A STUDY OF BOVINE SYNOVIAL FLUID FROM THE CLINICALLY NORMAL TIBIO-TARSAL ARTICULATION

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ABSTRACT

A STUDY OF BOVINE SYNOVIAL FLUID FROM THE CLINICALLY NORMAL TIBIO-TARSAL ARTICULATION

by Rollo Winslow Van Pelt, Jr.

An investigation has been made of certain aspects and properties of synovial fluid from the clinically normal bovine tibio-tarsal articulation. Studies of blood and plasma sugars in relation to synovial fluid sugars in fasting slaughter cattle and non-fasting cattle of mixed breeds, ages, and sexes have been conducted. A laboratory analysis and observation of the following synovial fluid properties has been made: (1) synovial fluid sugar levels; (2) total, absolute, and differential counts of normal synovial fluid leukocytes; (3) relative viscosity; (4) quality of mucin clot as determined by precipitation of synovial fluid with 7N glacial acetic acid; (5) nature of the synovia at the time of collection, viz., color, absence and/or presence of flocculant material in the synovia, and degree of opacity.

A review of the literature revealed few references on the properties of bovine synovial fluid. References in veterinary literature were limited primarily to gross

observations. The diagnosis, prognosis, and evaluation of synovial fluid from diseased joints in man, as well as the evaluation of anti-arthritic and anti-bacterial agents, has been reported to be of practical importance.

Blood and synovial fluid samples were obtained from two sources: (1) cattle that had been fasted 12 to 18 hours prior to the time of slaughter; (2) cattle that were on full feed at the time of sample collection. All blood, plasma, and synovial fluid sugars were determined by a modification of the Folin-Wu method. Total synovial fluid leukocyte counts were made with the use of a standard hemacytometer. Differential cell counts for synovia were made from smears stained with Wright's stain. The relative viscosity of synovial fluid was determined at 101.5 degrees fahrenheit in a Krebs Constant Temperature Bath employing either the Cannon-Fenske routine or Cannon-Ubbelohde semi-micro dilution viscometers. Quality of synovial fluid, i.e., degree of polymerization, was determined by a method of precipitation with 7N glacial acetic acid.

Blood, plasma, and synovial fluid sugar levels were determined for both the fasting slaughter cattle and non-fasting cattle in this study. From a diagnostic standpoint, it is the relationship between blood and/or plasma sugar levels to synovial fluid sugar levels that form a useful criteria in the diagnosis and prognosis of joint

diseases. The results revealed that whole blood sugars in both groups of cattle most consistently paralleled synovial fluid sugars and that plasma sugars greatly exceeded those of blood and synovia, as well as exhibiting a greater degree of fluctuation in relation to the latter.

Cellular studies brought to light the low total number of leukocytes in the synovia of the tibio-tarsal articulation. Differential studies made on stained smears of synovia in this investigation reveal that lymphocytes and monocytes constitute the major cellular elements, followed by clasmatocytes and neutrophils, with eosinophils rarely observed.

Age and sex of the individual influenced the results of blood, plasma, and synovial fluid sugars, as well as the relative viscosity and quality of mucin clot.

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Dedicated to my wife,

Karen

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CHAPTER I

INTRODUCTION

To date there has been little or no research in veterinary medicine designed to arrive at either a partial or complete analysis of bovine synovial fluid. Therefore, this study was designed to determine certain aspects of normal bovine synovial fluid. Once a true picture of the findings in normal synovial fluid is obtained, it could then be used to evaluate the various pathological processes encountered in diarthrodial articulations.

The synovia of diarthrodial articulations serves not only as a source of nourishment to the articular surfaces, but also plays a mechanical role by the very nature of its mucopolysaccharide content. It is conceivable then that normal synovial fluid reflects a joint that is functioning in a normal mechanical and physiological manner. On the other hand, joint fluid would serve to reflect changes produced in the synovial membrane and articular cartilages by disease processes. An abnormality in the exchange of substances between blood and/or lymph and the joint fluid would be reflected in the fluid as an intra-articular disturbance of normal metabolism.

The information gained from analysis of synovial

fluid would be of value in diagnosis and prognosis of joint disease as well as a means of evaluating anti-arthritic (particularly the adrenocortical steroids and their synthetic analogs designed for intra-articular use) and antibacterial agents.

The combination of clinical and laboratory findings would be of unequivocal value in making a differential diagnosis of the various arthridites encountered in bovine practice.

With these thoughts in mind, this study was conducted in an effort to find a series of laboratory tests that could eventually be used by the bovine practitioner as an aid to making a diagnosis and evaluation of the pathological state of the joint or joints in question. These tests should be designed so that they could be easily and inexpensively carried out without the use of extensive laboratory facilities.

This study was then designed to determine the following values for bovine synovial fluid in animals of all sexes, ages, and breeds, from the clinically normal tibio-tarsal articulation: (1) blood, plasma, and synovial fluid sugar levels in both fasting slaughter cattle and non-fasting cattle; (2) total, absolute, and differential count of normal synovial fluid leukocytes; (3) relative viscosity; (4) quality of mucin clot as determined by

precipitation of synovial fluid with 7N glacial acetic acid; (5) nature of the synovia, i.e., color, absence or presence of flocculant material, and degree of opacity.

CHAPTER II

REVIEW OF THE LITERATURE

Introduction

A review of veterinary literature on the subject of synovial fluid serves only to reveal the paucity of information on this subject. To date the work done in this field has been scant and inconclusive. The review to follow will serve to acquaint the reader with the basic fundamentals essential for a better understanding of the role synovial fluid plays in maintenance of a normal physiological balance in diarthrodial articulations.

Gross and Microscopic Anatomy

Diarthrodial articulations have been defined by Sisson and Grossman (1950) as those joints characterized by the presence of a joint cavity with a synovial membrane in the joint cavity and by their mobility. They are often called movable or true joints.

The articular surfaces of the bones are covered by a layer of hyaline cartilage which provides a smooth gliding surface when movement occurs. The bones of the joint are maintained in direct apposition by ligaments which

pass from bone to bone around the joint. This relationship thus forms a cavity bounded on the ends by the articular cartilages of the bones and surrounded by the synovial membrane.

A negative intra-articular pressure aids in maintaining the articular surfaces in apposition and drawing the villous projections of the synovial membrane in around them.

Ham (1957) described the synovial membrane as smooth, glistening, and well supplied with blood vessels, lymphatics and nerves. The lining cells are called synovial cells and are of mesenchymal origin. Due to the smooth continuity of the synovial membrane, it has the appearance of being a continuous membrane; however, there are numerous collagenous fibers which lie among the synovial cells and help make up the membrane, especially in the deeper structures.

Not all authors classify or term the cells lining the membrane as synovial cells. Arey (1957) described this tissue as a loose vascular layer, surfaced discontinuously with fibroblasts, sometimes called a false epithelium because it is a layer made of white fibers and scattered, flattened fibroblasts, hence he terms it a connective tissue rather than epithelium.

Trautmann and Fiebiger (1957) likewise described the synovial membrane as a layer of flattened fibroblasts resting on a collagenous connective tissue.

Synovial villi and Haversian fringes are descriptive terms applied by Copenhaver and Johnson (1958) to the synovial processes that project into the joint cavity. Some of these are non-vascular and consist mainly of stellate cells similar to those of the synovial membrane. Others have a distinct core of fibrous tissue containing blood vessels and covered with stellate connective tissue cells. From the primary villi, small secondary non-vascular villi are frequently given off. The larger blood vessels of the joint capsule lie in the outer layer of the stratum synoviale where smaller vessels and capillaries pass to the inner layer and some to the villi. The inner layer is richly supplied, and the outer layer poorly, with lymph capillaries. Non-medulated nerve fibers are found in the connective tissue, some of them ending in Pacinian corpuscles.

Samuel (1950) made a study of twenty normal human knee joints and revealed that the nerves accompanying the blood vessels in the articular capsule migrate from one to another, forming a coarse network of nerve bundles in the capsular ligament and a more delicate network of individual fibers in the synovial membrane. His observations showed that the synovial membrane is only slightly sensitive to pain and does not give rise to pressure sensations. He concluded that it would then appear that almost all sensory impressions from the articular capsule arise in the fibrous capsular ligament.

Bunn and Burch (1955) observed intra-articular pain in horses, lasting from eight to twenty-four hours following arthrocentesis. They attributed pain to increased intra-articular pressure as a direct result of air intake due to a greater negative pressure following withdrawal of synovial fluid.

The hyaline cartilage that covers the ends of the bones is free of vascular supply and receives its nourishment by osmosis. Maximow and Bloom (1960) have shown that articular cartilages are intimately adhered to a layer of compact bone, lacking haversian systems, possessing large lacunae, and free of canaliculi. At the base of the hyaline cartilage, a small area of perichondrium is reflected backward into the membrane of the joint capsule. At this point there are many cartilagenous cells extending into the synovial membrane. They noted that there is an increase in size and number of villi with age, with new islets of cartilage being formed in them mainly by metaplasia of the synovial fibroblasts. Johnson (1959) noted that tiny granules and threads from the superficial cartilage zone were commonly found in normal synovial fluid.

Reformation of synovial membranes following synovectomy in the rabbit was demonstrated by Key (1925). It was shown that following removal of a portion of the synovial lining that there was a rapid deposition of fibrin which was quickly organized by young connective tissue cells

from the fibrous capsule. These were soon differentiated into synovial cells so that in sixty days, the newly formed synovial lining could not be differentiated from the undamaged area.

Synovial tissues apparently play an active role in joint metabolism. Wilson, et al. (1956) in a study of the metabolites of intra-articular cortisone and hydrocortisone found this tissue to be quite active. Cortisone in the joint must be converted to hydrocortisone in order to develop anti-inflammatory activity and this reaction cannot be affected to a sufficient degree by the synovial tissues. An alternate to this theory would be that both cortisone and hydrocortisone must be transformed into available products in order to exert their effects and that synovial tissue can make this substance from hydrocortisone, but not cortisone. The metabolites of cortisone are different than those of hydrocortisone.

Harris, et al. (1958) injected radioactive sodium into the knee joint of patients with rheumatoid arthritis and showed that reduced clearance rates occurred in the presence of hydrocortisone. These falls in clearance rates usually coincided with good clinical response when compared with subjects who showed no significant fall in clearance rates of radioactive sodium.

The author (1960) has stated previously that intra-articular adrenocortical steroids exert their greatest effect

upon the synovial membrane and as a consequence change the character of the synovial fluid. Changes in the state of the synovial fluid act as an index to determine the efficacy of the particular steroid employed.

Physiology of Synovial Structures

Gardner (1959) observed that the term synovia was introduced in the sixteenth century by Paracelsus (1493-1541) who used it to refer to various body fluids. Its use is now confined to the fluid present in certain joints (presumably in bursae and synovial tendon sheaths also), which are consequently termed synovial.

In 1887, McFadyean, (Hughes and Dransfield, 1953) an English veterinary anatomist, described synovial fluid as the glairy, straw-colored fluid which plays the part of a lubricant to the articular surfaces. Although somewhat oily in appearance, due to traces of fat, its glairy character was probably due to mucin and albumin.

Synovial fluid, as might be expected from the hypothesis of Ropes and Bauer (1953), is a tissue fluid, showing changes with disease which reflect alterations in the synovial tissues and intra-articular metabolism. The normal exchange of substances between the vascular and lymphatic system may be disturbed or there may be altered utilization, formation or destruction of various constituents

within the joint. Examination of synovial fluid should therefore yield information concerning the type and degree of change in the joint.

Davies (1944) in studies of synovial fluid from the tibio-tarsal articulation of clinically normal cattle, observed that approximately 80 per cent were colorless and that the remainder showed a slight tinge of yellow pigment, though a deep yellow coloration was most unusual. No attempt was made to determine the nature of the pigment or pigments present.

The volume of the synovial fluid of cattle taken immediately after slaughter is quite large and Meyer (1947) reported volumes sometimes over 50 cc. per joint. He suggested the possibility of the fluid actually being a mixture of synovial and edematous fluids because of long periods of standing and trauma (rough handling) of animals before slaughter.

Normal human synovial fluid does not clot as Ropes, et al. (1940) discovered. They postulated that this was presumably due to the absence of fibrinogen, since no fibrinogen was found by precipitation with 1.1 M phosphate solution at pH 6.5.

Synovial fluid has been defined by Bauer, et al. (1940) as a protein-containing dialysate of blood plasma to which mucin, secreted by the synovial cells, is added as the plasma water diffuses through the synovial tissue

spaces into the larger tissue space, the joint cavity. Ropes, et al. (1939) in making a comparison of joint fluid with that of plasma indicated that non-electrolytes diffused readily in either direction between blood and synovial fluid and that electrolytes were distributed in accord with the Gibbs-Donnan theory of membrane equilibrium.

Synovial membrane permeability was defined by Moffett (1954) as the capacity of synovial tissues to allow the passage of fluids and colloids in either direction between the joint cavity and the synovial blood vessels, lymphatics and surrounding tissues. Sunderman and Boerner (1950) maintained that synovial fluid keeps in close equilibrium with the blood stream, owing to the fact that the synovial membrane is very permeable as compared with the choroid plexus or the glomerular membrane.

The properties which distinguish synovial fluid from dialysates of blood plasma are attributable to mucin. This characteristic component of synovial fluid has been found to be a protein-polysaccharide complex or hyaluronic acid, that is produced by the synovial cells lining the membranous portion of the joint cavity. Kling, et al. (1955) observed the presence of hyaluronic acid in about two-thirds of the supernates of tissue cultures from synovium. This was demonstrated by precipitation reactions with acetic acid, enzymatic reactions, viscosity determinations, tests for metachromasia, and electrophoretic patterns. Control

cultures of periarticular connective tissue were negative for hyaluronic acid and sulfonated mucopolysaccharides.

Castor (1959) has demonstrated the in vitro synthesis of hyaluronic acid by cultures of human synovial cells. Paul (1959) stated that synthesis of a few structural elements in vitro can occur, particularly that certain cultured cells can synthesize large amounts of mucopolysaccharides.

Asboe-Hansen (1954) in previous work does not agree with this concept. His work has shown that whenever mast cells are found in the body, there is hyaluronic acid production; thus with an increase in number and size of these cells, there is a corresponding increase in the secretion of the mesenchymal muco-polysaccharide-hyaluronic acid complex. In conclusion he postulated that secretion of this complex was influenced by a heparin-like precursor. The protein content of bovine hyaluronic acid (protein-polysaccharide) complex has been identified by Curtain (1955) as being derived from serum and composed largely of alpha globulin, with smaller amounts of beta and gamma globulin as determined by immunological and electrophoretic studies.

Neuhaus and Letzring (1958) in a later study observed that the proportion of albumin is greater in human and bovine synovial fluids than in their respective serums, suggesting that a slight generalized permeability (or perhaps selective permeability) exists for molecules of a

molecular weight of 65,000 or less.

In cases of arthritis, Platt, et al. (1957) have shown that the proteins of synovial fluid may resemble those of the blood serum and that the electrophoretic behavior during the active stages of the disease more closely approach that of blood.

The polysaccharide component has been identified by Meyer, et al. (1939) as hyaluronic acid, a non-ramified polymer of D-glucuronic acid and N-acety-D-glucosamine in equimolar amounts, connected through beta-glucosidic 1-3 linkages. These workers determined that this polymer, i.e., the polysaccharide, was composed of equimolar parts of hexosomine, a hexuronic acid and acetyl. The hemosamine has been demonstrated as D-glucosamine, and the uronic acid as glucuronic acid.

Meyer (1947) in later work stated that the gel-like consistency that hyaluronic acid produced in normal joint fluid, also acts to protect surfaces and forms a viscous barrier which also serves to regulate the exchange of metabolites and water.

Spector (1956) reports an average pH of 7.31 with a range of 7.27 to 7.43 for synovial fluid from the tibiotarsal articulation of normal cattle. In an extensive study involving the canine species, Joseph, et al. (1946) showed that the pH of joints or venous blood is more acid during exercise and that blood pH falls more rapidly than that

of the joint during exercise. Their studies involving intravenous administration of acidic and basic solutions to dogs brought to light several facets of ion exchange between blood and the joint fluid. Following intravenous injections of weak solutions of Na_2CO_3 the blood pH rose rapidly but soon returned to normal, while joint pH rose more slowly and reached a maximum later than blood pH. Studies on the effect of acids revealed the fact that an intravenous injection of 65 cc. of a five per cent solution of NH_4Cl caused blood pH to drop from 7.4 to 7.08 in four minutes, with recovery occurring in twenty minutes. Meanwhile the joint pH fell from 7.5 to 7.25 in ten minutes and did not recover in twenty minutes. In conclusion, they observed that exercise played a major role in the pH of synovial fluid and that exercise apparently facilitated the exchange of ions across the synovial membrane since the pH dropped more rapidly within the joint than it did following intravenous injection of the electrolyte solutions.

The fact that the composition of protein varies with the pH and ionic strength of the solution was demonstrated by Curtain (1955) who also demonstrated that the binding power of synovial proteins was greater with a lowering of pH. At pH 5.8, alpha globulins predominate; at pH 7 to 8, alpha and beta globulins predominate and at pH 10 the protein content is considerably reduced. At neutrality, increasing ionic strength leads to a marked increase in

the amount of the protein complex.

In a study on intra-articular temperature, Horvath and Hollander (1949) reported that the amount of synovial fluid present appeared to make little difference in the joint temperature, except to alter the rate of metabolic exchange. A close correlation was noted between the internal joint temperature and the clinical activity of the disease. This indicated that the temperature of the synovium was directly dependent on the relative hyperemia of the synovium. These workers observed that joint temperature in degenerative joint disease was higher than anticipated from relative clinical activity. External heat over a joint was usually, but not necessarily, related to joint temperature. They concluded that this was a factor that might be related to greater joint friction effects and to retarded cooling.

Hollander, et al. (1951) later evaluated the effects of anti-arthritic agents to reduce joint temperature. Following administration of cortisone and adrenocorticotropic hormone, serial synovial fluid counts showed a pronounced decrease in leukocytes which paralleled the fall in joint temperature. A distinct parallelism was noted by a prompt clinical improvement followed by the fall in joint temperature. This fall in joint temperature was found to be a more constant determination of clinical effect than a drop in cell counts.

Employing the knee joint of the cat, Hunter and

Whillams (1951) reported that exposure to zero and subzero ambient temperatures, resulted in a significant fall in joint temperature. A fall in temperature was associated with an increase in friction of the joint which was freed from muscular attachments, with the exception of cruciate ligaments. They concluded that a physiologically important component of joint stiffness resulting from exposure to cold must therefore result from a local physical change in the joint tissues.

Cytology

Cytological studies of synovial fluid from the tibio-tarsal and carpo-metacarpal joints of healthy young beef cattle by Warren, et al. (1935) showed that a variation occurred in the total number of nucleated cells and individual cell types. The widest variation in cell types was observed in the tibio-tarsal synovial fluid. At the time, these workers stated that they knew of no real value in subdividing the large mononuclear phagocytes of synovial fluid.

Observations made on 29 synovial samples in man by Bauer, et al. (1940) failed to reveal either eosinophils or basophils, indicating that they are rarely present in normal synovial fluid. These workers further noted that synovial fluid cytology was never a reflection of blood cytology.

Robinson, et al. (1955) made a study of synovial fluid changes in patients suffering from rheumatoid arthritis and could find no correlation between total cell count and viscosity of the synovia. Likewise there was no apparent correlation between the level of the cell count and a less satisfactory mucin clot formation. Erythrocyte and leukocyte cell counts were made, using the same technique as for blood counts, except that normal saline was used as the diluting fluid. Here again, no attempt was made in counting the mononuclear cells to differentiate between lymphocytes, monocytes, clasmatocytes and synovial cells.

Their reasons for not differentiating the various mononuclear cells may be based on work by Coggeshall (1953) who concluded that from a diagnostic standpoint, the most significant information comes from the total leukocyte count and the percentage of polymorphonuclear leukocytes. Polymorphonuclear leukocytes should not exceed 25 per cent of the synovial fluid leukocytes in normal fluids.

Davies (1945) observed in cattle that high nucleated cell counts generally occur in highly viscous fluids, in joints characterized by freedom from disease and degenerative changes. Erythrocytes did not normally appear in the synovial fluid and he attributed their presence in small numbers to contamination during aspiration. This worker reported clasmatocytes and monocytes as the predominate cell type in synovial fluid. Phagocytic type cells

predominate in the more highly viscous fluids, with less viscous fluids such as found in the tibio-tarsal articulation showing a predominance of lymphocytes. This coincided with the finding of less cellular debris noted in joints that are more acellular and exhibit a predominance of lymphocytes.

The work of Coggeshall, et al. (1940) was substantiated by these findings, since they had previously determined that the function of the synovial fluid leukocyte in man is the removal of the wear and tear debris incident to daily use. They postulated that variations in total and individual cell types are probably dependent upon the inconsistent tissue trauma resulting from daily use of the joint.

Gardner (1950) stated that the cellular constituents of synovial fluid were predominantly mononuclear and included phagocytic and non-phagocytic types. These cells were undoubtedly derived mainly from synovial tissue and reflected its cellular content and that the findings for one joint were not necessarily similar to those of others. Anderson (1952) noted that in septic arthritis of man, the nature of the synovial fluid was at first serous, but later purulent. The synovial membrane being greatly congested, swollen, and infiltrated with inflammatory cells. Fowler and Kingrey (1956) reported that aspiration of synovial fluid reveals its purulent character in cases of pyemic tarsitis

of cattle. However, they made no mention of further examination of the synovia. One could conclude from these observations that the synovial membrane is the source of the inflammatory cells observed in septic synovial fluid.

Working with rabbits, Cunningham, et al. (1924) differentiated two different types of phagocytic cells in the spleen. These two cell types were designated as clasmatocytes, macrophages or histiocytes, and the large mononuclear elements of blood, the so-called monocytes or transitional cells, which they felt were closely related if not identical.

Sabin, et al. (1924) also separated the phagocytic cells of the peritoneal exudate into two distinct types. They were able to demonstrate that the first reaction to take place after injection of any substance into the peritoneal cavity was the emigration of the polymorphonuclear leukocytes, which appeared in the exudate at the end of four to six hours. In later work, Sabin, et al. (1925) noted that clasmatocytes were outnumbered by monocytes in irritated connective tissue areas because they were slower to become phagocytic, as these cells must maturate before becoming functional. In advanced cases of degenerative arthritis in the bovine, Shupe (1959) noted an increased nucleated cell count, but apparently neglected to ascertain both the total number and type of nucleated cells in this disease. Studies by Mikkelsen, et al. (1958) suggest that

the morphologic features of rheumatoid arthritis may persist even though the joint fluid has become essentially normal. They concluded that there was often a poor correlation between synovial fluid characteristics and histologic changes in the synovial membrane.

DeGara (1943) reported on the bactericidal properties of synovial fluid in man and observed that it contained phagocytic cells, but that only a few effusions were streptoccidal. Thus he concluded that most of the synovial fluids were practically free of streptoccidal antibodies.

Sugars

Cajori, et al. (1926) reported synovial fluid sugar values in man as high as those of blood and as an argument in favor of the view that its function was partly nutritional. These workers conducted experiments in which the blood and synovial fluid were analyzed before and after administration of 100 grams of glucose orally. Results revealed a communication of the synovial fluid and alimentary tract that was novel and heretofore unsuspected. The rise in blood sugar following glucose ingestion promptly caused a rise in sugar concentration within the joint, with no evidence of a lag. In two experiments, the concentration of sugar in the synovial fluid, half an hour and one hour after administration of the sugar, was higher than the concentration of sugar in blood at these times. This may mean

that there is a tendency for sugar to accumulate in the joint cavity, though it seemed more likely that it represents an equilibrium with respect to sugar between arterial blood and synovial fluid. The analysis was made on venous blood, which usually has a much lower sugar content, following sugar ingestion, than has arterial blood.

Lower concentrations of synovial fluid glucose in relation to blood glucose were reported by Davies (1946). He felt that this fact was partly explained by a glucose consumption in the joint.

Working with calves, Zeller, et al. (1941) noted that the initial rise of synovial fluid sugar lagged behind that of blood at least 20 minutes following intravenous administration of glucose solutions. Blood sugar in two of their experiments returned to almost pre-injection levels in less than two hours. When serum glucose fell, synovial fluid glucose again showed a lag. They concluded the rate of utilization of glucose by articular tissues and the rate of diffusion from the joint space into the synovial capillaries is not sufficiently rapid to keep synovial sugar at or below that of serum.

