid contains a higher proportion of albumen than globulins.

Altered permeability is readily observed by comparing Infected serum and synovial fluid A/G ratios. The values for these two fluids are almost the same, revealing reduced selectivity of the synovial membrane as a result of M. hyorhinis infection.

Intergroup comparisons

Total protein A comparison of total serum protein in the three groups (Table 3) revealed a weight effect and an effect produced by infection with M. hyorhinis. The heavier Slaughter animals had higher total serum protein levels than the lighter Control animals. This finding is in agreement with observations made in normal swine at various levels of maturity by Miller et al. (1961) and Tumbleson et al. (1967). Infected serum was also higher in total protein levels than Control serum reflecting increases in both anabolic and catabolic processes during the course of disease (Owen, 1967).

Comparison of synovial fluids from the three groups (Table 3) also reflect weight and infection effects. As expected, synovial fluid (a plasma dialysate) reflected the same changes seen in serum in response to weight differences and infectious disease.

Protein fraction distribution A comparison of Slaughter group and Control group serum (Figure 20) for weight effect reveals a great deal of similarity in sera of the two weight groups. Although statistical significance was not achieved, the minor differences observed should be noted. Higher albumen and γ globulin levels were present in the heavier Slaughter group. This finding is in agreement with Rutqvist (1958), Lecce et al. (1961), Miller et

al. (1961) and Tumbleson et al. (1969) who described albumen and γ globulin elevation in pigs during postnatal development. Differences in the remaining protein fractions of these two groups were minor.

Comparison of the protein fraction distribution of Control and Infected sera (Figure 22) revealed that M. hyorhinis infection produced a significant drop in serum albumen and elevation of serum γ globulin levels. Barden et al. (1968) have also reported elevations in serum γ globulin levels of M. hyorhinis infected swine. A very slight elevation in α_2 globulin was also noted in sera of Infected animals. Reduction of albumen and elevation of γ globulin have also been reported by Papp and Sikes (1964) and Sikes et al. (1966) in sera of swine with erysipelatous arthritis, edema disease (Vesselinovitch, 1955) a variety of acute swine diseases (Boguth, 1954) and in man with early rheumatoid arthritis (Bernstein and Allerhand, 1964). It is readily seen, that these nonspecific changes occur in a variety of disease conditions. Peterman (1961) ascribes these changes to an acute adrenal influence during infection. He states that adrenal hormones are probably responsible for shifts in protein fraction distribution during stressful situations such as disease and reflect the host response to disease. Elevation of γ globulin levels may also result from increased antibody production but generally the response is more marked and occurs later

in the disease process.

Slaughter and Control synovial fluid comparisons (Figure 25) revealed differences in the fluids of these two weight groups. Although not statistically significant, a moderate reduction in α_2 globulin and moderate elevation in γ globulin of Slaughter synovial fluid had occurred. These differences may reflect the effect of increasing weight on synovial fluid protein fraction distribution.

Comparison of Control and Infected synovial fluid (Figure 26) revealed a significant decrease in albumen with infection. This may reflect reduced serum levels of this fraction. Although not statistically significant, differences in β and γ globulin fractions were moderate. Elevated β and γ globulin levels were present in synovial fluid of Infected animals. Similar elevations of β globulins have been observed in human rheumatoid synovial fluid and attributed to degradation products of fibrinogen that migrate in the β region (Schur and Sandson, 1963) Riddle et al. (1965) have found fibrin to be a major factor in the acute inflammatory process seen in rheumatoid arthritis. Elevated γ globulin levels in synovial fluid from arthritic joints originate in plasma, but at least part of the γ globulin elevation may come from infiltrating lymphocytes and plasma cells present in inflamed synovial tissues (Wilkinson and Jones, 1962).

Comparison of A/G ratios (Table 4) in Slaughter and Control sera revealed that with increasing weight, A/G ratios decreased. The reduced A/G ratio of Slaughter serum probably reflects the decrease in serum albumen and increase in γ globulin levels that occurs with physiologic development of the animal.

A reduction in the A/G ratio of Infected serum also occurred. The A/G ratio of Infected serum was approximately $1/3$ that of the control. This again reflects the reduction in albumen and elevation of γ globulin that occurs during infectious disease.

Slaughter and Control synovial fluid A/G ratios were nearly equal. Weight evidently had no effect on the A/G ratio of synovial fluid.

Control and Infected A/G ratios were distinctly different. Infection reduced the A/G ratio considerably. This reduction reflects the decrease in albumen and elevation in globulins.

Clinical Chemistry-Glucose

Intra and intergroup comparisons of total glucose levels were made via plasma/synovial fluid ratios (Table 5). Slaughter and Control ratios were greater than 1.0 indicating the presence of higher glucose levels in plasma than in synovial fluid. Ropes and Bauer (1953) and Ropes et al. (1960) indicate that plasma and synovial fluid glucose levels

are the same in man and animals (a plasma/synovial fluid ratio of 1.0). The lack of agreement between these two studies is not completely understood although species and dietary factors play an important role in plasma (and probably synovial fluid) glucose levels. In this study weight had no apparent effect on plasma/synovial fluid ratios.

Infection with M. hyorhinis had a marked effect on the plasma/synovial fluid ratios. The Infected group ratio, being less than 1.0, revealed a marked reduction in plasma levels and a moderate reduction in synovial fluid levels. The plasma/synovial fluid ratio of Infected animals was approximately $\frac{1}{3}$ the Control ratio. A reduction in glucose levels of synovial fluid to $\frac{1}{2}$ or $\frac{1}{3}$ that of blood levels has been observed in rheumatoid arthritis of man (Hamerman et al., 1963). Crimmins and Sikes (1965) and Bollwahn (1967) reported no changes in synovial fluid glucose of swine with erysipelatous arthritis. The disparity between these studies is not understood, but as a result of work by Dingle and Page Thomas (1956) on human rheumatoid arthritis, alterations in glucose levels may be expected. They report an appreciable increase in glucose utilization (aerobic and anaerobic) in rheumatoid synovial membrane and theorize that elevated glucose utilization occurs as a result of an increased need for energy rich phosphate bonds required for

synovial proliferation. As a result of this information and the observations in this study, reduced plasma/synovial fluid glucose ratios are plausible in rheumatoid-like arthritis.

Clinical Chemistry-Hyaluronic Acid

Hyaluronic acid levels in synovial fluid of the three groups of swine (Table 6) were compared. No statistically significant difference was observed. Decker et al. (1959a) and Castor et al. (1966) indicate that hyaluronic acid levels are sensitive indicators of altered synovial tissue function, and Holland (1960) and Hollander et al. (1966) describe synovial fluid as a "liquid biopsy" that is too often neglected in diagnosis. Dintenfass (1963) described a reduction in viscosity of human rheumatoid synovial fluid, probably a result of reduced levels of functional hyaluronic acid.

Further work should be done with arthritic swine synovial fluid before negative findings are acceptable. Synovial fluid from acute M. hyorhinitis arthritis should be re-examined and synovial fluid from chronic M. hyorhinitis arthritis should be investigated, since alterations may not occur during the acute phase of this disease.

Histology and Histochemistry

Microscopic study of normal synovial tissue from Control and Articulation Study group swine of varying weight classes, disclosed an architectural pattern which did not vary significantly with intra-articular location or weight. Castor (1960) in a comparable study of normal human synovial tissue reported that neither age, sex nor intra-articular location had a significant influence on its architectural pattern.

Histochemical studies revealed no significant differences in localization of oxidative enzymes such as DPNH and TPNH-diaphorases, lactic, succinic or isocitric dehydrogenases with weight or intra-articular location. In each section of synovial membrane from Control and Articulation Study groups, the most prominent localization of these oxidative enzymes was in the cytoplasm of synovial lining cells. The lysosomal hydrolytic enzyme, acid phosphatase, was also prominently localized in synovial lining cell cytoplasm of normal swine synovial membrane. Histochemical localization of these enzymes closely paralleled findings by Hamerman and Blum (1959), Hamerman et al. (1961) and Shaw and Martin (1962) in normal human synovial membranes. The localization of the hydrolytic enzyme, alkaline phosphatase, was not reported by these authors, but its localization in normal swine synovial membrane was confined to endothelial cells lining capillaries and small blood vessels. The

morphology and histochemistry of normal swine synovial membrane in the light of these findings are quite similar to that of man. This similarity may be extended to synovial membrane activity. In man synovial membrane is quite active metabolically, as evidenced by high levels of enzyme activity in synovial tissue components, particularly the synovial lining cells.

Microscopic studies of normal swine articular cartilage have disclosed developmental changes. The progressive degeneration of endochondral vasculature paralleling an increase in body weight was observed in articular cartilages of the Articulation Study, Control and Infected groups and is probably a normal developmental alteration. Histochemical localization of DPNH and TPNH-diaphorases, lactic, succinic and isocitric dehydrogenases, and acid and alkaline phosphatases in swine cartilage was confined almost exclusively to chondrocyte cytoplasm. This localization was quite similar to that observed in other species including man (Follis and Berthrong, 1949; Balogh et al., 1961; Shaw and Martin, 1962; and Greenspan and Blackwood, 1966). Localization of these enzymes in chondrocytes as in synovial lining cells is reflective of the level of metabolic activity present in this tissue.

Acid mucopolysaccharide localization in normal articular cartilage varied only slightly. This variation was char-

acterized by a heterogeneity of anion group localization in the various morphologic strata of articular cartilage and a reduction in sulfation of matrix surrounding endochondral vasculature. These variations were attributed to weight differences and varying levels of tissue maturity.