Hepler (1957) stated that all that is reported as blood sugar is not glucose, but the sum total of reducing substances encountered during the determination. This author listed the non-fermentable portion of the reducing substances as being composed of glutathione, ergothioneine, creatinine

and unknown substances. She concluded that the concentration of the non-fermentable portion varied from 10 to 30 milligrams per cent.

Hawk, et al. (1954) stated that sugars owe their reducing ability to their free or potentially free aldehyde or ketone groups and were thus able to reduce in the presence of alkaline solutions, the ions of certain heavy metals. They contended that certain organic compounds, particularly those containing one or more alcoholic hydroxy groups in the molecule, e.g., tartaric acid, citric acid, glyerol, and even sugars themselves, react in alkaline solution metalic hydroxides to form a soluble complex ion, which though relatively little ionized, nevertheless dissociates to yield sufficient ions of the metal for reduction to occur.

To designate this group of non-fermentable copper reducing materials present in blood, Benedict (1931) proposed the term "saccharoids". The word saccharoid has been used as an adjective, and use of the word as a noun did not seem objectionable to him. He stated that the saccharoid content of plasma was almost as high as for whole blood and concluded that saccharoids are found in both plasma and corpuscles in readily detectable quantities, but that in many samples of blood the analysis of unlaked blood by the usual methods may fail to reveal their presence. His observations on blood indicated that a considerable portion of the saccharoid fraction is represented by glutathione.

In their work on the determination of blood sugar, Folin and Wu (1920) concluded that non-glucose reducing substances may enhance glucose values, but this saccharoid fraction appeared to be relatively constant and therefore its presence does not influence unduly the interpretation of variations in the blood sugar.

Fashena (1933) determined that glutathione accounts for 37 per cent of the total saccharoid content in human blood by the Folin-Wu method and that oxidized and reduced forms of glutathione exert the same degree of reducing power towards the Folin-Wu reagent. She believed the results of her work establish that 63 per cent of the saccharoid fraction of human blood, as determined by Folin-Wu method is composed of reducing compounds other than glutathione. It is probable that different results would be obtained through the use of reagents other than Folin-Wu for measurements of the reduction, when the reducing value of glutathione in terms of glucose assumes different properties. Her work seemed to establish positively the presence of a large saccharoid fraction in blood, the composition of which was undetermined.

Later Fashena and Stiff (1941) presented data that appeared to indicate that glucuronic acid or its compounds were responsible for practically all of the residual reducing power of tungstic acid blood filtrates not accounted for by glucose or glutathione. A very small part of the

reduction was due to creatinine, uric acid, and ascorbic acid; however, the total reducing value of these compounds, based on experimental determinations of glucose probably does not exceed two or three milligrams per cent in normal blood as determined by Folin-Wu methods.

Cajori and Pemberton (1928) demonstrated the glycolytic activity of cell free synovial fluid. This was much less marked than was that of the uncentrifuged material. Rapid glycolysis occurred in a portion of synovial fluid containing leukocytes, whereas the glucose level did not change for several hours in cell free fluids. Coincident with the loss of glucose, the lactic acid levels rose markedly. This work demonstrated that in synovial fluid containing leukocytes, conditions are highly favorable for the rapid disappearance of glucose and the formation of lactic acid. The presence or absence of leukocytes was of first importance in determining the level of synovial fluid sugar.

The presence of glycolytic enzymes in synovial fluid appeared to be present in the polymorphonuclear leukocyte and confined almost entirely to these cells. This was demonstrated by Hubbard and Porter (1943). In no experiment in which glucose or fructose was added to cell-free fluid was there a significant change in concentration after incubation. On readdition of cells to a cell-free fluid, glycolytic activity was noted. Interestingly enough,

glucose destruction was almost twice that of fructose. In these experiments, both glucose and fructose were added in measured amounts to the synovial fluids under trial.

Viscosity and Mucin

Newlander (1949) defined viscosity of a substance as its resistance to flow, due to internal friction between molecules as they shear each other. Hausman and Slack (1939) stated that when a liquid flows over a surface, the layer of liquid particles in contact with the surface remains stationary because of adhesion, the next layer of particles moves slowly over the first, the third layer moves with respect to the second, and so on, the speed of each layer increasing with its distance from the solid surface. Shearing then occurs in the uppermost layers or layers furthest from the surface. There being a resultant decrease in viscosity with a rise in temperature. Eckles, et al. (1936) concluded that viscosity is a property of all fluids, and that it can be expressed only in relative terms. For convenience, the viscosity of any fluid is therefore expressed in comparison with water.

Repeated shearing or stirring of synovial fluid was reported by Blair, et al. (1954) to cause a small but definite increase in viscosity, the cause of which is unknown.

Gortner, et al. (1953) determined the rate of flow through a capillary as the basis for an important method for the measurement of viscosity. In this method, the time of flow of a known volume of liquid through a capillary under the influence of gravity is determined. The apparatus used in measurement was the Ostwald viscometer. For determination of relative viscosity, Bingham (1922) recommended the Ostwald viscometer. It consists essentially of a U-tube with a capillary in the middle of one limb above which is placed a bulb. A given volume of liquid is placed in the instrument and the time required for the liquid, which is only under the influence of hydrostatic pressure, to pass between two marks, one above and one below the bulb, is determined (or measured).

To reduce error in the mean head caused by deviation of the viscometer from the vertical, Cannon and Fenske (1935) devised a modified Ostwald viscometer in which the upper and lower bulbs lie on the same vertical axis. Such a viscometer is in common use today.

Cannon and Fenske (1938) later reported that efflux volume is used to measure efflux time. They further stated that efflux volume and capillary diameter should be made small in order to reduce kinetic energy corrections. This factor was also reduced by increasing the driving fluid head, i.e., the distance between the efflux bulb and the lower reservoir. Drainage errors arise from the fact that

all liquids do not drain from a surface with equal ease; hence, the measured efflux volume for one liquid may be different than another. They attributed sources of error to too much fluid in the lower reservoir and reduction of the driving head by that amount. In conclusion they stated that relative viscosities are very accurate and if the reference basis is clearly defined, little confusion will exist and reported values of viscosities may be readily compared. In later work, Cannon and Manning (1959) concluded that kinetic energy correction is insignificant in the great majority of cases.

In order to remedy errors encountered in other types of viscometers, e.g., the Ostwald, Ubbelohde (1937) introduced the phenomenon of suspended level. He theorized that because of suspended level, smaller amounts can be used and capillary viscosity is therefore independent of the charge in the reservoir. In other type viscometers, the effective pressure driving the liquid through the capillary is influenced by the surface tension of the film lying on the surface of a liquid at a higher level, and by the surface tension effect either on the stream emerging from the capillary or on the surface of the liquid in the lower reservoir. Application of the principle of suspended level thus allows one to balance corrections for these factors against each other and to design an instrument whose dimensions are so proportioned as to avoid others. Suspended level

is the term used to designate the hanging surface of a liquid and to distinguish it from recumbant level forms by the film lying on the surface of a liquid. In the case of Ubbelohde's viscometer, this film is formed by a hemispherical surface, concave downward. It is formed at the point where a vertical capillary expands into a relatively large tube by the layer of a liquid spreading upon the interior surface at this junction. This principle therefore reduces the need for surface tension correction. Since all liquids wet the walls of this upper bulb, the meniscus formed exerts upward pull against the vessel walls because of surface tension, thus tending to diminish the effective height of the liquid column. Suspended level, by a properly curved surface acts to exert traction in the opposite direction forming an inverse meniscus to the surface tension on the surface of the liquid in the upper bulb and balancing it. Ubbelohde felt conclusively that this principle eliminated loading and surface tension errors.

Barnett (1958) ascertained that synovial fluid behaves as an extremely thick oil when joint surfaces are being sheared very slowly, one upon the other, but become progressively less viscous as the rate of shear increases.

In a study on the physiological function of hyaluronic acid in synovial fluid, the results of Ogston and Stanier (1953) suggested that a major function of hyaluronic acid in synovial fluid may be its contribution to the

lubricant properties of the synovial fluid. These workers reasoned that a solution of hyaluronic acid is peculiarly suitable for lubricating a bearing surface of this type, required to carry a load at varying rates of movement. The viscosity is high at the lowest rates of shear, so that the joint is enabled to support a heavy load even at a low rate of movement. At higher rates of movement, the viscosity falls so that the drag of the joint is relatively lessened, the load which it will bear is not reduced, because the greater rate of movement more than offsets the fall in viscosity. A normal (Newtonian) fluid would not give these advantages. Furthermore hyaluronic acid produces high viscosity at low concentration, without any large osmotic effect such as would be produced, for example, by protein at concentrations needed to give a similar viscosity. These properties of hyaluronic acid are gained from its molecular weight and its random-chain structure.

The rheological and pathological behavior of synovial fluids provided Levine and Kling (1956) with valuable information about the physiological and pathological nature of joints. They concluded that the concentration of hyaluronic acid determines the intrinsic viscosity of a fluid and that this in turn reflected the mean polymerization as an index to this factor. Polymerization is of prime importance since even in the presence of increased production of hyaluronic acid, if this is low, so will be the

relative viscosity of the fluid. In reviewing their work, they felt that relative viscosity appeared to be as valuable as any other single viscosity determination.

Depolymerization of hyaluronic acid occurs in the presence of hyaluronidase a process that Harrow and Mazur (1954) observed, led to a decrease in viscosity of the acid and eventual hydrolysis. The effect of hyaluronidase on the hyaluronic acid of bovine synovial fluid did not alter the hyaluronic acid glucosamine but Ogston and Stanier (1953) did show that it affected the character of the mucin precipitate. They suggested a method of judging degradation of mucin based on measurements of viscosity alone. Since glucosamine precipitated as mucin is not affected, even when the viscosity is strongly reduced and when the character of the mucin precipitate has been profoundly changed. Therefore viscosity may be compared with a normal sample having the same content of mucin, neither the weight of mucin nor its nitrogen content should be used in place of glucosamine content. The sum total of the effects of hyaluronidase on hyaluronic acid is its degradation or depolymerization and resultant variation of viscosity with velocity gradient and the elastoviscosity of bovine synovial fluid.

Moffett (1954) made a study on the permeability of synovial membranes in the rat and found that the addition of hyaluronidase caused a greater than two-fold increase

in the iodopyracet (3,5-diiodo-4-pyridone-N-acetic acid)* diffusion rate in both live and dead rats. Removal time measurements were unaffected, however, by addition of hyaluronidase. He concluded that synovial permeability in the rat could be altered by two means: (1) a change in the synovial ground substances; (2) a change in the synovial blood supply.

A quantitative study of metachromasy in synovial fluid and mucin by Hamerman and Schubert (1953) revealed that the fluid is not metachromatic even when the protein content has been reduced to levels found in mucin solutions. Hyaluronate that has been depolymerized by hyaluronidase is not metachromatic, since it is chiefly high polymers that are metachromatic and the higher the state of polymerization, the greater the degree of metachromasia. They went on to state that hyaluronate is not free in synovial fluid and that native synovial fluid with a viscosity higher than the mucin, or the hyaluronate derived from it, is not metachromatic. Addition of alkali has been noted to free the hyaluronate and to produce a fall in viscosity. Alkali also causes synovial fluid to become metachromatic, thus the altered state of the hyaluronate is correlated with a fall in viscosity and appearance of metachromasy. The evidence presented indicates that in its native state in synovial

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*Diodrast, Winthrop Laboratories, New York, N.Y.

fluid, hyaluronates are either bound or their anionic groups are not entirely free.

It was Gunter (1947) who described a method for assay of hyaluronidase based on the fact that the action of the enzyme on synovial fluid prevented stringiness or the formation of fibers around a rotating rod. He termed this fiber forming capacity "spinnbarkeit". Whether the spinnbarkeit of synovial fluid depended solely on the presence of polysaccharide, or a protein in combination with polysaccharide, was not in his opinion altogether clear. The slight fall in spinnbarkeit after tryptic digestion suggested that this property was due mainly to the long-chain polysaccharide molecule, with protein playing only a minor role. The addition of an equal volume of undiluted normal serum had no effect on the spinnbarkeit of synovial fluid. This argument was supported by the destruction of spinnbarkeit by enzymes prepared from the testis (testicular hyaluronidase) and from Clostridium welchi, both of which are well recognized sources of hyaluronidase and therefore capable of breaking down long-chain polysaccharide molecules. Carpenter (1959) stated that hyaluronidase was formed by Clostridium perfringens as well as streptococci, pneumococci, certain micrococci, and other organisms.

According to Wintrobe (1956), blood viscosity was dependent upon a number of factors, the most important of which was the quantity of erythrocytes. The quantity

of leukocytes was of significance only when they were greatly increased in number. This was particularly true in cases of myelocytic leukemia because the myeloid leukocytes were larger than lymphocytes. Furthermore, he showed that the influence of various factors which determine blood viscosity were rapidly intensified in hemoconcentration.

Mann (1948) attributed variations in viscosity of human plasma to qualitative changes in protein.

CHAPTER III

MATERIALS AND METHODS

Samples of blood and synovial fluid obtained for this study were collected from cattle, noted as to breed, sex, age, and state, i.e., either ante mortem or post mortem. Specimens taken from slaughter cattle were recorded as coming from fasted animals, since these individuals were as a rule taken off feed the afternoon prior to the morning kill. Samples taken from other than slaughter cattle were recorded as coming from non-fasting animals, in view of the fact that they were on full feed at the time of specimen collection. Determination of differences between blood and synovial sugars and plasma and synovial sugars in nonfasting cattle was deemed necessary in order to evaluate the effects of fasting in relationship to synovial fluid sugar in the slaughter animals.

Synovial fluid was aspirated from the tibio-tarsal articulation. This joint is readily accessible and sufficient quantities may be obtained for a complete examination. Color and quality of the synovial fluid was noted, but no effort was made in the course of this study to determine the average quantity this articulation contained.

Cattle for this study were selected after clinical examination revealed no gross enlargements of the hock joint

and no manifestations of pain or impaired gait. All individuals appeared clinically to be free of disease. This procedure applied also in the case of cattle presented for slaughter. With slaughter cattle it was possible to examine the synovia, articular cartilages, and synovial membrane for pathological changes as recommended by Jones and Gleiser (1954).

Age determination of animals presented for slaughter were made by examination of the teeth as correlated to the work of Sisson and Grossman (1950). Records kept on experimental animals not intended for slaughter verified their ages.

A. Collection Procedures:

1. Blood

All samples were obtained from the jugular vein, either by venipuncture in ante mortem cases or following incision of the jugular vein at slaughter time. Approximately 10 milliliters of blood was collected directly into screw cap vials containing ammonium potassium oxalate in a ratio of 0.1 milliliter of the anticoagulant solution to 1.0 milliliter of blood. Thus 1 cc. of the anticoagulant solution contains 1.2 per cent ammonium oxalate and 0.8 per cent potassium oxalate or in

round numbers a 2 per cent solution of anticoagulant. The water in the anticoagulant solution had previously been evaporated to dryness in vials at 70 degrees centigrade as recommended by Hepler (1957).

2. Synovial Fluid

All samples were obtained from the tibiotarsal articulation in a manner similar to the technique used by the author (1960) for arthrocentesis of the carpus in horses. Arthrocentesis to obtain ante mortem samples was performed with the animal in the standing position. The hair over the anteromedial surface of the joint was clipped closely and then the area cleansed with 70 per cent ethyl alcohol. Using a 26 gauge, ½ inch needle, the area was subcutaneously infiltrated with three to five milliliters of a two per cent solution of lidocaine hydrochloride* containing 1:100,000 epinepherine hydrochloride. While waiting for the anesthetic to exert its maximum effect, the area was scrubbed

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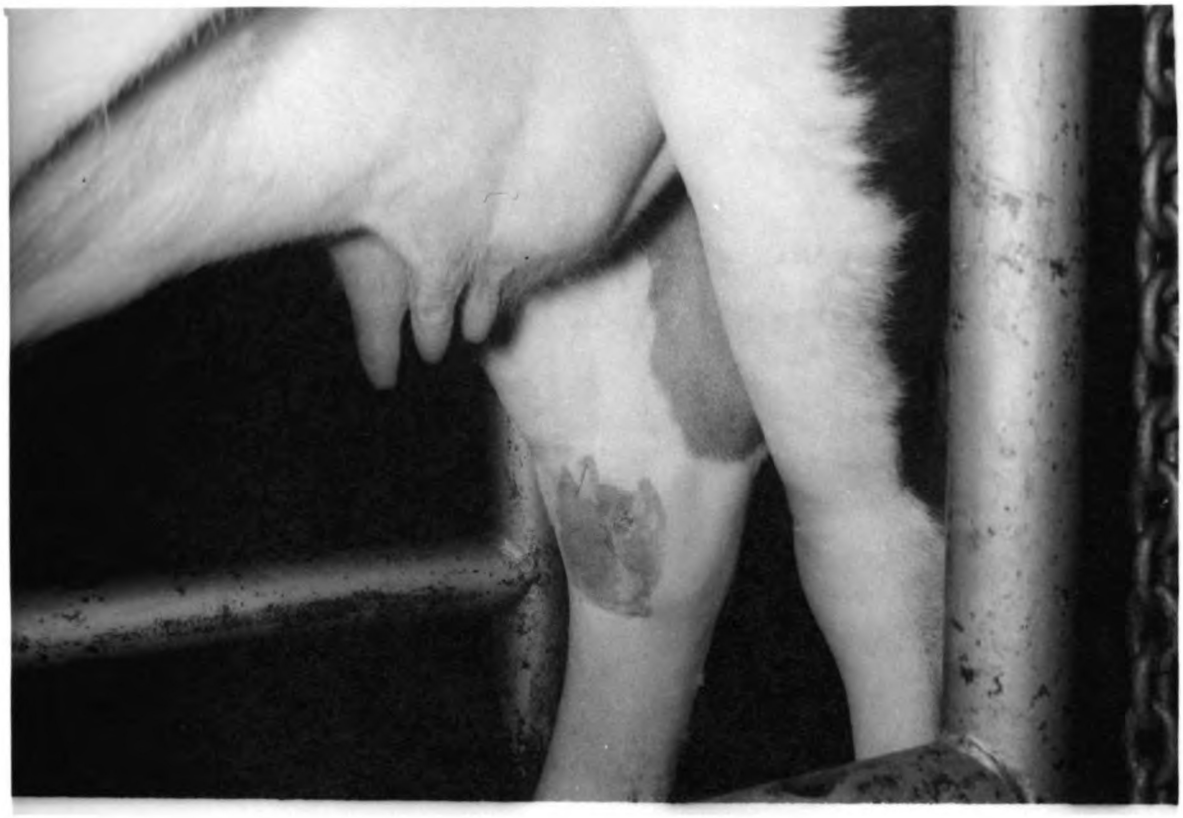
*2% Xylocaine Hydrochloride, Astra Pharmaceutical Products, Inc., Worcester, Mass.

Figure 1. The anteromedial aspect of the hock joint has been clipped and anesthetized, then scrubbed with surgical soap, and painted with Strong Tincture of Iodine, N.F., prior to arthrocentesis of the tibio-tarsal articulation.

Figure 2. A 16 gauge, two-inch needle has been directed into the tibio-tarsal joint cavity from the anteromedial position.



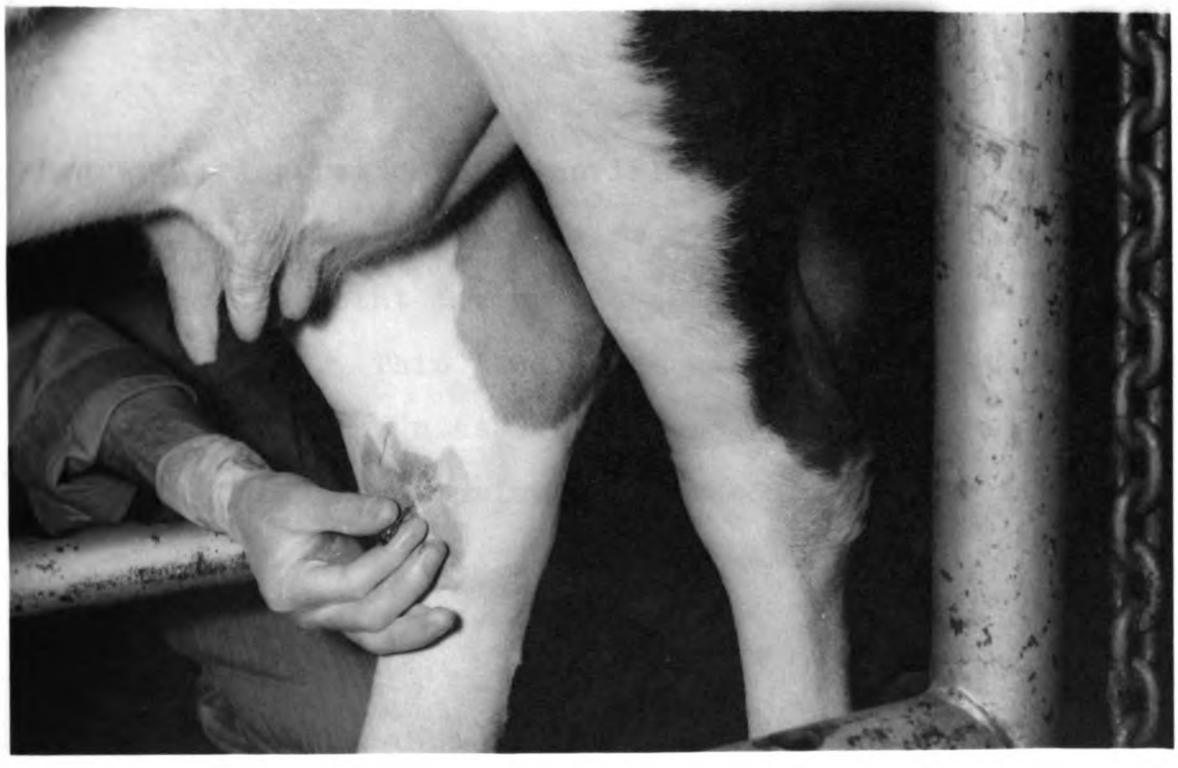
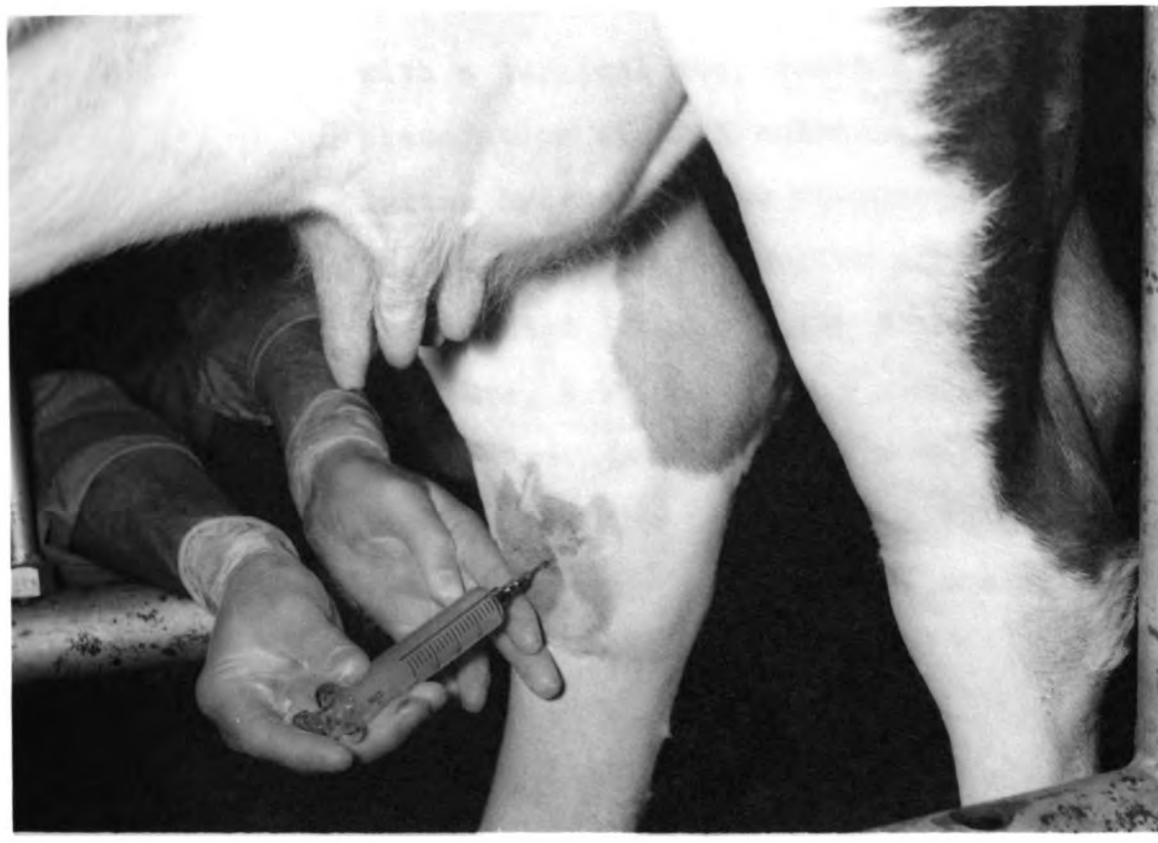
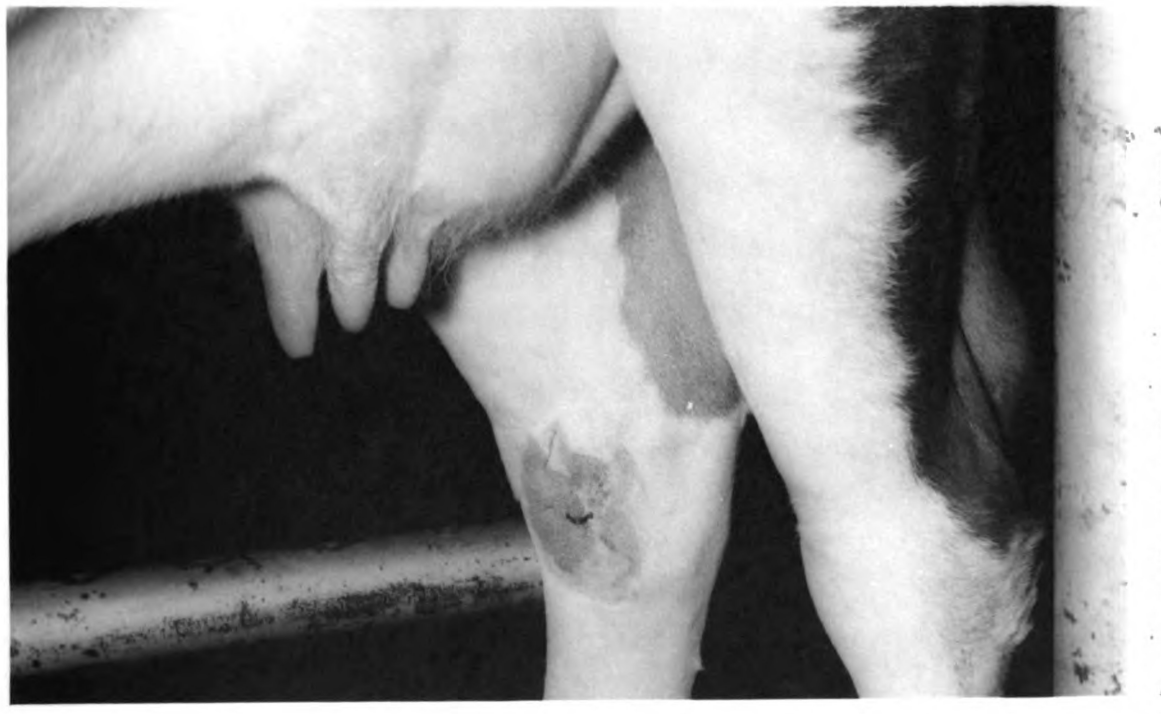