One of the earliest changes observed in M. hyorhinis infected swine synovial membrane was hyperemia and edema. During the septicemic phase of this infection, alterations in capillary permeability occur. The specialized nature of the synovial microvasculature and its relatively large fenestrations (Duncan and Ross, 1969 and Schumacher, 1969) permits the escape of high molecular weight proteins and possibly mycoplasmal organisms during these initial inflammatory stages of the disease. Fibrinogen and numerous enzymes gain entrance to the synovial fluid bathing articular cartilage and synovial lining cells. The next most prominent changes observed in M. hyorhinis infected synovial membrane are synovial lining cell hypertrophy and hyperplasia. Similar proliferation has been experimentally induced by intra-articular inoculation of large molecular weight polysaccharides such as carageenin (Thomas et al., 1960). Degradation products of large molecular weight polysaccharides such as hyaluronic acid, chondroitin sulfate or keratan sulfate from synovial tissue, synovial fluid or articular cartilage may in part be responsible

for synovial lining cell proliferation. Other materials may also stimulate lining cell hyperplasia. Weissman et al. (1965) induced synovitis (including lining cell hyperplasia) with lysosomal enzymes. Cells damaged during the exudative phase of inflammation may have released lysosomal enzymes resulting in a hyperplastic effect on the synovial lining.

Inflammatory exudate appears to play an important role in arthritis. Phillips et al. (1966) injected synovial fluid from acutely arthritic rabbits into normal rabbits and produced acute arthritis. Whatever the materials, degradation products or enzymes, they play an important role in synovial membrane alterations during early arthritis.

The fine structure of M. hyorhina infected synovial lining cells is altered (Duncan and Ross, 1969) and not unlike that observed in rheumatoid arthritis of man (Wyllie, et al., 1966). Fibrin-like material is often observed within these altered cells (Norton and Ziff, 1966 and Duncan and Ross, 1969) and probably has an active role in inflammation of the synovial membrane. Riddle et al. (1965) discussed the role of fibrin in eliciting an augmented emigration of neutrophils into rheumatoid arthritic tissues.

Neutrophils, although present in the later stages (after fibrin is visually present in tissues and synovial fluid) of M. hyorhina arthritis, are preceded by emigration

of lymphocytes, histiocytes and plasma cells, again not unlike the response observed in human rheumatoid arthritis.

Monocytes and lymphocytes have been shown to phagocytize mycoplasmal organisms in vitro, and these organisms have been shown to survive unchanged in these cells for up to 3 hours (Zucker-Franklin et al., 1966b). Neutrophils on the other hand have been shown to degranulate and destroy phagocytized mycoplasmas in vitro (Zucker-Franklin et al., 1966a). Although these studies were conducted in vitro, the findings may have significant pathologic implications and lend themselves to the following hypothetical discussion.

The early lymphocytic and mononuclear response may introduce mycoplasmal antigens to antibody producing mechanisms, thus initiating early antibody production. The presence of fibrin in the tissues following the mononuclear phase elicits a neutrophilic response. Mycoplasmal organisms remain viable in lymphocytes while lymphocytes continue to produce antibody and phagocytize organisms. Degradation products of fibrin and other materials from the inflammatory process are also phagocytized. Interaction of mycoplasmal antigens and products of inflammation may occur. Lymphocytes and plasma cells may now produce antibody against a compound antigenic structure part of which may be "self". Neutrophils that have phagocytized mycoplasmal organisms and fibrin

contain the necessary enzymes to degrade these engulfed materials. In the light of these findings possibly a self perpetuating chronic lesion may arise on the basis of an autoimmune-like response to the inflammatory exudate irrespective of the initial cause of the inflammation.

Concurrent with exudation of leukocytes and fibrin, proliferation of fibroblasts in the sublining cell connective tissue occurs. Smyth et al. (1962) suggest materials such as serotonin and naturally occurring amines play a role in connective tissue proliferation in joints.

Histochemical studies indicated a more intense localization of DPNH and TPNH-diaphorases lactic, succinic and isocitric dehydrogenases and alkaline and acid phosphatase in synovial lining cells of M. hyorhinis infected synovial membranes than in normal membranes. This finding is not unlike that described in human rheumatoid synovial membrane (Hamerman et al., 1961 and 1963). Increased enzyme localization of proliferated lining cells is probably the result of increased metabolic activity of these cells.