Figure 3. A 20 milliliter metal-tip glass syringe has been attached to the needle for withdrawal of synovial fluid.

Figure 4. Aspiration of synovial fluid has been completed and the external site of needle puncture into the joint cavity closed with a Michel's suture clip. This final step will minimize external hemorrhage as well as reduce the chance entry of microorganisms into the tissues.





with a surgical soap containing an aqueous preparation of high molecular weight alkylamine hydrochlorides composed of benzethonium chloride* for five minutes, dried, and painted (Fig. 1) with Strong Iodine Tincture, N.F., which was allowed to dry. A 16 gauge, two-inch needle was then directed into the joint cavity (Fig. 2) from the anteromedial position at the tibiotarsal articulation and synovial fluid then aspirated with a sterile, dry, 20 milliliter metal-tip glass syringe (Fig. 3). Following withdrawal of the needle, many cattle exhibited external hemorrhage at the site of puncture. In order to minimize this, the needle openings were closed with either a large (22 mm.) or medium (15 mm.) size Michel's suture clip (Fig. 4). The synovial fluid was then injected directly into dry, sterile, screw cap vials containing no anticoagulant. In the case of samples collected at slaughter, arthrocentesis of the tibiotarsal articulation was performed in

*Liquid Germicidal Detergent, Parke, Davis and Co., Detroit, Mich.

essentially the same manner, with the exception that the animal was in dorsal recumbancy and aseptic procedures were not followed.

B. Laboratory Procedures

1. Blood Sugar Determinations

Whole blood was used to determine the sugar levels of fasting and non-fasting animals. Protein-free filtrates of the oxalated whole blood were made within two hours after collection.

2. Plasma Sugar Determinations

Following removal of two milliliters of the oxalated whole blood to make a protein-free filtrate for blood sugar determinations, the remainder of the sample was centrifuged for 30 minutes at 3,000 revolutions per minute to obtain the plasma. Protein-free filtrates of the plasma were then made.

3. Synovial Sugar Determinations

The non-oxalated synovial fluid was centrifuged for 30 minutes at 3,000 revolutions per minute to obtain a sample free of its cellular content as well as any organic debris accumulated in the process of collection.

4. Blood, plasma, and synovial sugar determinations were ascertained by a modification of the macromethod of Folin and Wu (1919) and Folin (1929). The unknown samples were read against a standard glucose solution using a Bausch and Lomb Spectronic 20 Colorimeter*.

   a. Preparation of a Protein-Free Filtrate

      (1) Place 16 milliliters of N/12 sulfuric acid in a 50 milliliter Erlenmeyer flask.

      (2) Using a two milliliter Ostwald-Folin pipette**, add slowly two milliliters of oxalated whole blood, plasma, or synovial fluid to the N/12 sulfuric acid solution.

      (3) Shake gently and allow to stand for two minutes.

      (4) Add, slowly and with constant shaking, two milliliters of 10 per cent sodium tungstate***. This will make a one to 10 dilution, i.e., in every 10 milliliters of filtrate

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*Bausch and Lomb Optical Co., Rochester, N.Y.
**Kimble Glass Co., Toledo, Ohio
***Hartman-Leddon Co., Philadelphia, Pa.

there is the equivalent of one milliliter of blood, plasma, or synovial fluid.

(5) Insert a number one rubber stopper and shake well, a metallic click and absence of foam on the mixture are indicative of complete precipitation of the protein.

(6) Allow the mixture to stand for five minutes.

(7) Filter, using a dry, folded number 12 Whatman Filter Paper*. The filtrate must be clear and free of any color. In the case of whole blood, a brown or brownish-yellow filtrate usually indicates that the filtrate is slightly too alkaline.

b. Preparation of a Folin-Wu Filtrate Blank

(1) The Folin-Wu blank is prepared by placing 16 milliliters of N/12 sulfuric acid in a 50 milliliter Erlenmeyer flask.

(2) Using a two milliliter Ostwald-Folin

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*A.S. Aloe Co., St. Louis, Mo.

pipette, add slowly two milliliters of distilled water.

(3) To this mixture is then added two milliliters of 10 per cent sodium tungstate.

c. Preparation of a Glucose Standard

(1) Using a one milliliter Ostwald-Folin pipette*, and a corresponding amount of a one per cent glucose stock solution** (Folin-Wu) in benzoic acid to a 100 milliliter volumetric flask and dilute to volume with distilled water (one milliliter equals 0.1 milligram).

(2) Two milliliters of the glucose standard is then equal to 0.2 milligram of glucose.

(3) The glucose standard may be refrigerated for future use.

d. Sugar Determinations

(1) Use five Folin-Wu blood sugar tubes*** graduated at 25 milliliters. This

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*Kimble Glass Co., Toledo, Ohio
**Hartman-Leddon Co., Philadelphia, Pa.
***Kimble Glass Co., Toledo, Ohio

number was necessary to determine the sugar levels of whole blood, plasma, and synovial fluid for each animal and at the same time run a glucose standard and filtrate blank.

(2) Into three of the tubes using a two milliliter volumetric pipette, place two milliliters of the Folin-Wu filtrates of whole blood, plasma, and synovial fluid.

(3) In two other tubes using a two milliliter volumetric pipette, add two milliliters of Folin-Wu blank and two milliliters of the glucose standard solution.

(4) To each of the five tubes add two milliliters of alkaline copper tartrate (Folin-Wu)*.

(5) Place each tube in boiling water and continue boiling for exactly eight minutes.

(6) Cool in cold running tap water for two minutes, avoiding agitation.

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*Hartman-Leddon Co., Philadelphia, Pa.

(7) To each add, two milliliters of phosphomolybdic acid reagent (Folin-Wu)* and shake until dissolved.

(8) Replace in boiling water for exactly five minutes.

(9) Repeat step number six.

(10) Dilute each to the 25 milliliter mark with distilled water and mix by inversion.

(11) Transfer to absorption tubes.

(12) Wavelength of the Bausch and Lomb Spectronic 20 Colorimeter is set at 430 mu; with the Folin-Wu blank, set per cent transmittance scale at 100 per cent.

(13) The standard glucose solution should then give a reading of 60 on the per cent transmittance scale which is then equal to 100 milligrams of glucose per 100 milliliters of the original one per cent stock solution.

(14) Replace standard with unknown and note the readings on the per cent

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*Hartman-Leddon Co., Philadelphia, Pa.

transmittance scale.

(15) Concentrations in milligrams of sugar per 100 milliliters of blood, plasma, or synovial sugar, are found on the table provided by Bausch and Lomb.

5. Cellular Studies

Total leukocyte counts were made on a non-centrifuged portion of the synovial fluid. To obtain an adequate number of cells for an accurate differential leukocyte count, the synovial fluid sample was centrifuged for 30 minutes at 3,000 revolutions per minute. The cellular studies were made by a modification of the techniques described by Coffin (1953). All cellular studies were done on either a Dialux* or Labolux IIIa* microscope. Cytological identifications were made according to the work of Sabin, et al. (1924 and 1925) and Doan, et al. (1925).

a. Differential Leukocyte Counts

Following centrifugation the supernatant fluid was poured off and smears of the

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*Ernest Leitz, Inc., New York, N.Y.

sediment made by compressing it between two microscope slides (75 x 25 millimeters), both smears were stained with Wright's stain. One hundred leukocytes were counted and differentiated for each sample of synovial fluid.

b. Total Leukocyte Counts

Total leukocyte counts were made by diluting the synovial sample in a standard white cell pipette. Two types of diluting fluid were used, both employing normal saline as a base. To one, two to three drops of methylene blue were added per 10 milliliters of normal saline. To the other, methyl violet crystals were added to saline, then filtered to make a final concentration of one per cent. Since normal synovial fluid is relatively acellular, the sample under test was drawn to the 1.0 mark on the pipette, making a one to ten dilution. The pipette was then shaken for five minutes on a Burton Pipette Shaker, model number 1406*.

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*Burton Manufacturing Co., Santa Monica, Calif.

Leukocytes were then counted on a standard hemacytometer* in the same manner as done in the standard leukocyte count and the total leukocytes per cubic millimeter determined. In some cases where cells were more numerous, only the four large corner squares on the hemacytometer were used and in others less numerous, all nine squares were used. For excessively low counts, the technique described by Ham (1950) was employed. In all instances the total number of leukocytes was determined in cubic millimeters.

c. Absolute Leukocyte Counts

These determinations were made to arrive at the actual increase or decrease in the number of cells as opposed to the relative increase or decrease. The formula used has been described by Miale (1958) to arrive at the absolute number of leukocytes per cubic millimeter of synovial fluid.

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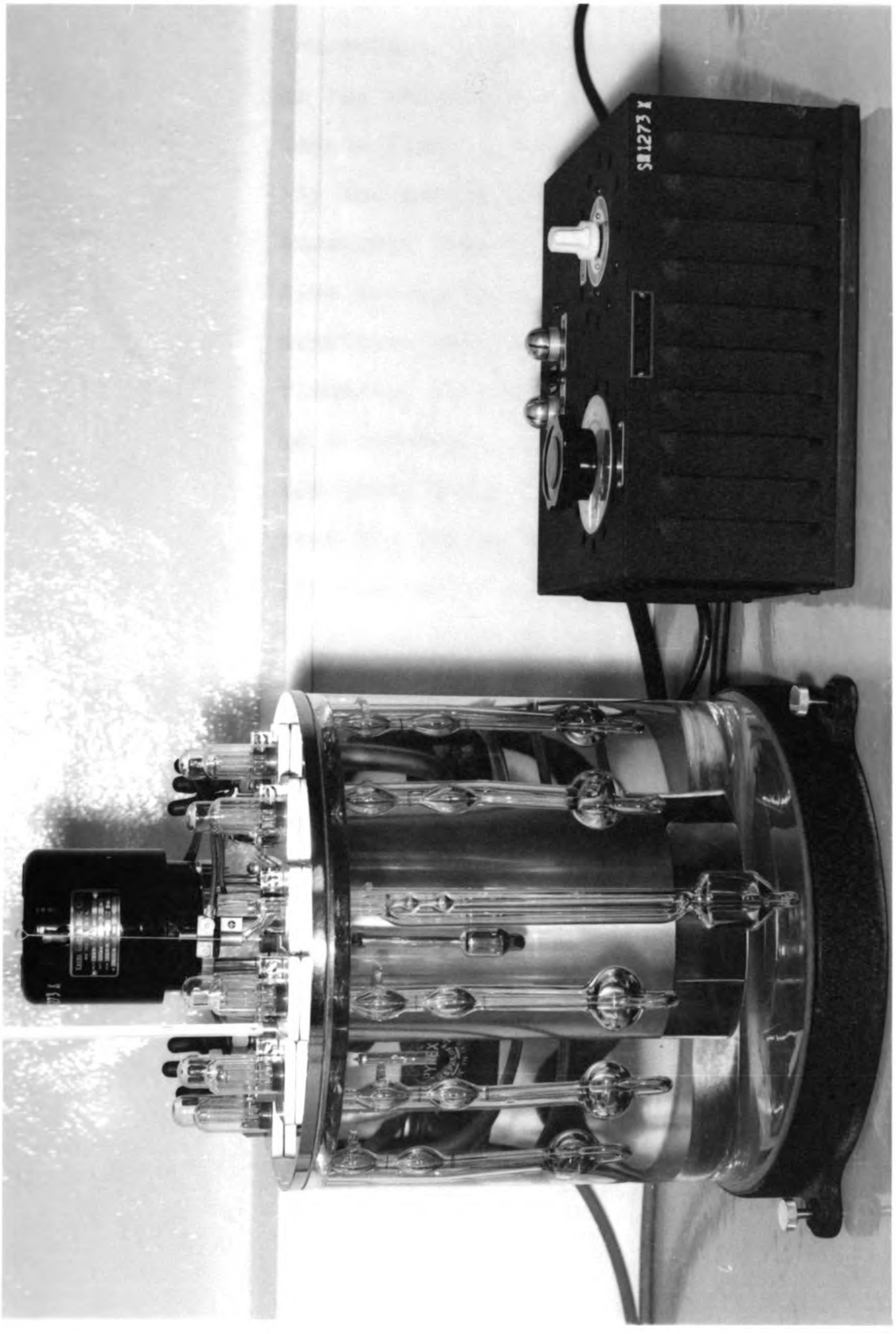
*Sharp Line Hemacytometer, Improved Neubauer Ruling, Chicago Apparatus Co., Chicago, Ill.

6. Relative Viscosity

Determination of the relative viscosity of synovial fluid was made on samples following centrifugation for 30 minutes at 3,000 revolutions per minute. The determinations were made using the Cannon-Fenske Routine Viscometers*, A.S.T.M. (American Society of Testing Materials) sizes 150 and 200, and the Cannon-Ubbelohde Semi-Micro Dilution Viscometers*, A.S.T.M. sizes 100 and 150. The viscometers were held in a Krebs Constant Temperature Bath** designed for use with capillary viscometers, A.S.T.M. D445-53T (Fig. 5). Dukes (1955) gives the average body temperature of the cow as 101.5 degrees fahrenheit; therefore, distilled water used as the bath liquid was maintained at 101.5 degrees fahrenheit plus or minus 0.1 degree, by a vapor type thermoregulator. Distilled water was used as the vapor source since it has an approximate sensitivity range of 92 to 104 degrees

*Cannon Instrument Co., State College, Pa.
**Krebs Electric and Manufacturing Co., New York, N.Y.

Figure 5. The Krebs Constant Temperature Bath (A.S.T.M. D445-53T) designed for use with capillary viscometers. Both Cannon-Fenske Routine Viscometers and the Cannon-Ubbelohde Semi-Micro Dilution Viscometer are suspended in this bath.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fahrenheit. Distilled water was used as the reference liquid as recommended by Cannon (1944), since its complete stability and general availability make it an excellent reference liquid for all relative viscosity measurements. The determinations were measurements of relative viscosity as compared to distilled water as a reference liquid, the conditions of the study being duplicated as closely as possible for water and synovial fluid. The viscometer tubes were flushed with distilled water following their use and then flushed with a solution of potassium dichromate and sulfuric acid in a concentration as recommended by Hepler (1957). This solution was left in the viscometer tube for a variable length of time, thus allowing digestion of any protein residue. Then the viscometer tubes were flushed with distilled water and dried in a hot air oven at 150 degrees centigrade until dry. Efflux times were measured by using a hand operated stop watch.

a. Determination of the Relative Viscosity Using the Cannon-Fenske Routine Viscometer

This procedure is a modification of the technique described by Manning (1960) and Hawk, et al. (1954).

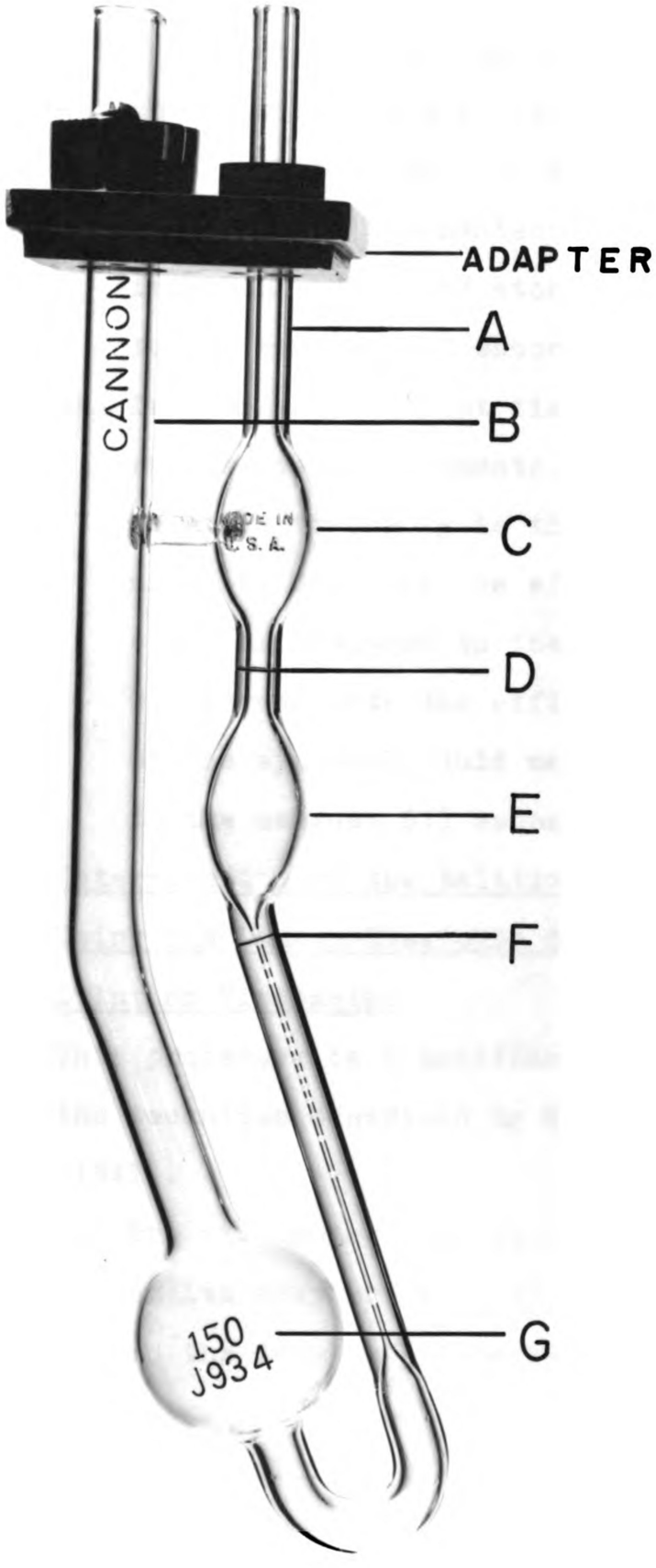
(1) Viscometer tubes were inserted in their adapters (Fig. 6) and placed in the constant temperature bath (Fig. 5) with the water level above bulb C and vertically aligned.

(2) A five, seven or 10 milliliter sample of synovial fluid is then pipetted into the lower reservoir of the viscometer (G) through tube B.

(3) The viscometer and synovial fluid sample are allowed 10 minutes to reach temperature equilibrium (101.5 degrees fahrenheit, plus or minus 0.1 degree).

(4) Suction is then applied to tube A until the liquid reaches the center of bulb C. Remove suction from tube A to measure the efflux time.

Figure 6. The Cannon-Fenske Routine Viscometer (A.S.T.M. 150) with adapter for the Krebs Constant Temperature Bath.


ADAPTER
A
B
C
D
E
F
G
CANNON
150
J934

(5) To measure the efflux time, allow the liquid sample to flow freely down past etch mark D, measuring the time for the meniscus to pass from etch mark D to etch mark E, to the nearest 0.1 second.

(6) Determine the efflux time for five successive measurements.

(7) Relative viscosity is then determined by dividing the efflux time of water measured to the nearest 0.1 second into the efflux time of the synovial fluid measured to the nearest 0.1 second.

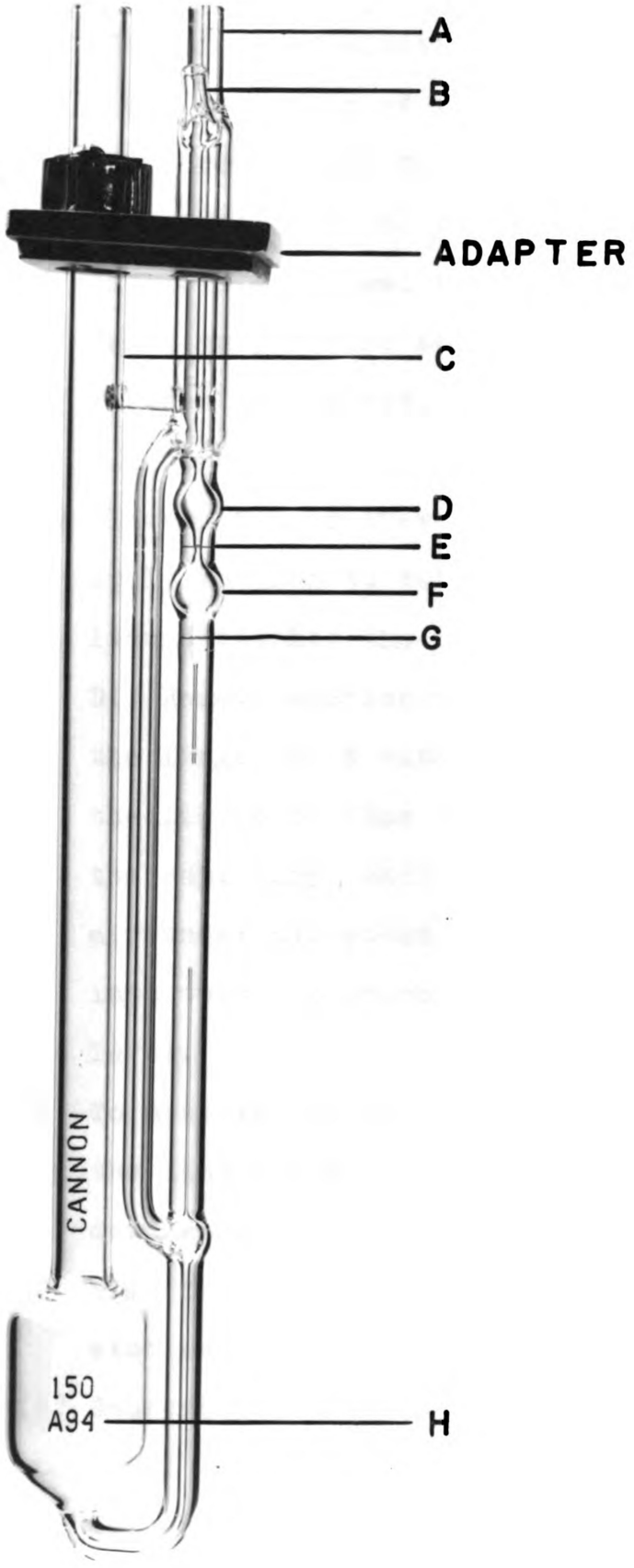
b. <u>Determination of the Relative Viscosity Using the Cannon-Ubbelohde Semi-Micro Dilution Viscometer</u>

This procedure is a modification of the technique described by Merrington (1941).

(1) The viscometer tube is inserted in its adapter (Fig. 7), placed in the constant temperature bath (Fig. 5) and vertically aligned.

(2) A one milliliter sample of synovial

Figure 7. The Cannon-Ubbelohde Semi-Micro Dilution Viscometer (A.S.T.M. 150) with adapter for the Krebs Constant Temperature Bath.


A
B
ADAPTER
C
D
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F
G
CANNON
150
A94
H

fluid is then pipetted into the lower reservoir of the viscometer (H) through tube C.

(3) The viscometer and the synovial sample are allowed ten minutes to reach temperature equilibrium (101.5 degrees fahrenheit, plus or minus 0.1 degree).

(4) Place a finger over tube B, and apply suction to tube A until the liquid reaches the center of bulb D. Remove suction from tube A and the finger from tube B, allowing the liquid to flow freely through the capillary, while the entry of air under atmospheric pressure into tube B produces the suspended level.

(5) To measure the efflux time, allow the liquid sample to flow freely down etch mark F, measuring the time for the meniscus to pass from etch mark G to the nearest 0.1 second.

(6) Because of the tendency for a synovial fluid sample to become turbulent and frothy on subsequent

attempts to measure efflux time, only one reading was taken.

(7) Relative viscosity is then determined by dividing the efflux time of distilled water measured to the nearest 0.1 second into the efflux time of the synovial fluid measured to the nearest 0.1 second.

c. Calibration of the Cannon-Fenske Routine Viscometer and the Cannon-Ubbelohde Semi-Micro Dilution Viscometer

(1) Since water is used as the reference liquid to determine the relative viscosity of synovial fluid, this then was used to calibrate the two types of viscometers used in this study.

(2) Cannon-Fenske routine viscometers were calibrated at 101.5 degrees fahrenheit, plus or minus 0.1 degree, with both five and ten milliliter charges. The same procedure was followed as outlined for measurement of synovial fluid efflux time.

(3) Sterile distilled water was used because it was free of either bacterial or mycotic growth, factors that could have a bearing on the efflux measurements.

(4) Cannon-Ubbelohde semi-micro dilution viscometers were calibrated at 101.5 degrees fahrenheit, plus or minus 0.1 degree with one milliliter charges. The same procedure was followed as outlined for measurement of synovial fluid efflux time, with the exception that five successive measurements were made. Lack of turbulence and froth with sterile distilled water made this possible.

7. Mucin Clot

Determination of mucin clot quality is made on a non-oxalated one milliliter sample of synovial fluid that has been centrifuged for 30 minutes at 3,000 revolutions per minute. The test was carried out in the following manner, a modification of the method described by Furey, et al. (1959).

a. 0.1 milliliter of 7/N glacial acetic acid is added to four milliliters of distilled water in a screw cap vial and thoroughly mixed.

b. Add one milliliter of the synovial fluid slowly, taking care that the sample does not come in contact with the glass as it is added.

c. Gently swirl the solution so as to mix the synovial fluid thoroughly.

d. Allow to stand for one hour and mix again by inverting the tube several times before results are determined.

e. Four grades of clot are recognized (Fig. 8), as described by Ropes and Bauer (1953).

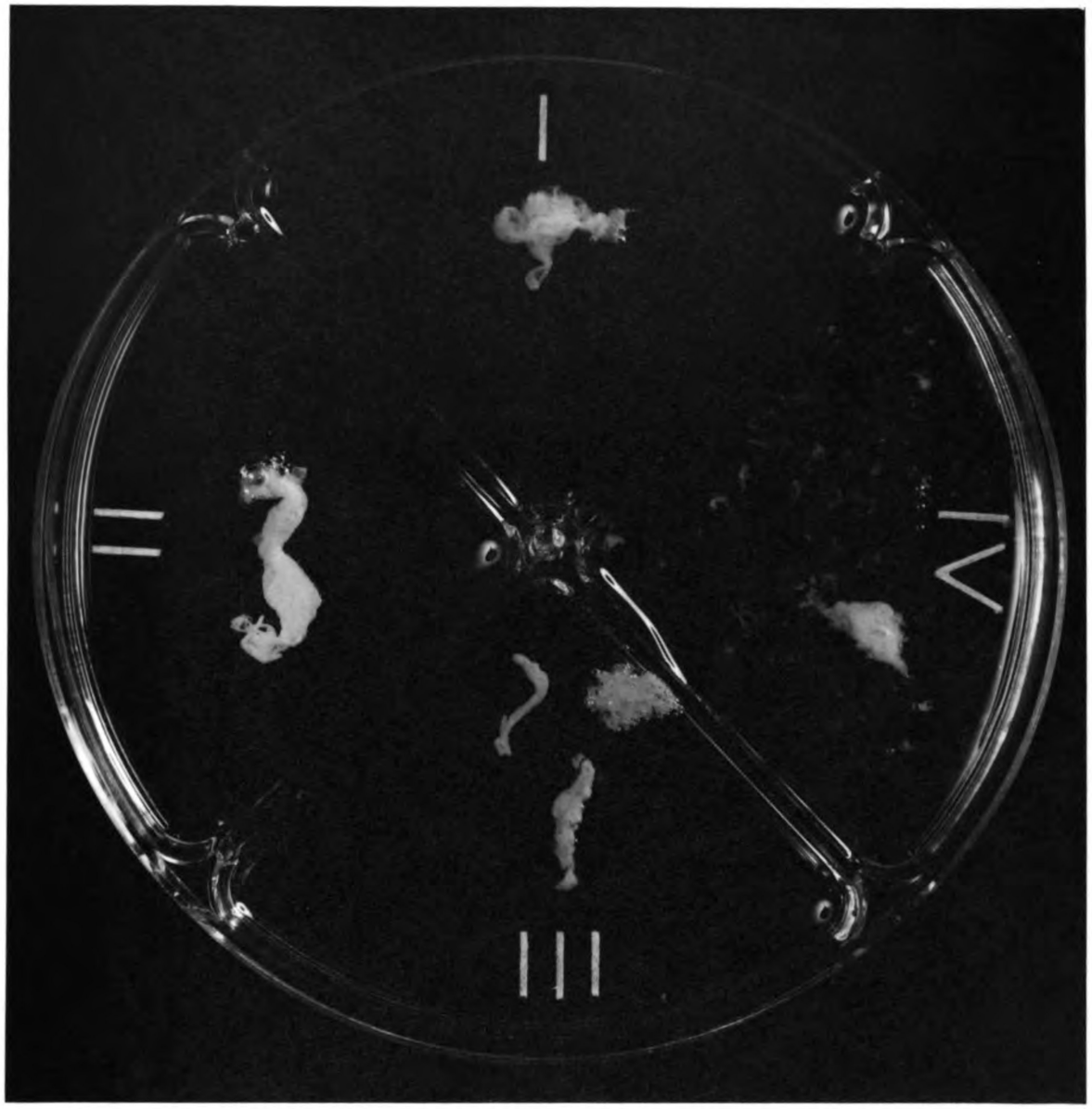
   (1) Normal - tight, ropy clump in a clear solution.

   (2) Fair - soft mass in a very slightly cloudy solution.

   (3) Poor - small, friable masses in a cloudy solution.

   (4) Very poor - few flecks in a cloudy solution.

Figure 8. Four grades of mucin clot obtained by precipitation with 7N glacial acetic acid: (I) normal; (II) fair; (III) poor; (IV) very poor.


I
III
IV

CHAPTER IV

RESULTS

General Considerations

In this study, each of the 83 animals are classified as clinically normal with respect to the tibiotarsal articulation and general health. They were selected after clinical examination failed to reveal any evidence of joint disease. The writer would like to state it is conceivable that some of the test animals may have undergone some form of joint disease prior to the collection date or were undergoing some insidious form of arthritis, inapparent at the time of sample collection. At no time did the author observe either clinical or post mortem evidence of arthritis in this study.

Because of the availability of slaughter animals that could provide unlimited material, this source was readily sought and hence accounts for the major portion of test animals in this study.

The results of laboratory determinations are presented in table form in their respective classifications. Averages and standard deviations are also included with the exception of Table I.

With regard to sugar determinations, these data are classified as to findings from fasting or non-fasting animals. Plasma sugar values were sought and compared with that of blood in relation to synovial fluid to arrive at a constituent that would most closely parallel or approximate synovial fluid sugar in either a fasting or non-fasting animal. In handling of animals, stress and excitement factors are also taken into consideration in relation to their influence on sugar determinations.

All samplings were taken from the tibio-tarsal articulation, in the recumbant position in the case of slaughter cattle and standing position in the balance of the group.

Table I identifies the animals in this study along with a notation as to the nature of the synovial fluid at time of collection. The animals designated by numbers from 1 to 83. Animals in the group from 1 to 52 comprise those from which samples were taken at post mortem. Because these animals were removed from feed 12 to 18 hours prior to slaughter it was necessary to make note of this fact, since interpretation and classification of data, particularly with respect to sugar values, will be made in this light. Water was available to this group of animals at all times. It was noted that the majority of the cows in this classification were in some stage of lactation at the time of slaughter. The group of animals from 53 to 83 make up the

balance and were individuals not intended for slaughter. Many of these cattle were in active lactation and samples from all individuals in the group were collected while on full feed. The Holstein breed accounted for 56.6 per cent of all animals tested. This figure was followed by 16.9 per cent Herefords, 4.8 per cent each of Guernseys and Galloways, 3.6 per cent each of Brahmas and Shorthorns, 2.4 per cent Angus and 4.8 per cent animals of mixed breeding, two of which were Holstein crosses followed by one Hereford cross and one Guernsey cross. The average age of the entire group was 3.51 years. When subdivided the average age of fasting slaughter cattle was 2.94 years and non-fasting cattle 4.48 years. Females comprised 57 per cent of the entire group, followed by 23 per cent steers and 20 per cent males. These figures break down to reveal that 36.5 per cent of slaughter cattle were females, 34.6 per cent steers and 28.8 per cent males. Females again dominated the picture in the non-fasting series with 80.6 per cent, there being more males at 12.9 per cent than steers which only accounted for 6.5 per cent. Observations on the nature of synovial fluid were as follows: 70 per cent colorless, 9.6 per cent a clear amber, 2.4 per cent slightly opaque, 2.4 per cent amber with flocculation and 1.2 per cent an opaque-amber with flocculation.

Table II gives the data for blood, plasma, and synovial fluid sugars for 52 animals in the fasting state.

All sugar values are expressed in milligrams per 100 milliliters of blood, plasma, or synovial fluid. Blood sugars averaged 80.58 ± 4.84 milligrams, with a low of 51 and a high of 154 milligrams of sugar per 100 milliliters of whole blood. The average plasma sugar level is considerably higher at 100.71 ± 8.21 milligrams per 100 milliliters. Plasma values range from a low of 60 milligrams to 236 milligrams of sugar per 100 milliliters. Sugar values for synovial fluid are intermediate in value with an average of 81.17 ± 4.37 milligrams per 100 milliliters. This average value is 0.59 ± 3.11 milligrams higher than the average value given for blood. Synovial fluid sugars range from a low of 42 milligrams to a high of 124 milligrams per 100 milliliters of fluid. From a diagnostic standpoint, emphasis is placed on the difference between blood or plasma in relation to the respective synovial fluid sugar for each animal. With this in mind, the blood-synovial fluid sugar difference was determined for each animal and for the entire group, with synovial fluid as previously mentioned averaging 0.59 ± 3.11 milligrams of sugar higher than that of blood. Synovial sugar differences range from a low of 42 milligrams of sugar less than to 22 milligrams greater than its blood counterpart. Plasma-synovial fluid differences show a reverse, with synovial fluid sugar averaging 19.89 ± 5.99 milligrams lower than the average values for plasma. This synovial fluid difference ranges from a low of 124 milligrams

of sugar less than to ten milligrams of sugar greater than its plasma counterpart.

Table III gives the data for blood, plasma, and synovial fluid sugars, in milligrams per 100 milliliters for each animal in the non-fasting state. All 31 animals in this series were on full feed at the time of sample collection. Most of the animals in this series were cows in some stage of lactation. Only four males and two steers were tested, as against 25 females. This factor undoubtedly accounts for the lower averages encountered in this test series. Blood sugar values average 63.22 $\pm$ 5.39 milligrams with a low of 45 milligrams and a high of 105 milligrams per 100 milliliters of whole blood. The average sugar value for plasma is 65.32 $\pm$ 6.33 milligrams with a low of 40 milligrams and a high of 112 milligrams per 100 milliliters. Synovial fluid sugar averaged 62.02 $\pm$ 6.20 milligrams, ranging from a low of 36 milligrams to a high of 105 milligrams per 100 milliliters. Here again from a diagnostic standpoint, value is placed on the difference between blood and plasma in relation to their respective synovial fluid sugars. In non-fasting cattle, the blood-synovial fluid sugar difference reversed the corresponding results observed in fasting slaughter cattle, as synovial fluid averages 1.13 $\pm$ 3.79 milligrams per 100 milliliters less than that of whole blood. These values range from a low of 35 milligrams of synovial fluid sugar less than to 15 milligrams of synovial

fluid sugar greater than that of the corresponding values for whole blood. Plasma-synovial fluid sugars in this series average 8.08 ± 3.36 milligrams of synovial fluid sugar less than that of plasma. Plasma-synovial fluid differences range from a low of 33 milligrams of synovial fluid sugar less than to eight milligrams greater than the corresponding plasma values.

Table IV is designed to show the relationship of blood, plasma, and synovial fluid sugars to age in the group comprising 52 fasted cattle presented for slaughter. Blood and/or plasma-synovial fluid differences in relation to age were not determined in compilation of this data. All sugar values are expressed in milligrams per 100 milliliters of blood, plasma, or synovial fluid sugar. The number of animals tested in each age group is given in parenthesis to the left of the corresponding sugar value. The animals in this series ranged from one to seven years of age, with the greatest number falling in the 1.5 year bracket. This latter fact can be accounted for by the fact that the majority of animals presented for slaughter usually fall in this age range. Blood sugar values range from a low of 63.00 milligrams for the one animal in the seven year bracket to a high of 89.72 for two year olds. Plasma sugar values again show a low of 68.00 milligrams for the seven-year old animal and range to a high of 117.88 for the two-year

olds. The values for synovial fluid sugar follow the pattern set by the two previous values in the lowest range with 63.00 milligrams for the seven-year old animal on up to a high of 88.33 milligrams of sugar per 100 milliliters for three animals in the four-year age bracket. In retrospect, one can see in this sampling, lower values for animals in the one-year age group rise with age to drop progressively after four years to lower levels.

Table V presents the data on the relationship of blood, plasma, or synovial fluid sugars to age in the group of 31 animals on full feed at time of sample collection. Blood and/or plasma-synovial fluid differences were not determined in compilation of the data presented here. All sugar values are reported in milligrams per 100 milliliters of whole blood, plasma, or synovial fluid. The number of animals tested in each age group is given in parenthesis to the left of the corresponding sugar value. Animals in this series showed a wider age range than those in the fasting group. This group ranged from 0.75 to 10 years of age; however, none were in either the 1.5 or nine-year age brackets. The majority were grouped in the four-year age bracket. This can be accounted for by the fact that the females in this series were lactating at the time samples were taken. Blood sugar values range from a low of 51.5 milligrams for two animals ten years of age to a high of 93.0 milligrams for one animal 0.75 years of age. As usual,

plasma sugar values ran higher than those of blood with a low of 60 milligrams for the two oldest animals to a high of 83.5 milligrams for two animals in the two year bracket. Plasma sugar was not determined for the one animal 0.75 years of age as this individual was tested early in the course of the study before it was decided to determine this value. Synovial fluid sugars again show an overall range intermediate between that of blood and plasma. The low value for synovial sugar at 43 milligrams is observed in one animal in the six-year age bracket and the high of 87 milligrams for the one 0.75 year old. As in the series of animals tested in the fasting group, the younger animals in this sampling show higher values that progressively drop off with advancing age.

Table VI is a comparison of the average blood, plasma, and synovial fluid sugars in fasting slaughter cattle and non-fasting cattle. The material is presented in this form so that a better understanding may evolve regarding the sugar levels in the two groups.

Table VII gives the relationship and comparison of blood, plasma, and synovial fluid sugars as to sex in fasting slaughter cattle and non-fasting cattle. The various sexes in the fasting group were more evenly distributed than in the non-fasting group in which females predominated. The data in this table are compiled to show the sex differences in respect to sugar levels.

Table VIII presents findings on the cellular studies of synovial fluid. It will be noted that a paradoxical situation seems to present itself in that the number of leukocytes per cubic millimeter may be given and yet no differential percentage of 100 leukocytes or vice versa. In the case of the former, an uncentrifuged sample of synovial fluid was diluted one to ten with the diluent and placed in the counting chamber of the hemacytometer. In these instances no leukocytes were observed, but following centrifugation of the sample, enough cells were collected to make possible a differential leukocyte count.

Conversely, instances occurred wherein no differential leukocyte counts were made. Microscopic examination of the stained smear failed to reveal cells with a morphology distinct enough to arrive at any differential classification. These smears were then discarded as the writer felt it would detract from the accuracy of this study.

It soon became apparent that the leukocytes observed in synovial fluid are of a highly fragile nature and therefore must be handled with due consideration if their identification is to be accurate. Occasionally synovial cells are encountered in the course of a differential leukocyte count. These are readily identified in that they more closely resemble fibroblasts than monocytes with which they can become confused. Total leukocytes range from 0 to 725 cells, with an average number of 114 per cubic

millimeter. The predominant cell types observed in the differential count are: lymphocytes 48.87 per cent, monocytes 39.31 per cent, neutrophils 5.67 per cent, clasmatocytes 6.13 per cent, and eosinophils 0.79 per cent. No basophils were observed in any of the stained smears. Erythrocytes were observed but not counted since they were attributed to hemorrhage at the time of aspiration in the living animal or extravasation of the cells in the course of agonal struggles at slaughter.

Table IX gives the absolute leukocyte values or number of leukocytes per cubic millimeter. These figures represent actual numbers, not per cent of 100 leukocytes. The absolute leukocyte count is arrived at by multiplying the number of leukocytes per cubic millimeter by the percentage values for the respective cell types as determined from a differential. Since an absolute leukocyte count is made from samples of synovial fluid from which both a total and differential leukocyte count are obtained, the average total number of leukocytes is slightly higher than the figure reported in the preceding table. This figure was 115.8 leukocytes per cubic millimeter. Total number of leukocytes ranged from 22 to 600 per cubic millimeter. The various cell types gave the following values per cubic millimeter: lymphocytes 53.7, monocytes 38.95, clasmatocytes 12.19, neutrophils 10.03 and eosinophils 0.91. No basophils were observed in these cellular studies.

Table X presents the data for the relative viscosity of synovial fluid at 101.5 degrees fahrenheit and quality of mucin clot for the respective sample. All relative viscosity determinations were made in a constant temperature bath, with the majority of determinations employing the Cannon-Fenske routine viscometer. Animal numbers marked with an asterisk indicate viscosity determinations arrived at with the Cannon-Ubbelohde semi-micro dilution viscometer. Relative viscosity for the tibio-tarsal articulation average 3.79 $\pm$ 0.55, with a range of 1.53 to 13.01 for 73 determinations. Normal mucin clots were observed in 91.7 per cent of fluids and only 8.2 per cent exhibited fair quality. Mucin clot serves as an indicator of quality, i.e., the degree of polymerization of the hyaluronic acid is reflected by the firmness observed in the clot, the higher the degree of polymerization the better the quality of mucin clot or vice versa.

Table XI is designed to show the relationship between the relative viscosity of synovial fluid and the age of the animal. The corresponding quality of mucin clot in per cent of normal is also given for each of the age groups. The number of animals tested in each bracket is given in parenthesis to the left of its respective value. One animal in the eight year bracket has the highest relative viscosity at 9.50 and a normal mucin clot. On the other end of the scale the lowest relative viscosity is

observed in the one 0.75 year old at 1.99, with a normal mucin clot. Relative viscosity is quite constant from one to four years of age and at this point begins to rise only to drop after eight years to a lower than pre-peak level in the one animal tested at ten years of age. Normal mucin clot quality drops with a corresponding rise in viscosity to attain its lowest level for the entire series at 67 per cent for animals seven years of age, only to return to normal for the two remaining individuals.

Table XII is a comparison of the relative viscosity of synovial fluid in relation to sex. Quality of mucin clot is also presented to ascertain the effect sex might have on the polymerization of the hyaluronic acid. While per cent of normal mucin clot quality runs consistently high, steers rose only one per cent above the value for males and females, while relative viscosity shows more variation. Steers exhibit the lowest relative viscosity values at 2.99 ± 0.40, almost that of males at 3.02 ± 0.61. The 37 females are markedly higher in value at 4.55 ± 0.99, a figure slightly more than half again as much for the 17 males and 19 steers.

TABLE I

IDENTIFICATION OF ANIMALS

AND COLLECTION DATA ON SYNOVIAL FLUID

FROM THE CLINICALLY NORMAL TIBIO-TARSAL ARTICULATION

Animal Number	Breed*	Age Yrs.	Sex	Fasting or Non-fasting	Ante Mortem or Post Mortem	Nature of Fluid**
1	BRA	1.5	Steer	Fasting	Post Mortem	O
2	BRA	1.5	Steer	Fasting	Post Mortem	O
3	BRA	2	Steer	Fasting	Post Mortem	O
4	HOL	5	Female	Fasting	Post Mortem	OP
5	HER	1	Steer	Fasting	Post Mortem	O
6	HOL	3	Male	Fasting	Post Mortem	O
7	HOL	1.5	Male	Fasting	Post Mortem	O
8	G	1	Male	Fasting	Post Mortem	O
9	HOL	5	Female	Fasting	Post Mortem	O
10	HOL	5	Female	Fasting	Post Mortem	O
11	HER-X	1.5	Steer	Fasting	Post Mortem	O
12	HER	2	Steer	Fasting	Post Mortem	O
13	SH	1.5	Steer	Fasting	Post Mortem	O
14	A	1	Steer	Fasting	Post Mortem	O

* A - Angus
BRA - Brahma
G - Guernsey
GAL - Galloway
HER - Hereford
HOL - Holstein
J - Jersey
SH - Shorthorn
X - Mixed Breed

** O - Colorless Synovial Fluid
OP - Slightly Opaque Synovial Fluid
A - Amber Synovial Fluid
F - Flocculation

TABLE I (continued...)