With the exception of perivascular cartilage and bone marrow changes associated with articular cartilage from Infected animals, no other alterations were observed either histologically or histochemically. Interesting pathologic alterations of cartilage surrounding areas of endochondral

vasculitis were found. Absence of enzyme activity was generally noted in chondrocytes surrounding the involved vessels. This may have occurred because the chondrocytes were no longer functional. AMPS were also reduced in these regions. A number of workers have shown the matrix depletion effect of leukocytes (Ziff et al., 1960 and Curtiss and Klein, 1963). Lysosomal enzymes are thought to be the main constituent of leukocytes which effects cartilage matrix breakdown (Dingle, 1962 and Weissman and Spilberg, 1968). Loss of AMPS have also been shown in traumatically damaged articular cartilage (Meachim, 1963).

Coupled with endochondral vascular changes inflammation of the bone marrow characterized by large numbers of neutrophils was observed. The inflammation extended into articular cartilage via endochondral perivascular extension and was the source of the inflammation described above. Slight osteoclastic activity was observed along the base of the articular cartilage but had no demonstrable effect on histochemical AMPS or enzyme localization.

The following goals have been achieved in this investigation:

1. Histologic, histochemical and certain clinical biochemical parameters of normal swine joints have been established.

2. A better understanding of the pathogenesis of M. hyorhinis arthritis in swine has been obtained.
3. A greater similarity between M. hyorhinis arthritis in swine and human rheumatoid arthritis has been realized.

With the achievement of these goals, it is hoped effective control of M. hyorhinis infections of swine may soon be a reality and that M. hyorhinis arthritis of swine may serve as a suitable model for studies on human rheumatoid arthritis.

SUMMARY

The characterization of normal and M. hyorhinis infected swine articular tissues and body fluids was accomplished by examination of several biochemical, histologic and histochemical parameters.

Total LDH levels of normal plasma were approximately 5-7 times that found in respective synovial fluid. The effect of increasing weight on total LDH was to produce an elevation in plasma and synovial fluid levels in the heavier animals. Infection with M. hyorhinis had no effect on total LDH of plasma but produced a considerable increase in total LDH activity of synovial fluid.

LDH isoenzyme distribution in normal plasma differed from that of synovial fluid. LDH₄ of synovial fluid was present at higher levels than in respective plasma. The effect of weight on isoenzyme distribution was to produce an increase in plasma LDH₄ and synovial fluid LDH₁ and LDH₃. Normal developmental changes in swine LDH are evidently composed of a shift from an aerobic type of plasma LDH to an anaerobic type. Developmental changes in synovial fluid LDH reflected a shift toward an aerobic type of LDH. Infection with M. hyorhinis resulted in an intense shift in plasma isoenzyme distribution toward an anaerobic type of LDH. Injury produced by M. hyorhinis infection may

result in an altered metabolic function of major LDH producing tissues. An increased emphasis on anaerobic metabolic activity appears to have taken place in synovial tissues as a result of M. hyorhinitis infection. The emphasis is characterized by a reduction in LDH₁ and increase in LDH₃ of synovial fluid from infected swine.

Total serum protein levels of normal swine were 5-8 times those observed in synovial fluid. The effect of weight on total protein was to produce an elevation in serum and synovial fluid with increasing weight. Infection with M. hyorhinitis resulted in elevations in both serum and synovial fluid protein levels..

Protein fraction distribution in normal serum differed from that of synovial fluid. Albumen and α_1 globulin levels of synovial fluid were higher and γ globulin levels were lower than those of normal serum. The effect of weight on serum fractions was slight. Albumen and γ globulin levels were only slightly higher in the heavier animals. Infection with M. hyorhinitis resulted in a reduction in albumen and elevations in γ globulin levels of serum not unlike the changes seen in a variety of other infectious diseases. Synovial fluid albumen levels decreased and β and γ globulin levels increased with infection.

Plasma/synovial fluid glucose ratios were quite similar in both weight groups. Reduction of this ratio in M.

hyorhinis infected swine to approximately 1/3 of normal levels was observed. The reduction reflects increased glucose utilization by inflammed synovial tissues. Hyaluronic acid levels of synovial fluid were the same regardless of weight or the existence of M. hyorhinis infection.

Histologic and histochemical alterations coincident with a change in weight were developmental in nature. The developmental changes occurred in articular cartilage and were characterized by progressive endochondral vascular degeneration and altered acid mucopolysaccharide localization of surrounding cartilage matrix.

Histologic and histochemical alterations in M. hyorhinis infected articular tissues were observed. The morphologic changes were characterized in the earliest stages by exudative inflammation followed by a mononuclear and then neutrophilic response in synovial membranes. Increased metabolic activity of synovial lining cells was evidenced by intensification of enzyme localization in histochemical preparations. Osteochondromyelitis and endochondral vasculitis were observed in articular cartilage. Alterations in enzyme and AMPS localization occurred in the affected regions of articular cartilage.

A marked similarity between histologic and histochemical changes observed on M. hyorhinis arthritis of swine and

human rheumatoid arthritis exists. This similarity can also be extended to several of the clinical biochemical parameters examined in this study.

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