Animal Number	Breed	Age Yrs.	Sex	Fasting or Non-fasting	Ante Mortem or Post Mortem	Nature of Fluid
15	HOL	3	Female	Fasting	Post Mortem	O
16	HOL	1.5	Steer	Fasting	Post Mortem	O
17	HOL	2	Steer	Fasting	Post Mortem	OP
18	HOL	2	Steer	Fasting	Post Mortem	O
19	HOL	6	Female	Fasting	Post Mortem	O
20	GAL	1.5	Male	Fasting	Post Mortem	A
21	G	2	Male	Fasting	Post Mortem	O
22	HOL	1.5	Male	Fasting	Post Mortem	O
23	GAL	5	Male	Fasting	Post Mortem	O
24	GAL	5	Male	Fasting	Post Mortem	O
25	GAL	1	Male	Fasting	Post Mortem	O
26	SH	1.5	Male	Fasting	Post Mortem	O
27	HER	2	Steer	Fasting	Post Mortem	O
28	HER	3	Male	Fasting	Post Mortem	O
29	HER	1.5	Male	Fasting	Post Mortem	O
30	G	1.5	Male	Fasting	Post Mortem	A
31	J	2	Male	Fasting	Post Mortem	A
32	HER	4	Female	Fasting	Post Mortem	O
33	HER	5	Female	Fasting	Post Mortem	O
34	HER	5	Female	Fasting	Post Mortem	O
35	HER	6	Female	Fasting	Post Mortem	O
36	SH	6	Female	Fasting	Post Mortem	O

continued...

TABLE I (continued...)

Animal Number	Breed	Age Yrs.	Sex	Fasting or Non-fasting	Ante Mortem or Post Mortem	Nature of Fluid
37	HER	1.5	Female	Fasting	Post Mortem	0
38	HER	1.5	Steer	Fasting	Post Mortem	0
39	HER	1.5	Steer	Fasting	Post Mortem	0
40	HOL	4	Female	Fasting	Post Mortem	0
41	HOL	5	Female	Fasting	Post Mortem	0
42	HOL	4	Female	Fasting	Post Mortem	0
43	HOL	2	Steer	Fasting	Post Mortem	A
44	HOL	6	Female	Fasting	Post Mortem	AF
45	HOL	7	Female	Fasting	Post Mortem	AF
46	HOL-X	6	Female	Fasting	Post Mortem	A
47	HOL	6	Female	Fasting	Post Mortem	AOP
48	HOL-X	2	Steer	Fasting	Post Mortem	A
49	HOL	1.5	Female	Fasting	Post Mortem	0
50	A	1.5	Steer	Fasting	Post Mortem	0
51	HER	1.5	Female	Fasting	Post Mortem	0
52	HER	1.5	Steer	Fasting	Post Mortem	0
53	HOL	5	Female	Non-fasting	Ante Mortem	A
54	HOL	0.75	Male	Non-fasting	Ante Mortem	0
55	G-X	1	Male	Non-fasting	Ante Mortem	0
56	HOL	3	Female	Non-fasting	Ante Mortem	0
57	HOL	4	Female	Non-fasting	Ante Mortem	0

continued...

TABLE I (continued...)

Animal Number	Breed	Age Yrs.	Sex	Fasting or Non-fasting	Ante Mortem or Post Mortem	Nature of Fluid
58	G	4	Female	Non-fasting	Ante Mortem	0
59	HOL	4	Female	Non-fasting	Ante Mortem	0
60	J	3	Male	Non-fasting	Ante Mortem	0
61	HOL	3	Female	Non-fasting	Ante Mortem	0
62	HOL	3	Male	Non-fasting	Ante Mortem	0
63	HOL	2	Female	Non-fasting	Ante Mortem	0
64	HOL	7	Female	Non-fasting	Ante Mortem	0
65	HOL	10	Female	Non-fasting	Ante Mortem	0
66	HOL	7	Female	Non-fasting	Ante Mortem	0
67	HOL	7	Female	Non-fasting	Ante Mortem	0
68	HOL	4	Female	Non-fasting	Ante Mortem	0
69	HOL	8	Female	Non-fasting	Ante Mortem	0
70	HOL	1	Steer	Non-fasting	Ante Mortem	0
71	HOL	1	Steer	Non-fasting	Ante Mortem	0
72	HOL	8	Female	Non-fasting	Ante Mortem	0
73	HOL	10	Female	Non-fasting	Ante Mortem	0
74	HOL	2	Female	Non-fasting	Ante Mortem	0
75	HOL	4	Female	Non-fasting	Ante Mortem	0
76	HOL	4	Female	Non-fasting	Ante Mortem	0
77	HOL	3	Female	Non-fasting	Ante Mortem	0
78	HOL	5	Female	Non-fasting	Ante Mortem	0

continued...

TABLE I (continued...)

Animal Number	Breed	Age Yrs.	Sex	Fasting or Non-fasting	Ante Mortem or Post Mortem	Nature of Fluid
79	HOL	6	Female	Non-fasting	Ante Mortem	0
80	HOL	7	Female	Non-fasting	Ante Mortem	0
81	HOL	4	Female	Non-fasting	Ante Mortem	0
82	HOL	4	Female	Non-fasting	Ante Mortem	0
83	HOL	4	Female	Non-fasting	Ante Mortem	A
	Ave.	3.51				

TABLE II

BLOOD, PLASMA, AND SYNOVIAL FLUID SUGARS IN THE FASTING STATE*

Sugar Values in Milligrams Per 100 Milliliters

Animal	Blood	Plasma	Synovia	Blood-Synovia Difference**	Plasma-Synovia Difference**
1	105	134	82	-23	-52
2	112	171	93	-19	-78
3	93	120	96	+ 3	-24
4	79	82	79	0	- 3
5	70	85	78	+ 8	- 7
6	90	105	87	- 3	-18
7	96	102	96	0	- 6
8	82	105	102	+20	- 3
9	73	79	70	- 3	- 9
10	73	85	65	- 8	-20
11	96	128	90	- 6	-38
12	68	83	85	+17	+ 2
13	82	93	79	- 3	-14
14	-	82	63	-	-19
15	63	70	68	+ 5	- 2
16	70	85	70	0	-15

*Animals in this series had been taken off feed at least 12 hours prior to time of sample collection. Only water was allowed during the interim.

**Milligrams of synovial fluid sugar greater (+) or less (-) than blood or plasma sugar.

continued...

TABLE II (continued...)

Animal	Blood	Plasma	Synovia	Blood-Synovia Difference	Plasma-Synovia Difference
17	65	79	63	- 2	-16
18	70	84	76	+ 6	- 8
19	68	79	70	+ 2	- 9
20	93	128	87	- 6	-41
21	79	96	87	+ 8	- 9
22	68	79	68	0	-11
23	76	90	68	- 8	-22
24	70	73	63	- 7	-10
25	82	93	79	- 3	-14
26	73	85	95	+22	+10
27	63	87	76	+13	-11
28	68	90	70	+ 2	-20
29	68	87	87	+19	0
30	109	162	112	+ 3	-50
31	70	93	82	+12	-11
32	87	112	90	+ 3	-22
33	87	112	96	+ 9	-16
34	82	100	87	+ 5	-13
35	87	109	93	+ 6	-16
36	87	109	93	+ 6	-16
37	154	236	112	-42	-124

continued...

TABLE II (continued...)

Animal	Blood	Plasma	Synovia	Blood-Synovia Difference	Plasma-Synovia Difference
38	85	85	90	+ 5	+ 5
39	82	109	96	+14	-13
40	85	102	85	0	-17
41	79	102	79	0	-23
42	85	102	90	+ 5	-12
43	57	60	48	- 9	-12
44	68	82	73	+ 5	- 9
45	63	68	63	0	- 5
46	115	145	124	+ 9	-21
47	60	79	42	-18	-37
48	51	73	55	+ 4	-18
49	79	115	70	- 9	-45
50	82	102	73	- 9	-29
51	79	109	76	- 3	-33
52	82	112	82	0	-30
Ave.	80.58	100.71	81.17	+ 0.59	-19.89
SD	±4.84	±8.21	±4.37	± 3.11	± 5.99

TABLE III

BLOOD, PLASMA, AND SYNOVIAL FLUID SUGARS

IN THE NON-FASTING STATE*

Sugar Values in Milligrams Per 100 Milliliters

Animal	Blood	Plasma	Synovia	Blood-Synovia Difference**	Plasma-Synovia Difference**
53	79	-	76	- 3	-
54	93	-	87	- 6	-
55	90	-	105	+15	-
56	73	-	85	+12	-
57	105	-	70	-35	-
58	65	82	68	+ 3	-14
59	63	76	70	+ 7	- 6
60	63	77	70	+ 7	- 7
61	62	76	70	+ 8	- 6
62	63	65	65	+ 2	0
63	82	112	79	- 3	-33
64	70	-	70	0	-
65	48	55	57	+ 9	+ 2
66	57	65	60	+ 3	- 5
67	55	68	65	+10	- 3
68	60	65	52	- 8	-13

*Animals in this series were on full feed at the time of sample collection.

**Milligrams of synovial fluid sugar greater (+) or less (-) than blood or plasma sugar.

continued...

TABLE III (continued...)

Animal	Blood	Plasma	Synovia	Blood-Synovia Difference	Plasma-Synovia Difference
69	60	63	50	-10	-13
70	60	65	63	+ 3	- 2
71	76	79	87	+11	+ 8
72	60	73	65	+ 5	- 8
73	55	65	52	- 3	-13
74	50	55	43	- 7	-12
75	45	55	43	- 2	-12
76	48	40	43	- 5	+ 3
77	50	50	44	- 6	- 6
78	48	40	36	-12	- 4
79	63	57	43	-20	-14
80	52	55	48	- 4	- 7
81	45	48	43	- 2	- 5
82	52	65	40	-12	-15
83	68	82	76	+ 8	- 6
Ave.	63.22	65.32	62.09	- 1.13	- 8.08
SD	±5.39	±6.33	±6.20	± 3.79	± 3.36

TABLE IV

RELATIONSHIP OF BLOOD, PLASMA, AND SYNOVIAL FLUID SUGARS TO AGE IN THE FASTING STATE*

Sugar Values in Milligrams Per 100 Milliliters**

Age: Yrs.	1.0	1.5	2.0	3.0	4.0	5.0	6.0	7.0
	(3)***	(18)	(9)	(3)	(3)	(8)	(6)	(1)
Blood:	78.00	89.72	68.11	73.66	85.66	77.00	80.83	63.00
	(4)	(18)	(9)	(3)	(3)	(8)	(6)	(1)
Plasma:	91.25	117.88	86.11	88.33	105.33	90.37	100.50	68.00
	(4)	(18)	(9)	(3)	(3)	(8)	(6)	(1)
Synovia:	80.50	86.55	74.22	75.00	88.33	75.87	82.50	63.00

*Animals in this series had been taken off feed at least 12 hours prior to time of sample collection. Only water was allowed during the interim.

**Sugar values measured to the nearest one-hundredth milligram.

***Number of animals tested in each age group.

TABLE V

RELATIONSHIP OF BLOOD, PLASMA, AND SYNOVIAL FLUID SUGARS TO AGE IN THE NON-FASTING STATE*

Sugar Values in Milligrams Per 100 Milliliters**

Age: Yrs.	0.75	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	10.0
	(1)***	(3)	(2)	(5)	(9)	(2)	(1)	(4)	(2)	(2)
Blood:	93.00	75.33	66.00	62.20	61.22	63.00	63.00	58.50	60.00	51.50
	(0)	(2)	(2)	(4)	(9)	(0)	(1)	(3)	(2)	(2)
Plasma:	00.00	72.00	83.50	67.00	61.44	00.00	57.00	62.66	68.00	60.00
	(1)	(3)	(2)	(5)	(9)	(2)	(1)	(4)	(2)	(2)
Synovia:	87.00	85.00	61.00	66.80	56.11	56.00	43.00	60.75	57.50	54.50

*Animals in this series were on full feed at the time of sample collection.

**Sugar values measured to the nearest one-hundredth milligram.

***Number of animals tested in each age group.

TABLE VI

COMPARISON OF THE AVERAGE BLOOD, PLASMA, AND SYNOVIAL FLUID SUGARS IN FASTING AND NON-FASTING ANIMALS*

Sugar Values in Milligrams Per 100 Milliliters**

	Fasting Animals 1-52	Non-fasting Animals 53-83
Blood:	80.58 ± 4.84	63.22 ± 5.39
Plasma:	100.71 ± 8.21	65.32 ± 6.33
Synovia:	81.17 ± 4.37	62.09 ± 6.20

*Fasting animals in this series had been taken off feed at least 12 hours prior to time of sample collection. Only water was allowed during the interim. Non-fasting animals in this series were on full feed at the time of sample collection.

**Sugar values measured to the nearest one-hundredth milligram.

TABLE VII

RELATIONSHIP AND COMPARISON OF BLOOD, PLASMA, AND SYNOVIAL FLUID SUGARS TO SEX IN THE FASTING AND NON-FASTING STATE*

Sugar Values in Milligrams Per 100 Milliliters**

	(15)***	(19)	(17)	(4)	(25)	(2)
Blood:	80.53	82.26	78.41	82.00	60.61	68.00
	(15)	(19)	(18)	(2)	(21)	(2)
Plasma:	100.00	103.42	98.44	71.00	65.08	72.00
	(15)	(19)	(18)	(4)	(25)	(2)
Synovia:	84.86	80.78	77.50	64.87	56.89	75.00

*Fasting animals in this study had been taken off feed at least 12 hours prior to time of sample collection. Only water was allowed during the interim. Non-fasting animals in this study were on full feed at the time of sample collection.

**Sugar values measured to the nearest one-hundredth milligram.

***Number of animals tested for the respective sexes.

TABLE VIII

CELLULAR STUDIES MADE ON THE SYNOVIAL FLUID OF THE CLINICALLY NORMAL TIBIO-TARSAL ARTICULATION

White Blood Cell and Differential Cell Counts

Animal	W. B. C. per cu. mm.	DIFFERENTIAL PERCENTAGES OF 100 LEUKOCYTES Neutro.	Lymph.	Mono.	Clasmato.	Eosino.
1	75	0	62	36	2	0
2	50	4	45	49	2	0
3	0*	1	40	54	5	0
4	125	8	58	27	6	1
6	100	-	-	-	-	-
9	50	0	70	24	3	3
10	25	0	28	72	0	0
11	25	0	60	40	0	0
12	25	5	62	33	0	0
13	25	33	47	20	0	0
14	0*	4	56	39	1	0
15	175	2	28	69	1	0
16	0*	1	56	43	0	0
17	200	2	25	66	6	1
18	200	2	71	27	0	0
19	200	5	62	32	0	1

*No cells were found at a 1 to 10 dilution, but following centrifugation for 30 minutes at 3,000 r.p.m., enough cells were collected to make a differential leukocyte count.

continued...

TABLE VIII (continued...)

Animal	W. B. C. per cu. mm.	DIFFERENTIAL PERCENTAGES OF 100 LEUKOCYTES Neutro.	Lymph.	Mono.	Clasmato.	Eosino.
20	75	9	35	56	0	0
21	25	1	24	73	2	0
22	75	0	35	65	0	0
23	50	3	33	64	0	0
24	50	13	68	18	0	1
25	25	1	26	70	3	0
26	100	4	70	26	0	0
27	25	0	33	62	5	0
28	25	8	80	12	0	0
29	0*	5	57	35	3	0
30	25	0	67	33	0	0
31	25	3	73	22	2	0
32	75	-	-	-	-	-
33	50	-	-	-	-	-
34	125	1	70	23	1	5
35	300	12	85	3	0	0
36	175	-	-	-	-	-
37	125	0	24	74	2	0
38	100	-	-	-	-	-
39	275	1	69	29	1	0
40	125	3	87	10	0	0

continued...

TABLE VIII (continued...)

Animal	W. B. C. per cu. mm.	DIFFERENTIAL PERCENTAGES OF 100 LEUKOCYTES Neutro.	Lymph.	Mono.	Clasmato.	Eosino.
41	100	-	-	-	-	-
42	250	1	69	29	0	1
43	250	8	40	28	24	0
44	600	26	16	44	13	1
45	275	23	18	28	30	1
46	75	2	38	10	43	7
47	325	1	31	20	48	0
48	125	1	28	26	45	0
49	50	3	6	58	31	2
50	50	-	-	-	-	-
51	225	2	6	55	37	0
52	125	-	-	-	-	-
53	100	-	-	-	-	-
54	25	-	-	-	-	-
55	725	-	-	-	-	-
56	25	-	-	-	-	-
57	0*	2	74	19	5	0
58	25	2	72	26	0	0
59	50	-	-	-	-	-
60	50	1	74	25	0	0
61	250	5	74	21	0	0

continued...

TABLE VIII (continued...)

Animal	W. B. C. per cu. mm.	DIFFERENTIAL PERCENTAGES OF 100 LEUKOCYTES Neutro.	Lymph.	Mono.	Clasmato.	Eosino.
62	150	1	81	18	0	0
63	25	0	92	4	4	0
64	125	0	81	10	0	9
65	0*	0	61	38	1	0
66	0*	0	53	47	0	0
70	44	-	-	-	-	-
71	177	-	-	-	-	-
72	44	-	-	-	-	-
73	89	-	-	-	-	-
74	44	0	42	50	8	0
75	44	6	47	47	0	0
76	22	13	43	40	0	4
77	11	-	-	-	-	-
78	22	2	2	81	13	2
79	33	18	50	24	1	7
80	11	-	-	-	-	-
81	156	69	14	16	1	0
82	-	1	10	86	3	0
83	44	11	6	79	4	0
Ave.	114	5.67	48.87	39.31	6.13	0.79

TABLE IX

CELLULAR STUDIES MADE ON THE SYNOVIAL FLUID OF THE CLINICALLY NORMAL TIBIO-TARSAL ARTICULATION

Total Leukocyte and Absolute Leukocyte Values

		ABSOLUTE LEUKOCYTE VALUES				
	W. B. C.	Number of Leukocytes per cubic millimeter				
Animal	per cu. mm.	Neutro.	Lymph.	Mono.	Clasmato.	Eosino.
1	75	0.00	46.50	27.00	1.50	0.00
2	50	2.00	22.50	24.50	1.00	0.00
4	125	10.00	72.50	33.75	7.50	1.25
9	50	0.00	35.00	12.00	1.50	1.50
10	25	0.00	7.00	18.00	0.00	0.00
11	25	0.00	15.00	10.00	0.00	0.00
12	25	1.25	15.50	8.25	0.00	0.00
13	25	8.25	11.75	5.00	0.00	0.00
15	175	3.50	49.00	120.75	1.75	0.00
17	200	4.00	50.00	132.00	12.00	2.00
18	200	4.00	142.00	54.00	0.00	0.00
19	200	10.00	124.00	64.00	0.00	2.00
20	75	6.75	26.25	42.00	0.00	0.00
21	25	0.25	6.00	18.25	0.50	0.00
22	75	0.00	26.25	48.75	0.00	0.00
23	50	1.50	16.50	32.00	0.00	0.00
24	50	6.50	34.00	9.00	0.00	0.50
25	25	0.25	6.50	17.50	0.75	0.00

continued...

TABLE IX (continued...)

Animal	W. B. C. per cu. mm.	ABSOLUTE LEUKOCYTE VALUES Number of Leukocytes per cubic millimeter Neutro.	Lymph.	Mono.	Clasmato.	Eosino.
26	100	4.00	70.00	26.00	0.00	0.00
27	25	0.00	8.25	15.50	1.25	0.00
28	25	2.00	20.00	3.00	0.00	0.00
30	25	0.00	16.75	8.25	0.00	0.00
31	25	0.75	18.25	5.50	0.50	0.00
34	125	1.25	87.50	28.75	1.25	6.25
35	300	36.00	255.00	9.00	0.00	0.00
37	125	0.00	30.00	92.50	2.50	0.00
39	275	2.75	189.75	79.75	2.75	0.00
40	125	3.75	108.75	12.50	0.00	0.00
42	250	2.50	172.50	72.50	0.00	2.50
43	250	20.00	100.00	70.00	60.00	0.00
44	600	156.00	96.00	264.00	78.00	6.00
45	275	63.25	49.50	77.00	82.50	2.75
46	75	1.50	28.50	7.50	32.25	5.25
47	325	3.25	100.75	65.00	156.00	0.00
48	125	1.25	35.00	32.50	56.25	0.00
49	50	1.50	3.00	29.00	15.50	1.00
51	225	4.50	13.50	123.75	83.25	0.00
58	25	0.50	18.00	6.50	0.00	0.00
60	50	0.50	37.00	12.50	0.00	0.00

continued...

TABLE IX (continued...)

Animal	W. B. C. per cu. mm.	ABSOLUTE LEUKOCYTE VALUES Number of Leukocytes per cubic millimeter Neutro.	Lymph.	Mono.	Clasmato.	Eosino.
61	250	12.50	185.00	52.50	0.00	0.00
62	150	1.50	121.50	27.00	0.00	0.00
63	25	0.00	23.00	1.00	1.00	0.00
64	125	0.00	101.25	12.50	0.00	11.25
74	44	0.00	18.48	22.00	3.52	0.00
75	44	2.64	20.68	20.68	0.00	0.00
76	22	2.86	9.46	8.80	0.00	0.88
78	22	0.44	0.44	17.82	2.86	0.44
79	33	5.94	16.50	7.92	0.33	2.31
81	156	107.64	21.84	24.96	1.56	0.00
83	44	4.84	2.64	34.76	1.76	0.00
Ave.	115.8	10.03	53.70	38.95	12.19	0.91

TABLE X

RELATIVE VISCOSITY OF SYNOVIAL FLUID AT 101.5° F.
AND QUALITY OF MUCIN CLOT
FROM THE CLINICALLY NORMAL TIBIO-TARSAL ARTICULATION

Animal	Relative Viscosity	Mucin Clot
1	4.52	Normal
2	2.45	Normal
3*	2.77	Normal
4	5.32	Normal
5*	2.05	Normal
6	2.42	Normal
7	1.64	Fair
8	2.74	Normal
9	4.18	Normal
10	5.45	Normal
11	2.44	Normal
12	2.06	Normal
13	2.73	Normal
15	3.67	Normal
16	3.92	Normal
17	3.53	Normal
18	3.31	Normal

*Determinations were made using the Cannon-Ubbelohde Semi-Micro Dilution Viscometer.

continued...

TABLE X (continued...)

Animal	Relative Viscosity	Mucin Clot
19	5.65	Normal
20	3.32	Normal
21	2.50	Normal
22	3.31	Normal
23	4.73	Normal
24	4.72	Normal
25	6.07	Normal
26	2.70	Normal
27	2.55	Normal
28	3.40	Normal
29	2.36	Normal
30	1.75	Normal
31	2.06	Normal
33*	8.59	Normal
34	2.00	Normal
35	3.40	Normal
36*	13.01	Normal
37	3.94	Normal
38	2.98	Normal
39	4.24	Normal
40	4.48	Normal
41	2.20	Normal

continued...

TABLE X (continued...)

Animal	Relative Viscosity	Mucin Clot
42	2.95	Normal
43	4.35	Normal
44	2.46	Normal
45	3.45	Fair
46*	12.94	Fair
47*	7.40	Normal
48	1.63	Normal
49	3.40	Normal
50	3.53	Normal
51	3.10	Normal
52	2.26	Fair
53	2.24	Normal
54	1.66	Normal
55	2.64	Normal
56	3.14	Normal
57	1.95	Normal
58	4.05	Normal
59	2.90	Normal
61	3.44	Normal
62	2.93	Normal
64	3.63	Normal
70	2.92	Normal

continued...

TABLE X (continued...)

Animal	Relative Viscosity	Mucin Clot
71	2.66	Normal
72*	9.50	Normal
73	2.52	Normal
74	2.83	Normal
76	3.47	Normal
77	3.13	Normal
78*	9.95	Normal
79	2.61	Fair
80*	9.23	Normal
81	2.35	Fair
82	3.29	Normal
83*	1.53	Normal
Ave.	3.79	
SD	±0.55	

TABLE XI

RELATIONSHIP OF AGE TO THE RELATIVE VISCOSITY OF SYNOVIAL FLUID AT 101.5° F. AND QUALITY OF MUCIN CLOT FROM THE CLINICALLY NORMAL TIBIO-TARSAL ARTICULATION*

Age: Yrs.	0.75	1.0	1.5	2.0	3.0	4.0	5.0	6.0	7.0	8.0	10.0
	(1)**	(6)	(18)	(10)	(7)	(9)	(10)	(7)	(3)	(1)	(1)
Viscosity:	1.99	3.18	3.03	2.76	3.02	3.00	4.94	6.78	5.43	9.50	2.52
	(1)**	(6)	(18)	(10)	(7)	(9)	(10)	(7)	(3)	(1)	(1)
Clot:	100%	100%	89%	100%	100%	89%	100%	71%	67%	100%	100%

*Per cent of normal mucin clots in each age group are given.

**Number of animals tested in each age group.

TABLE XII

COMPARISON OF THE RELATIVE VISCOSITY OF SYNOVIAL FLUID AT 101.5° F. AND QUALITY OF MUCIN CLOT FROM THE CLINICALLY NORMAL TIBIO-TARSAL ARTICULATION* IN RELATION TO SEX

Sex:	Males	Females	Steers
	(17)**	(37)	(19)
Viscosity:	3.02 ±0.61	4.55 ±0.99	2.99 ±0.40
	(17)**	(37)	(19)
Clot:	94%	94%	95%

*Per cent of normal mucin clots for each sex are given.

**Number of animals tested for the respective sexes.

CHAPTER V

DISCUSSION

General Considerations and Observations

Several factors that may or may not have influenced the final outcome of this study will be considered and discussed, as well as certain proposals for future work of this nature.

The literature pertaining to human synovial fluid has revealed that study of this fluid is still in its embryo stages and veterinary literature reveals that it has barely reached the stage of conception. Therefore, any information derived through a constructive study of this tissue should be considered an integral part in the steps leading to the final solution of joint disorders.

It is unfortunate that in the selection of animals for this study, there were limitations on the specific number of individuals, *viz.*, with respect to age and sex, i.e., males, females, and steers. It is the author's opinion that the influence of age and sex merit further evaluation with respect to the picture these two variables may reflect on further synovial fluid analyses. It is known that certain forms of human arthritis have been shown to be sex-linked and certainly more prevalent in older individuals.

Collection of synovial fluid from diarthrodial articulations other than the tibio-tarsal would be of value in the eventual evaluation of disorders that arise in joints other than the one previously mentioned. To arrive at a complete picture of the normal in smaller articulations, semi-micro and micro techniques would have to be worked out, in view of the fact that generally speaking, considerably smaller quantities of synovia are obtained from articulations other than the tibio-tarsal.

With respect to the influence fasting plays on the sugar levels of blood, plasma, and synovial fluid, it would be desirable to utilize cattle not intended for slaughter, that could be taken off feed for varying lengths of time. It is understandable that except in the interests of research, lactating cattle would be difficult to obtain for work of this nature, in view of the effects fasting would have on milk production.

Total quantity of synovial fluid in the tibio-tarsal articulation was not in this author's opinion an important determination, since it was observed to vary markedly with the age, sex, size, and general bone structure of the individual. Small fine boned milk cows provided the smallest total quantity. Quality of the fluid is unquestionably important, since it reflects the physiological status of the joint. Qualitative determinations on the

mucopolysaccharide were carried out by observations on its relative viscosity and formation of clot by a method of glacial acetic acid precipitation.

The cytological picture appears to be an accurate reflection of joint status, since in this study, few inflammatory cells were noted. The high preponderance of synovial fluid lymphocytes is attributable to the large percentage of young animals with healthy joints. What few eosinophils that were observed are unaccounted for at this time. The fact that leukocytes were so scarce, necessitated the use of high centrifugation speeds in order to make differential counts and morphology studies. This procedure appeared to cause a more rapid breakdown of the already fragile leukocytes. In the course of completing a differential leukocyte count, many cells had to be passed over because distorted morphology made positive identification impossible.

Blood, Plasma, and Synovial Fluid Sugars

The sum total of reducing agents present in the tissues or fluid studied will be reported and/or referred to as sugar, since these values as determined by the macro-method of Folin-Wu do not represent glucose *per se*. It was Folin (1926) who made reference to the term blood sugar, because of the non-glucose reducing agents in whole blood.

From a diagnostic standpoint, it is the blood or plasma-synovial difference that is important in the interpretation of intra-articular metabolism and related physiological function. Synovial fluid generally reflects changes produced in synovial tissues by disease. Since quantitative changes in synovial sugars aid in a differential diagnosis of joint diseases, it was decided to seek out an average sugar value from blood or plasma that would most closely parallel that of the synovia, hence in this study whole blood and whole plasma are evaluated in this light.

Hall (1959) stated that glucose is present in normal human blood in concentrations which vary between 60 milligrams per 100 milliliters in a fasting state and 120 milligrams per 100 milliliters after a meal rich in carbohydrates. Furthermore the level of blood glucose at any given time represents the outcome of a dynamic equilibrium between glucose entering the blood from the alimentary canal, and the liver on the one hand, and glucose leaving the blood to enter the liver, brain, and muscle on the other. He noted that a number of factors affect the level of the renal threshold. For example, it varies from one individual to another; prolonged hyperglycemia causes a rise in the renal threshold for glucose, and thyroid hormone also appears capable of elevating the level of this threshold.

Since the animals used in this study were ruminants, it was decided after determining the blood, plasma,

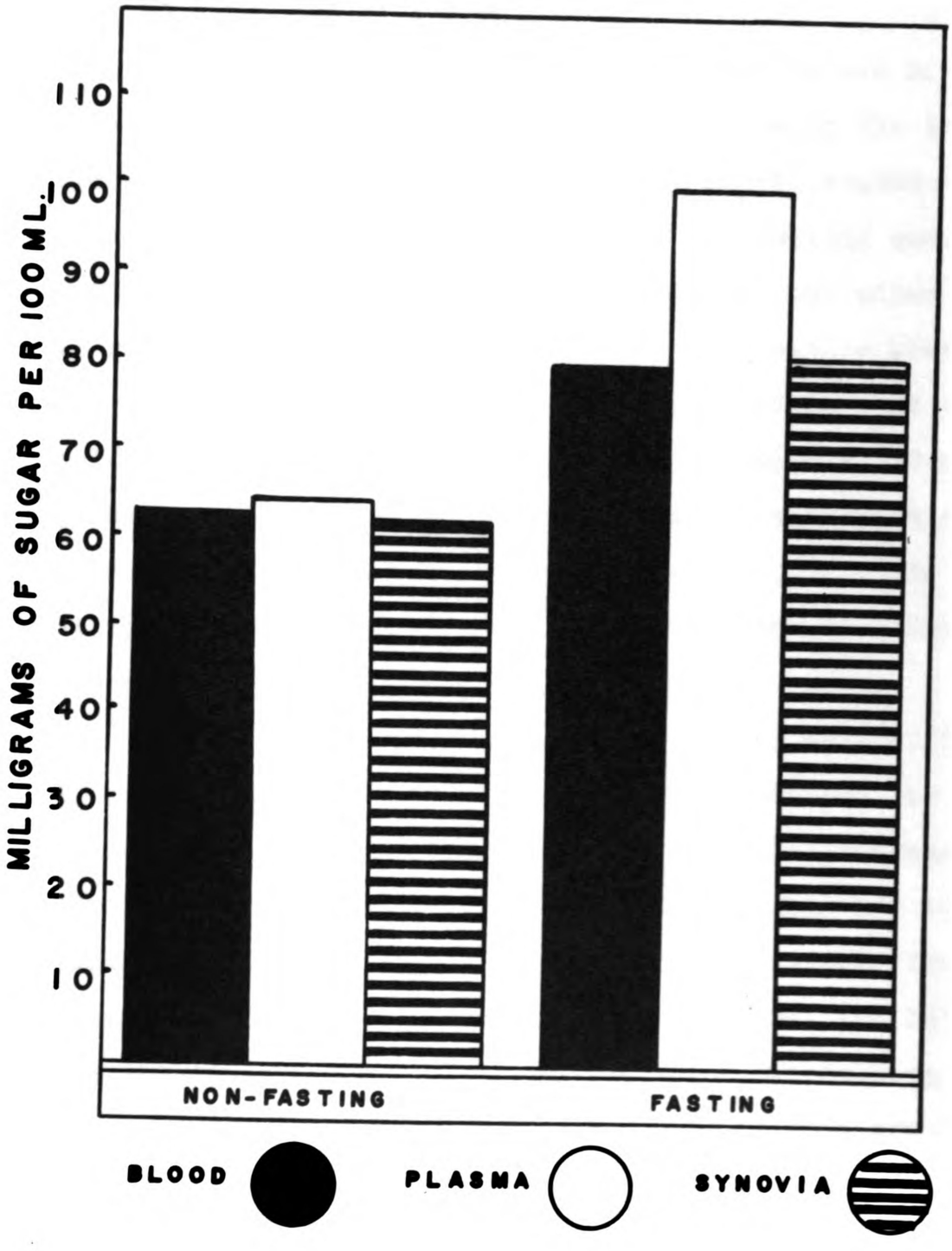
and synovial fluid sugar of fasting slaughter cattle, to measure these respective values in a group of non-fasting cattle. It is conceivable that ruminants may react to periods of fasting (12 to 18 hours) in a manner quite unlike that observed in man or animals possessing a simple stomach, e.g., dog, cat, and pig.

The average whole blood sugar value for non-fasting cattle (Table III) was 63.22 ± 5.39 milligrams per 100 milliliters. Individual values in this series ranged from a low of 45 to a high of 105 milligrams of sugar per 100 milliliters. A considerably higher average value was observed in fasting slaughter cattle (Table II) at 80.58 ± 4.84 milligrams of sugar per 100 milliliters. A wider variation existed between individuals in this group, with a low of 51 and a high of 154 milligrams per 100 milliliters. These figures represent a wider range of values than those reported by Dukes (1955) who gives a range of from 40 to 70 milligrams of sugar per 100 milliliters in the whole blood of cattle without stating sex, age, lactation status, or level of food intake.

Plasma sugar values (Table III) in non-fasting cattle were only slightly higher than their reported values for whole blood. The animals in this group averaged 65.32 ± 6.33 milligrams of sugar per 100 milliliters of whole blood, with a range from 40 to 112 milligrams. Fasting slaughter cattle (Table II) were considerably higher with

CHART I

COMPARISON OF THE AVERAGE SUGAR VALUES IN TWO GROUPS OF CATTLE



an average plasma sugar of 100.71 ± 8.21 milligrams per 100 milliliters. These animals also exhibited a wider individual variation in plasma sugar levels, ranging from a low of 60 to a high of 236 milligrams.

The average blood and plasma sugar values listed in the preceding paragraphs do not coincide with the observations made by another worker. Turner (1955) stated that the usual blood sugar concentration in the fasting animal was 60 to 80 milligrams per 100 milliliters, but after a meal rich in carbohydrates, the blood sugar may be elevated to 110 or 140 milligrams. In addition, he noted that the renal threshold for blood sugar was approximately 250 milligrams, and that concentrations exceeding this value resulted in glycosuria. He neglected, however, to state just what species of animal or what method was used to arrive at these blood sugar levels.

Synovial fluid sugar in non-fasting cattle (Table III) gave lower average values than those reported for either blood or plasma in this series of animals. The average synovial fluid sugar was 62.09 ± 6.20 milligrams per 100 milliliters, with a range lower than that of blood, from a minimum of 36 to a maximum of 105 milligrams, the latter value paralleling the high value for blood but falling below the high of 112 milligrams for plasma in this group. In fasting slaughter cattle (Table II), synovial fluid sugars dropped into an intermediate position between that

of blood and plasma, with an average of 81.17 ± 4.37 milligrams per 100 milliliters. Synovial sugar in fasting cattle ranged from 42 milligrams, a value lower than that of blood to a high of 124 milligrams, considerably lower than the high value reported for blood and lower than either the low or high values for plasma sugar in this series. These variations are best visualized in Chart IV which gives the total average sugar values for blood, plasma, and synovial fluid in non-fasting cattle and fasting slaughter cattle. Spector (1956) reported the average synovial fluid sugar from the tibio-tarsal articulation of cattle as 65 milligrams per 100 milliliters, with a range of 45 to 95 milligrams. He neglected, however, to state by what method these determinations were made or the nutritional status of the animal, i.e., fasting or non-fasting. Ropes, et al. (1939) determined the average serum sugar (Folin-Wu method) of slaughter cattle as 91 milligrams per 100 milliliters, with a minimum of 58 milligrams and a maximum of 176 milligrams. The figures for the average synovial fluid sugar in their study showed this value to average 66 milligrams per 100 milliliters, with a minimum of 45 milligrams and a maximum of 93 milligrams. They attributed the lower concentration in the synovia as in part due to the fact that the animals were not fasting, and in part to the fact that they struggled considerably when sacrificed, thereby raising the concentration of the sugar in the serum just before

the samples were collected and not allowing time for the fluid to come to equilibrium with the serum. Their high serum sugar values are undoubtedly due to the fact that arterial blood from the carotid arteries served as the source for serum sugars.

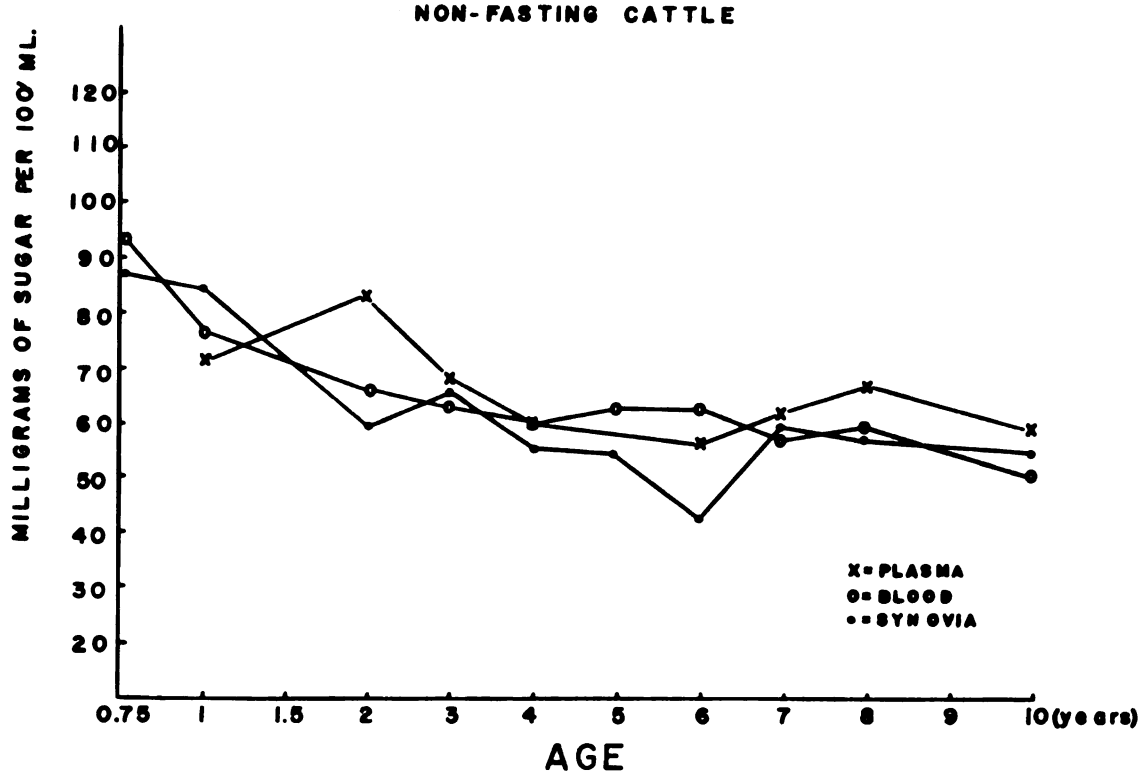
Comparison of data on blood, plasma, and synovial fluid sugars brings to light the fact that in both non-fasting and fasting cattle (Tables II and III) of all ages and sexes, blood sugar averages most closely parallel the average values for synovial fluid sugars. Plasma sugar averages were consistently greater than the corresponding average synovial fluid sugar, especially in fasting slaughter cattle and considerably higher than their corresponding blood sugar values for these animals.

In regard to age, the blood, plasma, and synovial fluid sugars in non-fasting cattle (Table V), almost without exception showed a linear decline with advancing age (Chart II). In fasting slaughter cattle, blood, plasma, and synovial fluid sugars (Table IV) reached their greatest height in the 1.5 year age group and then showed a progressive decline with advancing age; however, this group of animals never reached the age limits or minimal sugar levels seen in the non-fasting series (Chart III).

When blood, plasma, and synovial fluid sugars were evaluated with respect to sex of the individuals concerned (Table VII), differences were readily noted (Chart IV).

CHART II

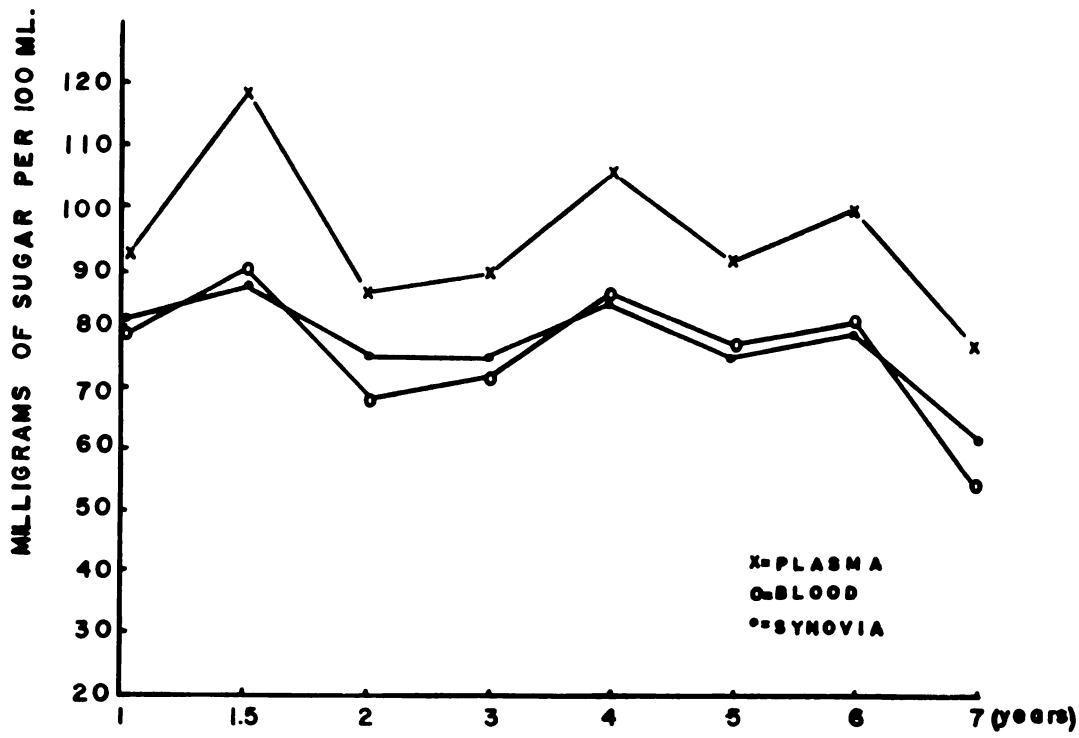
RELATIONSHIP OF AVERAGE SUGAR VALUES TO AGE IN NON-FASTING CATTLE



AGE

CHART III

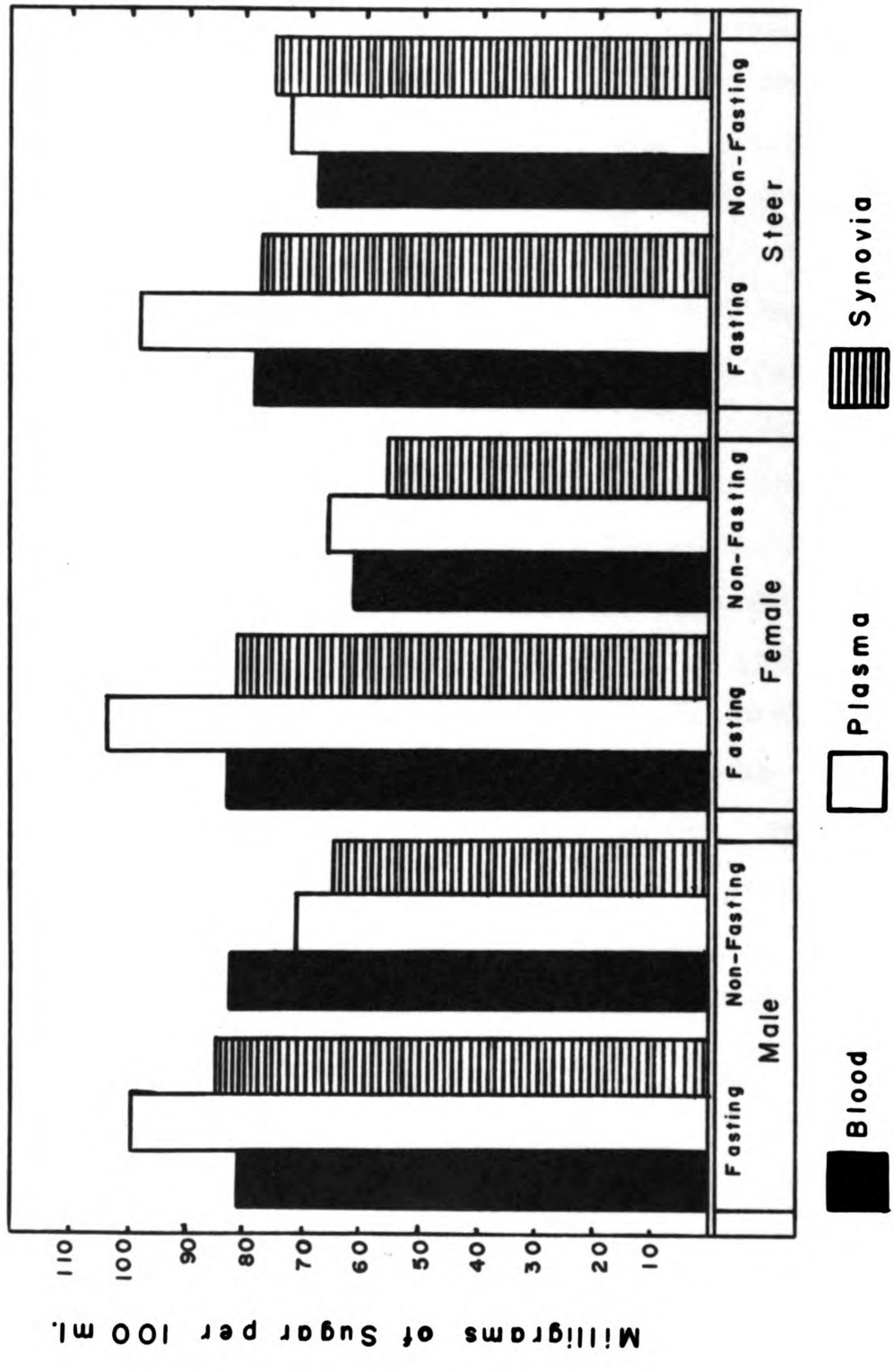
RELATIONSHIP OF AVERAGE SUGAR VALUES TO AGE IN FASTING CATTLE



AGE

CHART IV

COMPARISON OF THE AVERAGE SUGAR VALUES IN TWO GROUPS OF CATTLE IN RELATION TO SEX



In non-fasting cattle, females gave the lowest values; however, this can probably be explained by the fact that the majority of these individuals were older animals and in various stages of lactation. Udall (1954) stated that a cow producing 30 quarts of milk per day loses three pounds of sugar and that the main part of the sugar must be produced as a result of cellulose fermentation in the rumen. This fact alone would account in part for lowered sugar levels in this group. Diurnal variations in the blood sugar level of the lactating cow were noted by Allcroft (1933) who concluded that, while there is no apparent diurnal rhythm in the blood sugar level of dry cows and dry sheep, there is some evidence of a decided trend of diurnal changes in the blood sugar levels of lactating cows. He also noted the fact that no such rhythm is apparent in the curves for dry cows or dry sheep, and would seem to imply that this rhythm is associated with the state of lactation. Males in the non-fasting series gave slightly higher values than females and steers.

Females in the fasting slaughter group of animals (Table VII) were diametrical to non-fasting females, in that they showed the highest average sugar values (Chart IV). Most of these animals were younger females than those of the previously discussed group and most were in some stage of lactation at the time of slaughter. In the fasting series, steers showed the lowest sugar values, while

males were intermediate. The overall higher sugar values for blood, plasma, and synovial fluid sugar in fasting slaughter cattle could very well be attributed to a younger age group, with a higher preponderance of males and steers, with this exception, females in this series showed the highest values. The effects of fasting may influence the results to some degree, since the greatest sugar values were recorded for plasma. What effect saccharoids have in producing elevations in sugar levels of fasting cattle was not determined in this study. Undoubtedly in the ruminant the influence of the saccharoid fractions of blood, plasma, and synovial fluid are exerted in some manner. Glucose portions of the reducing fraction were apparently quite high in plasma; since the erythrocytes can carry only so much of this element, the balance would have to be borne by plasma. Allcroft and Strand (1933) were able to show that a seven day fast did not appreciably alter the blood sugar or lactic acid of ruminants.

A factor to consider is excitement in slaughter animals and apprehension prior to the bleeding of non-fasting cattle as well as the ensuing struggle at the time of venipuncture. Daugherty, et al. (1956) listed three factors which influence the blood glucose levels in cattle and sheep: (1) the kind of animal; (2) temperament of the experimental animal used; (3) methods of handling these animals. Their work indicated that the method of handling an animal prior

to venipuncture did not influence blood glucose levels to any great extent. If the influence of excitement did enter into the concentration of blood sugar at any given time, then this factor would have to have been induced for extensive periods of time. Zeller, et al. (1941) have shown that the initial rise of synovial fluid sugars lagged behind that of the blood for at least 20 minutes. Blood-synovial sugar equilibrium remained relatively constant in both fasting slaughter cattle and non-fasting cattle. The only apparent difference in blood and synovial sugar levels in the two groups was one of total concentration, not one of altered equilibrium. It can be stated that in both the fasting slaughter cattle and non-fasting cattle, synovial fluid sugars have always remained constant in relation to that of their respective blood sugar levels.

Cellular Studies

The average number of leukocytes in synovia (Table VIII) from the tibio-tarsal articulation for 69 animals was 114 per cubic millimeter. In many instances no leukocytes were observed during the routine microscopic examination, but following centrifugation of these samples, a smear of the sediment contained enough cells to make a differential leukocyte count. Individual samples gave cell counts ranging from 11 to 725 leukocytes per cubic millimeter. Ropes,

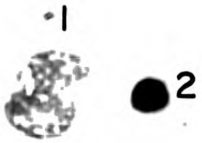
et al. (1939) reported an average leukocyte count of 131 per cubic millimeter of synovia from the tibio-tarsal articulation of young cattle. Their values for individual samples ranged from a minimum of 65 to a maximum of 250 leukocytes per cubic millimeter.

Dilution of the uncentrifuged portion of synovial fluid with physiological sodium chloride solution to which either methylene blue or methyl violet had been added, facilitated identification of leukocytes. The nuclear material of the cell absorbed the stain readily and thus aided in rapid differentiation between cellular constituents and synovial debris. Of the two solutions used in this study, a one per cent saline-methyl violet diluent proved to be the most satisfactory.

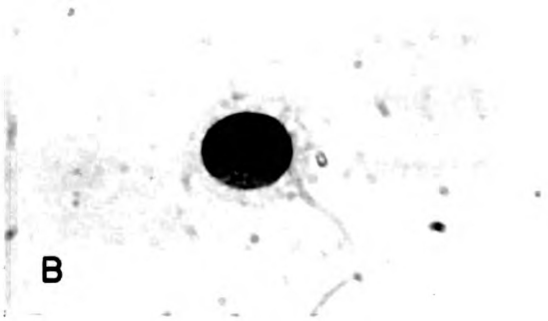
Differential leukocyte counts (Table VIII) were made from fixed smears stained with Wright's stain. This method proved to be quite satisfactory for differentiation (Fig. 9) of the cellular elements in synovial fluid. Murdock and Well (1959) made differential cell counts on the synovia of man with thin smears, fixed with heat, and stained with Leishman's stain. In this study it was not deemed advantageous to heat fix smears for Wright's staining.

Lymphocytes were the preponderant cell type constituting 48.87 per cent of all leukocytes followed closely by monocytes with 39.31 per cent. These two cellular types accounted for the major portion of the synovial fluid

Figure 9. The cells of normal bovine synovial fluid from the tibio-tarsal articulation. Plate A - clasmatocyte (1) showing constriction of its nucleus and phagocytosis of oxalate crystals; a small lymphocyte (2) with scant cytoplasm is adjacent to the clasmatocyte. Plate B - a typical synovial fluid monocyte. Plate C - a polymorphonuclear leukocyte. Plate D - a small lymphocyte (1) and synovial lining cell (2) associated with some degenerating mononuclear leukocytes (3).

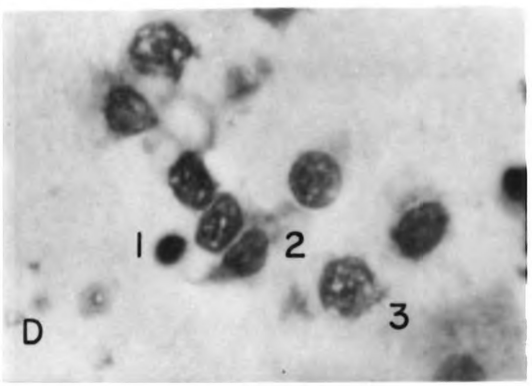


A



B

C



D

leukocytes. Lymphocytes (Fig. 9) are especially characterized by their intense basophilic staining and relatively small size in comparison to mononuclear phagocytes and polymorphonuclear leukocytes. Huddleson, et al. (1947) reported that Brucellosis in man produced a synovitis with pain, swelling, and a joint fluid that was sterile and contained an excess of lymphocytes. Monocytes (Fig. 9) followed closely the description given by Bauer, et al. (1930) who observed and described these cells from the normal synovial fluid of cattle. They observed that monocytes were spherical or oval in shape, containing eccentrically placed nuclei, usually kidney-shaped and sometimes oval, with considerable amounts of chromatin. These workers observed that 90 to 95 per cent of all nucleated cells were phagocytic for particulate matter, cells, and fragments of cells, and that their function was the removal of the products of wear and tear from articular cartilages and synovial membranes. The author observed monocytes in fixed smears arrested in stages of phagocytosis; however, these cells did not approach the degree of phagocytosis exhibited by clasmatocytes. Monocytes are easily confused with synovial cells (Fig. 9), which they closely resemble. In the case of the latter, the kidney-shaped to ovoid nucleus is lacking; however, the nucleus may appear elongated. The cytoplasm of the synovial cell is usually drawn out at the poles of the nucleus and may form branching processes. As a rule the

nuclear material is more homogenous in nature and does not exhibit the more intense basophilic, rough granular chromatin observed in synovial fluid monocytes. Sikes (1959) observed a rheumatoid-like arthritis in swine, the acute stages of which were due to Erysiplothrix rusiopathiae, exhibiting a serosanguinous to mucinous synovial fluid. In chronic arthritis of six months' duration or longer, he noted, the synovial effusions were non-purulent, and contained mostly monocytes, lymphocytes, and a few synovial lining cells. In advanced chronic cases the joints contained little or no synovial fluid.

In the synovia of clinically normal tibio-tarsal articulations, clasmatocytes (Fig. 9) accounted for only 6.13 per cent of the total cells. Underitz (1952) stated that these cells are sometimes called histiocytes or macrophages. In stained smears, clasmatocytes were observed to be arrested in the processes of phagocytosis, athrocytosis, and pinocytosis. These large leukocytes apparently perform a large part of the chore of removing debris from the synovial fluid. Lewis (1931) observed in macrophages, that by the mechanism of pinocytosis, cells were able to take in substances which cannot diffuse into them or be taken in by ordinary phagocytosis of semisolid particles. Using living amoeba and stained sections fixed during pinocytosis, Brandt (1958) reported that pinocytosis served as a mechanism for the transport of solute across the cell

membrane, and to a lesser extent the role it may have in the formation of cell organelles. The author has concluded from his observations that the vacuoles noted in the clasmatocytes of fixed and stained smears are probably a result of this process.

Neutrophils (Fig. 9) accounted for only 5.67 per cent of the total average differential leukocyte counts. Cordy (1959) made the following observations on the synovia of ruminants with arthritis due to pleuropneumonialike organisms (PPLO). In early cases the synovial fluid was yellowish and turbid, but only moderately increased in volume and frequently clotted quickly after being collected. Joint fluid from the more severe cases contained large amounts of fibrin. Microscopically he noted that the masses of fibrinous exudate were composed of compacted fibrin enmeshing many disintegrating neutrophils. He also observed that the synovial membrane was edematous and hyperemic with a heavy infiltration of both mononuclear and neutrophilic leukocytes. McNutt (1959) observed in swine arthritis associated with pleuropneumonialike organisms (PPLO) that the synovial membrane was infiltrated with inflammatory cells, largely mononuclear in nature, not abundant, and located perivascularly. His work showed that the infection seemed not to result in polymorphonuclear infiltration here or elsewhere, unless necrosis took place.

Eosinophils were rarely observed in the synovial

specimens in this study and accounted for only 0.79 per cent of the total average differential leukocyte count.

Ropes, et al. (1940) stated that erythrocytes are not normal constituents of synovial fluid. Occasionally synovial fluid in this study exhibited erythrocytes, which were not recorded since they were attributed to capsular hemorrhage as a result of needle puncture or, in the case of slaughter cattle, trauma from agonal struggles. Swanton (1959) reported on hemophiliac arthropathy in dogs. These animals exhibited blood in the synovial fluid in about half the joints in each animal, with fresh hemorrhage usually in sufficient quantity to color the fluid, which was present in increased amounts. If more bleeding occurred, the joint cavity contained chiefly dark red, viscous fluid.

Absolute leukocyte counts (Table IX) were made to determine the actual number of cells per cubic millimeter of synovial fluid. It will be noted that the average number of 115.8 leukocytes per cubic millimeter is slightly higher than the figure reported for this determination in Table VIII. This can be accounted for by the fact that the only specimens which could be used were those wherein it was possible to make both a total and differential count. The absolute differential count per cubic millimeter for this study was: 53.70 lymphocytes; 38.95 monocytes; 12.19 clasmatocytes; 10.03 neutrophils; 0.91 eosinophils.

No attempt was made to categorize synovial

leukocyte counts with respect to fasting, non-fasting, age, or sex.

Viscosity and Mucin Clot

The average relative viscosity (Table X) for synovial fluid from the tibio-tarsal articulation was 3.79 ± 0.55 for 73 animals of all sexes, ranging in age from nine months to ten years. Relative viscosity ranged from a low of 1.53 to a high of 13.01. Spector (1956) gives the average relative viscosity of synovial fluid from the bovine tibio-tarsal articulation as five, with a range of from two to twelve. These determinations were made at 20 degrees centigrade employing a Scott-Blair viscometer. Ropes, et al. (1939) reported an average relative viscosity of 3.72 at 25 degrees centigrade with variations between 2.84 to 4.15. Synovia in their study was also obtained from the tibio-tarsal articulation of young cattle.

The majority (86.3 per cent) of relative viscosity determinations were determined by a macro-method employing the Cannon-Fenske routine viscometer (Fig. 6). The balance (13.7 per cent) were determined by a semi-micro method using the Cannon-Ubbelohde semi-micro dilution viscometer (Fig. 7). The viscometers were suspended in a water bath (Fig. 5) maintained at 101.5 degrees fahrenheit. Since viscosity changes with temperature, Cannon (1957) stated, it is

important that a constant bath temperature be maintained in order to keep the resulting viscosity readings within desired limits. He also noted that the greater the viscidity of a fluid, the greater will be the rate of change in viscosity with temperature variations and that the greatest quantity of friction is developed in the capillary tube.

With regard to the effects of temperature on viscosity measurements, Geist and Cannon (1946) observed that the volume of a liquid in the viscometer will increase in going from room temperature to test temperature of the bath. Depending on the viscometer employed, charges of one, five, and ten milliliters were used to determine the relative viscosity of synovial fluid in this study. Temperature no doubt was an influencing factor in measurement of efflux time, since it would tend to cause an increase in fluid volume; this in turn would increase the driving head, the result being a decrease in the viscosity reading because of increased capillary pressure. The author has concluded from this study that while bath temperature was a constant factor, variations in room temperature from day to day may have altered the volume of the initial charge when brought to test temperature; however, all other factors being constant, this was one facet that is next to impossible to control and when dealing with non-volatile liquids it is probably negligible in the final analysis.

Jebens and Monk-Jones (1959) stated that there

are two factors which determine the viscosity of any particular synovial fluid: (1) the concentration of the hyaluronic acid in the fluid and (2) the state of polymerization of the hyaluronic acid molecule. Ragan (1946) concluded that the high viscosity of normal human synovial fluid would indicate these fluids contain either a large amount of hyaluronic acid per unit volume or that the polysaccharide is in a highly polymerized state or in a state of high aggregation. In a later study, Ragan and Meyers (1949) observed that there is evidence that the long chain molecule of the polysaccharide component, hyaluronic acid, is responsible for certain physical properties of synovial fluid such as its viscosity.

Asboe-Hansen (1950), in a study on the variability in the hyaluronic acid content of dermal connective tissue, concluded that thyroid hormone regulated the amount of polysaccharide formed.

With the aforementioned statements in mind, a discussion of synovial fluid viscosity becomes meaningful in relation to polymerization of the hyaluronic acid molecules and the physiological state of a diarthrodial articulation.

It was noted in the course of this study that many of the synovial fluid specimens exhibited properties of thixotropism. At first glance it would seem that a paradoxical situation exists in the physiological function

of synovial fluid. Samples that were thixotropic in nature returned to their normal fluid state following vigorous agitation of the test tube. It would seem that the reverse might occur since Blair, et al. (1954) reported that agitation or stirring produces an increased shearing of molecules with a resultant increase in the viscosity of synovial fluid. These properties are apparently related to the degree of polymerization of the hyaluronic acid complex. Robertson, et al. (1940) using enzymes from anaerobic cultures of Clostridium perfringens, demonstrated an enzyme, mucinase, which decomposes mucin and causes a loss in viscosity of synovial fluid. They were unable to demonstrate such an enzyme in sterile normal or pathological synovia, allowed to stand for varying periods of time at 25 and 37 degrees centigrade. In no instance was any decrease in mucin noted, nor did any change in mucin occur in the sterile fluids. These workers suggested that the enzyme mucinase worked in two steps: (1) degradation of mucin or its polysaccharide as catalized by the bacterial enzyme mucinase or hyaluronidase, a depolymerization or (2) liberation of free amino sugars and reducing substances, a hydrolysis. They concluded that the action of the mucinase or hyaluronidase on hyaluronic acid prevents the formation of the characteristic mucin clot when hyaluronic acid is mixed with albumen at an acid pH.

In the course of the rheological determinations,

these factors were taken into consideration, since efflux time would vary within a sample between the first and fifth measurements when employing macro determinations, i.e., use of a Cannon-Fenske viscometer. There seemed to be no consistency from specimen to specimen, e.g., the efflux time of the first measurement may be less than that of the last or vice versa. These qualities are apparently related to the quantity of hyaluronic acid and its degree of polymerization. Therefore, a specimen exhibiting thixotropism would have greater efflux time on the first timing and which would gradually become shorter with each successive measurement. Other specimens would give the reverse results with longer efflux times as each measurement is repeated due to increased shearing which results in an increased viscosity. Efflux measurements made with the Cannon-Ubbelohde did not present these time variations, since the suspended level principle eliminates the influence of factors other than those directly concerned with the capillary tube, i.e., the charge in the reservoir bulb and driving head and their respective menisci, and the shearing forces associated with these factors. With the use of the Cannon-Fenske viscometer, charges of five milliliters would often exhibit signs of rheotaxis or eddying at the entrance of the capillary tube into the reservoir bulb. This phenomenon was especially noticeable with fluids of a lower than average viscosity and could be attributed to reduced shearing of the molecules

associated with increased velocity all of which indicated a reduction in viscosity. Fessler, et al. (1954) observed that the viscosity of synovial fluid was due to the presence of hyaluronic acid and that slight changes in the hyaluronic acid affected the velocity gradient noticeably. These changes in the hyaluronic acid complex undoubtedly account for the variation in viscosity observed in this study.

The Cannon-Ubbelohde semi-micro dilution viscometer was employed because in many instances the five and ten milliliter amounts necessary to charge the Cannon-Fenske viscometer were not available. Many of the complications arising with the use of the latter viscometer were avoided in the suspended level Cannon-Ubbelohde viscometer; however, only one efflux measurement was made due to the turbulence of synovia caused by negative pressure in subsequent attempts to recharge the driving head.

Quality of mucin clot (Table X) was determined for each sample of synovia in an attempt to correlate viscosity with the quality of mucin. In the author's opinion, character of the mucin clot as determined by precipitation with an aqueous solution of 7N glacial acetic acid has proven to be a representative indication of its viscous properties and usually correlated with the relative viscosity of the respective sample. Mucin clot was classified as normal or fair (Fig. 8), since in this study the poorer grades

of clot were not observed, e.g., poor and very poor. In compilation of data on quality of mucin clot, averages were determined as per cent of animals in each age or sex bracket (Charts V and VI), that had a normal clot.

Age and sex of the animals were taken into consideration to ascertain what effect they might have on viscosity and quality of mucin clot. Since there was only one animal in the nine-month old age bracket, the relative viscosity reading of 1.99 cannot be considered representative of animals in this age group. Likewise only one animal was tested at the upper end of the age scale, a ten-year old female with a relative viscosity of 2.52. Both the young and old animal exhibited normal quality mucin clots. The highest relative viscosity was observed in an eight-year old female at 9.5, with a normal mucin clot. Animals of mixed sexes from one to four years ranged in viscosity from 2.76 to 3.18, and 89 per cent of them had mucin clots that were classified as normal. Beginning with the four-year age bracket there was a linear rise in relative viscosity to a maximum of 9.5 at eight years of age and a corresponding drop in the percentage of them that had a normal mucin clot. One explanation for this might be that the majority of animals in the latter age groups were females in some stage of lactation and as a rule much less volume of synovia was obtained from the joint.

Bauer, et al. (1930) made observations on the

RELATIVE VISCOSITY AND QUALITY OF MUCIN CLOT
IN RELATION TO AGE

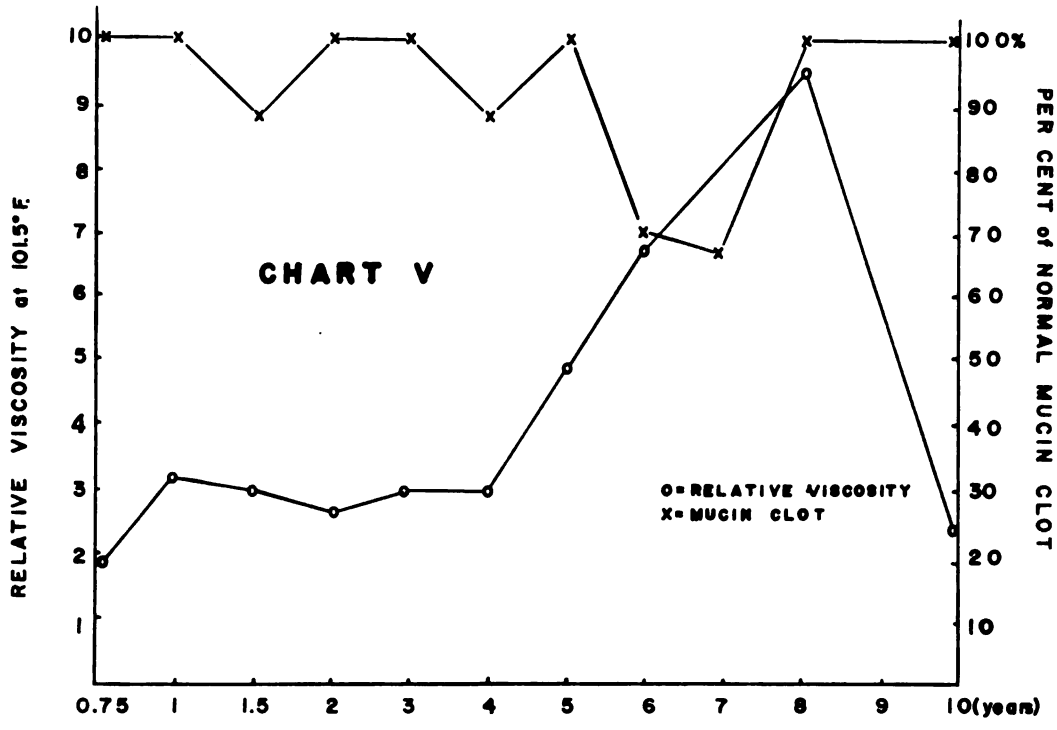
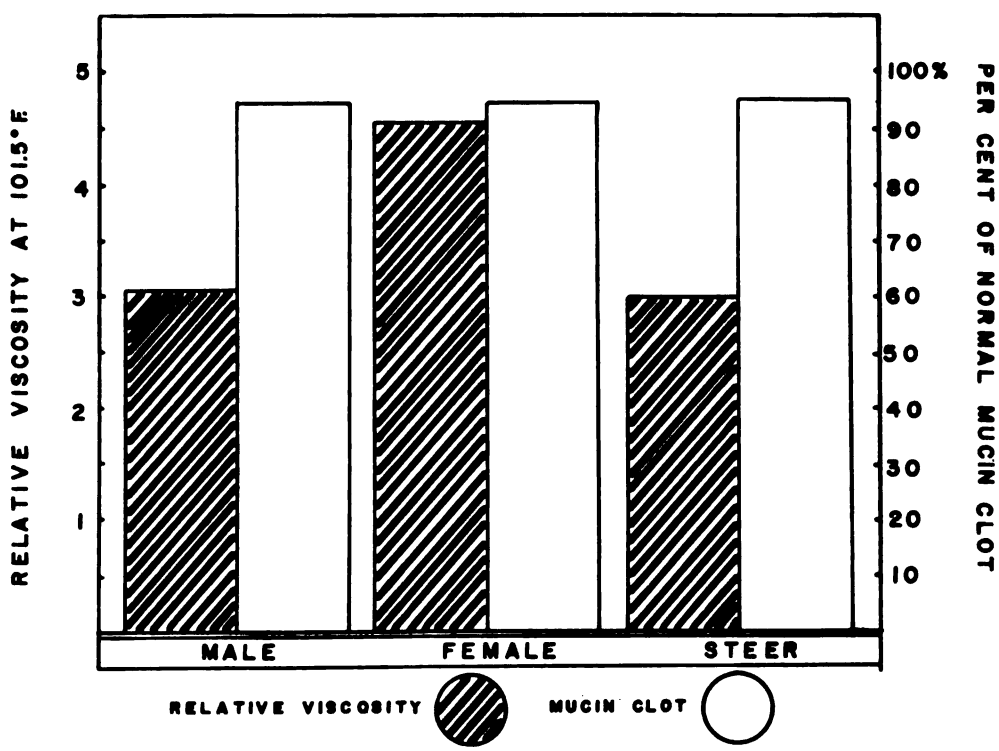


CHART VI

RELATIVE VISCOSITY AND QUALITY OF MUCIN CLOT
IN RELATION TO SEX



normal synovial fluid of cattle and reported lower quantity of fluid from milking cows compared with young beef cattle. They obtained five to ten cubic centimeters from the tibiotarsal and two to three cubic centimeters from the carpometacarpal articulation of milk cows. Perhaps in these instances there is greater concentration of the hyaluronic acid complex, associated with a lesser degree of molecular polymerization as noted by poorer grades of mucin clot in relation to correspondingly high relative viscosity values of the synovia. This may explain why younger animals have greater total fluid volumes and more of them have a normal mucin clot but still have lower relative viscosity values. One might conclude from these findings that volume of synovia and concentration of mucopolysaccharide are inversely proportional as reflected by a lower viscosity, in animals with greater total fluid volume. Since intrinsic viscosity was not ascertained in these studies, speculation and correlation of results obtained leave the only alternatives. Therefore, in instances wherein viscosity is high, it seems probable that lesser quantities of synovia would be needed for normal physiological function of the joint.

Gardner (1959) has shown that the viscosity of hyaluronic acid increases exponentially relative to its concentration and that solutions (nonsulfated mucopolysaccharide or hyaluronic acid) of over one per cent form gels. Conversely he stated that viscosity decreased exponentially

as synovial fluid is diluted. The viscosity of synovial fluid is decreased to that of water if the hyaluronic acid is removed by precipitation with acid or hydrolized by enzymes, or if its polymerization is destroyed by enzymes.

Sex seemed to have some influence on viscosity (Table XII and Chart VI) with females exhibiting an average relative viscosity of 4.55 ± 0.99. Males were next with an average relative viscosity of 3.02 ± 0.61 and steers were lowest at 2.99 ± 0.40. Ninety-four per cent of the males and females had a normal mucin clot, with steers only one per cent higher. It is difficult to explain why the females in this study were higher in viscosity values than either males or steers, except as previously discussed, females were generally comprised of an older group of animals exhibiting lesser quantities of fluid, undoubtedly higher in concentration and polymerization of the hyaluronic acid complex. In retrospect it would be difficult to assign age as an influencing factor since the majority of females were older animals. Whether or not there is a reduction in synthesis or concentration of the mucopolysaccharide, or an enzymatic factor concerned with reduced synovial fluid, quality was not determined in this study. Neher and Tietz (1959) observed in aged bulls from a bull stud, that in about one-third of the pathologic joints, the synovial fluid was excessive in volume and of a less viscous nature than normal. These workers failed to mention how they arrived

at their viscosity findings as did Moulton, et al. (1953) who aspirated 100 milliliter quantities of synovial fluid from the hock joints of calves infected with Erysipelothrix rhusiopathiae and observed that the fluid was tan to pink in color, more viscid than normal synovial fluid, and cloudy.

It is conceivable that the enzyme hyaluronidase may play a role in depolymerization of the hyaluronic acid complex. Colowick and Kaplan (1955) stated that the action of hyaluronidase on hyaluronic acid when present in synovia resulted in a decrease in viscosity, the rate of which is a function of enzyme concentration. Furthermore they noted that hyaluronidase is widely distributed in nature, although it has conclusively been demonstrated only in testicular tissue of mammals. In human patients with rheumatoid arthritis, Bollet (1956) observed there was no correlation between lowering of the intrinsic viscosity of synovia and the clinical aspects of the disease or the severity of inflammation or the degree of cartilage and bone destruction as estimated on x-ray findings. He further observed that intrinsic viscosity and relative viscosity both increased following treatment with either oral or intra-articular adrenocortical steroids.

CHAPTER VI

SUMMARY AND CONCLUSIONS

The blood, plasma, and synovial fluid from 83 animals of mixed ages, sexes, and breeds has been analyzed. Of the total number, 52 of these animals comprised individuals that had been fasted 12 to 18 hours prior to the time of slaughter. The remaining 31 animals in the series was made up of individuals not intended for slaughter, many of whom were in some stage of lactation.

In the overall picture, breed was not considered an important factor; however, sex and age did exert some influence on blood, plasma, and synovial fluid sugar levels. Age and sex also influenced the relative viscosity of synovial fluid.

From the standpoint of blood-synovial fluid sugar difference, as opposed to plasma-synovial fluid sugar difference, the former relationship proved to be the most consistent, in that both groups, i.e., fasting slaughter cattle and non-fasting cattle, showed minimal changes in a comparison of values. From a diagnostic standpoint, the results of this study suggest that the blood-synovial fluid sugar differences have shown the most consistent relationship, and that plasma-synovial fluid sugar differences are too great, and too fluctuant to be of value. The total blood

and synovial fluid sugars in fasting slaughter cattle were considerably higher than in non-fasting cattle but this does not seem to alter the respective relationship between these constituents in the same individual. These factors are likewise applicable when sex and age of an individual are taken into consideration.

Cellular studies tend to point to lymphocytes and monocytes as the predominant leukocytes of synovial fluid from the tibio-tarsal articulation. These findings can undoubtedly be attributed to the relatively young age of the animals in this study, and accounts for the fact that few clasmatocytes were observed. The erythrocytes encountered were attributed to hemorrhage at the time of aspiration in the living animals or extravasation of the cells into the joint cavity at slaughter due to agonal struggles. The low total number of leukocytes per cubic millimeter of synovial fluid from the tibio-tarsal articulation is in accord with that reported by other workers and serves to reflect the overall normal state of the joints analyzed in this study.

The average relative viscosity of synovia at 101.5 degrees fahrenheit as determined by the Cannon-Fenske routine and Cannon-Ubbelohde semi-micro dilution viscometers, was 3.79 ± 0.55. Age and sex of the individuals apparently influenced the relative viscosity, since females gave the highest values, followed by males and steers, which presented

almost parallel results in this respect. Determination of mucin clot by precipitation with 7N glacial acetic acid serves as an index to the degree of polymerization of the hyaluronic acid complex, i.e., the more closely the clot approaches that of "normal", the greater the degree of molecular polymerization.

BIBLIOGRAPHY

Allcroft, W. M., and Strand, R.: Studies on the Lactic Acid, Sugar and Inorganic Phosphorus of the Blood of Ruminants. Biochem. J., 27, (1933): 512-521.

Allcroft, W. M.: Diurnal Variations in the Blood Sugar Level of the Lactating Cow. Biochem. J., 27, (1933):1820-1823.

Anderson, W. A. D.: Synopsis of Pathology. 3rd ed., C. V. Mosby Co., St. Louis, Mo., 1952.

Arey, L. B.: Human Histology - A Textbook in Outline Form. W. B. Saunders Co., Philadelphia, Pa., 1957.

Asboe-Hansen, G.: International review of cytology. 3rd ed., New York Academy Press, New York, N. Y., 1954.

Asboe-Hansen, G.: The Variability in the Hyaluronic Acid Content of the Dermal Connective Tissue under the Influence of Thyroid Hormone. Acta Dermato-Venereal, 30, (1950):221.

Barnett, C. H.: Measurement and Interpretation of Synovial Fluid Viscosities. Ann. Rheumat. Dis., 17, (1958):229-233.

Bauer, W., Bennett, G. A., Marble, A., and Claflin, D.: Observations on the Normal Synovial Fluid of Cattle. I. The Cellular Constituents and Nitrogen Content. J. Exp. Med., 52, (1930):835-848.

Bauer, W., Ropes, M. W., and Waine, H.: The Physiology of Articular Structures. Physiol. Rev., 20, (1940):272.

Benedict, S. R.: The Analysis of Whole Blood. II. The Determination of Sugar and of Saccharoids (non-fermentable copper reducing substances). J. Biol. Chem., 92, (1931):141-159.

Bingham, E. C.: Fluidity and Plasticity. 1st ed., McGraw-Hill Book Co., New York, N. Y., 1922.

Blair, G. W., Williams, P. O., Fletcher, E. T. D., and Markham, R. L.: On the Flow of Certain Pathological Human Synovial Effusions through narrow Tubes. Biochem. J., 56, (1954):504-508.

Bollet, A. J.: The Intrinsic Viscosity of Synovial Fluid Hyaluronic Acid. J. Lab. & Clin. Med., 48, (1956):721-728.

Brandt, P. W.: A Study of the Mechanism of Pinocytosis. Exp. Cell Res., 15, (1958):300-313.

Bunn, C. E. E., and Burch, J. E.: Hydrocortisone in the Treatment of Traumatic Arthritis in Thoroughbreds N. Am. Vet., 36, (1955):458-461.

Cajori, F. A., Crouter, C. Y., and Pemberton, R.: The Physiology of Synovial Fluid. Arch. Int. Med., 37, (1926):92-101.

Cajori, F. A., and Pemberton, R.: The Chemical Composition of Synovial Fluid in Cases of Joint Effusion. J. Biol. Chem., 76, (1928):471-480.

Cannon, M. R., and Fenske, M. R.: Viscosity Standardization of Petroleum Lubricating Fractions. Oil & Gas J., 33, (1935):52-55.

Cannon, M. R., and Fenske, M. R.: Viscosity Measurement. Ind. Eng. Chem., Anal. Ed., 10, (1938):297-301.

Cannon, M. R.: Viscosity Measurement. Ind. Eng. Chem., 16, (1944):708-710.

Cannon, M. R.: Derivation of Viscosity Equation and Sources of Error in Viscometry. Cannon Instrument Co., State College, Pa., Report No. 1 for A.S.T.M., (1957):1-10.

Cannon, M. R., and Manning, R. E.: Viscosity Measurement - The Kinetic Energy Correction. Cannon Instrument Co., State College, Pa., Report No. 2 for A.S.T.M., (1959):1-8.

Carpenter, P. L.: Immunology and Serology. W. B. Saunders Co., Philadelphia, Pa., 1959.

Castor, W. C.: The Rate of Hyaluronic Acid Production by Human Synovial Cells Studied in Tissue Culture. Arthritis & Rheumat., 2, (1959):259-265.

Coffin, D. L.: Manual of Veterinary Clinical Pathology. 3rd ed., Comstock Publishing Associates, Ithaca, N. Y., 1953.

Coggeshall, H. C., Warren, C. F., and Bauer, W.: The Cytology of Normal Human Synovial Fluid. Anat. Rec., 77, (1940):129-144.

Coggeshall, H. C.: Comroe's Arthritis and Allied Conditions. Edited by J. L. Hollander. 5th ed., Lea and Febiger, Philadelphia, Pa., 1953.

Colowick, S. P., and Kaplan, N. O.: Methods in Enzymology. Vol. I. Academic Press, Inc., New York, N. Y., 1955.

Copenhaver, W. M., and Johnson, D. D.: Bailey's Textbook of Histology. 14th ed., The Williams and Wilkins Co., Baltimore, Md., 1958.

Cordy, D. R.: Arthritis in Ruminants Due to Pleuropneumonia-like Organisms. Lab. Invest., 8, (1959):1432-1434.

Cunningham, R. S., Sabin, F. R., and Doan, C. A.: The Differentiation of Two Distinct Types of Phagocytic Cells in the Spleen of the Rabbit. Proc. Soc. Exp. Biol. and Med., 21, (1924):326-329.

Curtain, C. C.: The Nature of the Protein in the Hyaluronic Complex of the Bovine. Biochem. J., 61, (1955): 688-696.

Daugherty, R. W., Klavano, C. S., Dickson, W. M., and Klavano, P. A.: Blood Sugar Levels in Cattle and Sheep Fed Various Amounts of Glucose. Cornell Vet., 46, (1956):397-409.

Davies, D. V.: Observations on the Volume, Viscosity, and Nitrogen Content of Synovial Fluid, with a Note on the Histological Appearance of the Synovial Membrane. J. Anat., 78, (1944):68-78.

Davies, D. V.: The Cell Content of Synovial Fluid. J. Anat., 79, (1945):66-73.

Davies, D. V.: Synovial Membrane and Synovial Fluid of Joints. Lancet, 251, (1946):813-819.

DeGara, P. F.: Studies on the Bactericidal Properties of the Synovial Fluid. J. Clin. Invest., 22, (1943): 131-136.

Doan, C. A., Cunningham, R. S., and Sabin, F. R.: Experimental Studies on the Origin and Maturation of Avian and Mammalian Red Blood-Cells. Contrib. Embryol., 83, (1925):163-226.

Dukes, H. H.: The Physiology of Domestic Animals. 7th ed., Comstock Publishing Associates, Ithaca, N. Y., 1955.

Eckles, C. H., Combs, W. B., and Macy, H.: Milk and Milk Products. 2nd ed., McGraw-Hill Book Co., Inc., New York, N. Y., 1936.

Fashena, G. J.: On the Nature of the Saccharoid Fraction of Human Blood. J. Biol. Chem., 100, (1933): 357-363.

Fashena, G. J., and Stiff, H. A.: On the Nature of the Saccharoid Fraction of Human Blood. II. Identification of Glucuronic Acid. J. Biol. Chem., 137, (1941):21-27.

Fessler, J. H., Ogston, A. G., and Stanier, J. E.: Some Properties of Human and Other Synovial Fluids. Biochem. J., 58, (1954):656-660.

Folin, O., and Wu, H.: A System of Blood Analysis. J. Biol. Chem., 38, (1919):81-110.

Folin, O., and Wu, H.: A System of Blood Analysis. Supplement I. A Simplified and Improved Method of Determination of Sugar. J. Biol. Chem., 41, (1920):367-374.

Folin, O.: The Determination of Sugar in Blood and in Normal Urine. J. Biol. Chem., 67, (1926):357-370.

Folin, O.: Two Revised Copper Methods for Blood Sugar Determination. J. Biol. Chem., 82, (1929):83-93.

Fowler, G. R., and Kingrey, B. W.: Diseases of Cattle. American Veterinary Publications, Inc., Evanston, Ill., 1956.

Furey, J. G., Clark, W. S., and Brine, K. L.: The Practical Importance of Synovial Fluid Analysis. J. Bone and Joint Surg., 41-A, (1959):167-174.

Gardner, E.: Physiology of Movable Joints. Physiol. Rev., 30, (1950):127-176.

Gardner, E.: Comparative Arthrology. Lab. Invest., 8, (1959):1160-1166.

Geist, J. M., and Cannon, M. R.: Viscosities of Pure Hydrocarbons. Ind. and Eng. Chem., 18, (1946):611-613.

Gortner, R. A., Gortner, R. A., Jr., and Gortner, W. A.: Outlines of Biochemistry. 3rd ed., John Wiley and Sons, Inc., New York, N. Y., 1953.

Gunter, G. S.: The Determination of "Spinnbarkeit" of Synovial Fluid and its Destruction by Enzymatic Action. Aust. J. Exp. Biol. Med. Sci., 27, (1947): 265-274.

Hall, P. F.: The Fundamentals of the Endocrine Glands. W. B. Saunders Co., Philadelphia, Pa., 1959.

Ham, A. W.: Histology. 3rd ed., J. B. Lippincott Co., Philadelphia, Pa., 1957.

Ham, T. H.: A Syllabus of Laboratory Examination in Clinical Diagnosis. Harvard University Press, Cambridge, Mass., 1950.

Hammerman, D. J., and Schubert, M.: A Quantitative Study of Metachromasy in Synovial Fluid and Mucin. J. Gen. Physiol., 37, (1953):291-300.

Harris, R., Mulard, J. B., and Banerjee, S. K.: Radiosodium Clearance from the Knee Joint in Rheumatoid Arthritis. Ann. Rheumat. Dis., 17, (1958): 189-195.

Harrow, B., and Mazur, A.: Textbook of Biochemistry. 6th ed., W. B. Saunders Co., Philadelphia, Pa., 1954.

Hausman, E., and Slack, E. P.: Physics. 3rd ed., D. VanNostrand Co., New York, N. Y., 1939.

Hawk, P. B., Oser, B. L., and Summerman, W. H.: Practical Physiological Chemistry. 13th ed., McGraw-Hill Book Co., Inc., New York, N. Y., 1954.

Hepler, O. E.: Manual of Clinical Laboratory Methods. 4th ed., Charles C. Thomas Publisher, Springfield, Ill., 1957.

Hollander, J. L., Stoner, E. K., Brown, E. M., and DeMoor, P.: Joint Temperature Measurements in the Evaluation of Anti-Arthritic Agents. J. Clin. Invest., 30, (1951):701-706.

Horvath, S. M., and Hollander, J. L.: Intra-Articular Temperature as a Measure of Joint Reaction. J. Clin. Invest., 28, (1949):469-473.

Hubbard, R. S., and Porter, R. C.: The Glycolytic Enzymes of Synovial Fluid. J. Lab. and Clin. Med., 28, (1943):1328-1334.

Huddleson, I. F., Hardy, A. V., Debono, J. E., and Giltner, W.: Brucellosis of Man and Animals. Rev. Ed., The Commonwealth Fund, New York, N. Y., 1947.

Hughes, H. V., and Dransfield, J. W.: McFadyean's Osteology and Arthrology of the Domesticated Animals. 4th ed., Bailliere, Tindall and Cox, London, England, 1953.

Hunter, J., and Whillams, M. G.: Effect of Cold on Joints. Fed. Proc., 10, (1951):68-69.

Jebens, E. H., and Monk-Jones, M. E.: On the Viscosity and pH of Synovial Fluid and the pH of Blood. J. Bone and Joint Surg., 41-B, (1959):388-400.

Johnson, L. C.: Kinetics of Osteoarthritis. Lab. Invest., 8, (1959):1223-1238.

Jones, T. C., and Gleiser, C. A.: Veterinary Necropsy Procedures. J. B. Lippincott Co., Philadelphia, Pa., 1954.

Joseph, N. R., Reed, C. I., and Hambuger, E.: An in Vitro Study of the pH of Synovial Fluid in Dogs. Am. J. Physiol., 146, (1946):1-11.

Key, J. A.: The Reformation of Synovial Membranes in the Knees of Rabbits after Synovectomy. J. Bone and Joint Surg., 7, (1925):793.

Kling, D. H., Levine, M. G., and Wise, S.: Mucopolysaccharides in Tissue Cultures of Human and Mammalian Synovial Membrane. Proc. Soc. Exp. Biol., 89, (1955):261-263.

Levine, M. G., and Kling, D. H.: Rheologic Studies on Synovial Fluid. J. Clin. Invest., 35, (1956):1419-1427.

Lewis, W. H.: Pinocytosis. Bull. Johns Hopkins Hosp., 49, (1931):17-28.

Mann, F. D.: A Clinical Viscometer. Am. J. Clin. Path., 18, (1948):79-83.

Manning, R. E.: Personal Communication. 1960

Maximow, A. A., and Bloom, W.: A Textbook of Histology. 7th ed., W. B. Saunders Co., Philadelphia, Pa., 1960.

McNutt, S. H.: Swine Arthritis Associated with Pleuropneumonialike Organisms (PPLO). Lab. Invest., 8, (1959):1427-1431.

Merrington, A. C.: Viscometry. 1st ed., Edward Arnold and Co., London, England, 1941.

Meyer, K., Smyth, E. M., and Dawson, M. H.: The Isolation of a Mucopolysaccharide from Synovial Fluid. J. Biol. Chem., 128, (1939):319-327.

Meyer, K.: The Biological Significance of Hyaluronic Acid and Hyaluronidase. Physiol. Rev., 27, (1947): 335-359.

Miale, J. B.: Laboratory Medicine - Hematology. C. V. Mosby Co., St. Louis, Mo., 1958.

Mikklesen, W. M., Duff, I. F., Castor, C. W., French, A. J., and Zevely, H. A.: Synovial Specimens Obtained by Knee Joint Biopsy. Acta Med. Scand., 341, (1958):15-29.

Moffett, B. C., Jr.: Studies on Synovial Permeability. I. Direct Measurement of Synovial Permeability in Rats. Anat. Rec., 118, (1954):813-823.

Moffett, B. C., Jr.: Studies on Synovial Permeability. II. Factors Influencing Synovial Permeability in the Rat. Anat. Rec., 118, (1954):825-839.

Moulton, J. E., Rhode, E. R., and Wheat, J. D.: Erysipelatous Arthritis in Calves. J. Am. Vet. Med. Assoc., 123, (1953):335-340.

Murdock, W. R., and Will, G.: Synovial Fluid Changes in Intra-Articular Therapy. Effect of Prednisolone Trimethylacetate. Brit. Med. J., May 16, 1959: 1271-1274.

Neher, G. M., and Tietz, W. J.: Observations on the Clinical Signs and Gross Pathology of Degenerative Joint Disease in Aged Bulls. Lab. Invest., 8, (1959):1218-1222.

Neuhaus, O. W., and Letzring, M.: Hexosamine-containing Glycoproteins of Normal Bovine Synovial Fluid. J. Biol. Chem., 232, (1958):177-184.

Newlander, J. A.: The Testing and Chemistry of Dairy Products. Rev. Ed., Olsen Publishing Co., Milwaukee, Wis., 1949.

Ogston, A. G., and Stanier, J. E.: The Physiological Function of Hyaluronic Acid in Synovial Fluid; Viscous, Elastic, and Lubricant Properties. J. Physiol., 119, (1953):244-252.

Ogston, A. G., and Stanier, J. E.: Some Effects of Hyaluronidase on the Hyaluronic Acid of Ox Synovial Fluid, and their Bearing on the Investigation of Pathological Fluids. J. Physiol., 119, (1953): 253-258.

Paul, J.: Cell and Tissue Culture. E. and S. Livingstone Ltd., Edinburgh, Scotland, 1959.

Platt, D., Halley, H. L., and Pigman, W.: Changes in the Electrophoretic Behavior of Arthritic Synovial Fluid Components Following Intra-Articular Steroid Therapy. J. Lab. and Clin. Med., 49, (1957):762-766.

Ragan, C.: Viscosity of Normal Human Synovial Fluid. Proc. Soc. Exp. Biol. and Med., 63, (1946):572-575.

Ragan, C., and Meyers, K.: The Hyaluronic Acid of Synovial Fluid in Rheumatoid Arthritis. J. Clin. Invest., 28, (1949):56-59.

Robertson, W. van B., Ropes, M. W., and Bauer, W.: Mucinase: A Bacterial Enzyme which Hydrolizes Synovial Fluid Mucin and Other Mucins. J. Biol. Chem., 133, (1940):261-276.

Robinson, W. D., Duff, I. F., and Smith, E. M.: Joint Fluid Changes in Rheumatoid Arthritis. J. Michigan State M. Soc., 54, (1955):270-291.

Ropes, M. W., Bennet, G. A., and Bauer, W.: The Origin and Nature of Normal Synovial Fluid. J. Clin. Invest., 18, (1939):351-372.

Ropes, M. W., Rossmeisl, E. C., and Bauer, W.: The Origin and Nature of Normal Synovial Fluid. J. Clin. Invest., 19, (1940):795-799.

Ropes, M. W., and Bauer, W.: Synovial Fluid Changes in Joint Disease. Harvard University Press, Cambridge, Mass., 1953.

Sabin, F. R., Doan, C. A., and Cunningham, R. S.: The Separation of the Phagocytic Cells of the Peritoneal Exudate into Two Distinct Types. Proc. Soc. Exp. Biol. and Med., 21, (1924):330-332.

Sabin, F. R., Doan, C. A., and Cunningham, R. S.: Discrimination of Two Types of Phagocytic Cells in the Connective Tissue by Supra Vital Techniques. Contrib. Embryol., 16, (1925):125.

Samuel, E. P.: An Histological and Sensory Investigation to Determine the Role of the Articular Capsule in Joint Sensation. J. Anat., 84, (1950):77.

Shupe, J. L.: Degenerative Arthritis in the Bovine. Lab. Invest., 8, (1959):1190-1196.

Sikes, D.: A Rheumatoidlike Arthritis in Swine. Lab. Invest., 8, (1959):1406-1415.

Sisson, S., and Grossman, J. D.: The Anatomy of the Domestic Animals. 3rd ed., W. B. Saunders Co., Philadelphia, Pa., 1950.

Spector, W. S.: Handbook of Biological Data. W. B. Saunders Co., Philadelphia, Pa., 1956.

Sunderman, W. F., and Boerner, F.: Normal Values in Clinical Medicine. W. B. Saunders Co., Philadelphia, Pa., 1950.

Swanton, M. C.: Hemophilic Arthropathy in Dogs. Lab. Invest., 8, (1959):1269-1277.

Trautman, A., and Fiebiger, J.: Fundamentals of the Histology of Domestic Animals. Trans. and Rev. by R. E. Habel and E. L. Biberstein. Comstock Publishing Associates, Ithaca, N. Y., 1957.

Turner, C. D.: General Endocrinology. 2nd ed., W. B. Saunders Co., Philadelphia, Pa., 1955.

Ubbelohde, L.: The Principle of the Suspended Level - Applications to the Measurement of Viscosity and Other Properties of Liquids. Ind. and Eng. Chem., 9, (1937):85-90.

Udall, U. H.: The Practice of Veterinary Medicine. 6th ed., Geo. Banta Publishing Co., Menasha, Wis., 1954.

Underitz, E.: Sandoz - Atlas of Haematology. Trans. by A. M. Woolman. Sandoz Ltd., Basle, Switzerland, 1952.

Van Pelt, R. W.: The Role of Intra-Articular Adrenocortical Steroids. M. S. U. Vet., 20, (1960):68-78.

Van Pelt, R. W.: Arthrocentesis of the Equine Carpus. Vet. Med., 55, (1960):30-34.

Warren, C. F., Bennett, G. A., and Bauer, W.: The Significance of the Cellular Variations Occurring in Normal Synovial Fluid. Am. J. Path., 11, (1935): 953-967.

Wilson, H., Fairbanks, R., Scialabba, D., McEwen, C., and Ziff, M.: Metabolites of Hydrocortisone and Cortisone in Synovial Fluid in Rheumatoid Arthritis. J. Clin. Endocr. Metab., 16, (1956):86-113.

Wintrobe, M. M.: Clinical Hematology. 4th ed., Lea and Febiger, Philadelphia, Pa., 1956.

Zeller, J. W., Bywaters, E. G. L., and Bauer, W.: The Passage of Thiocyanate and Glucose from the Blood Stream into the Joint Spaces. Am. J. Physiol., 132, (1941):150-156.

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