OBSERVATIONS ON NORMAL SYNOVIAL FLUID OF CATTLE

I. THE CELLULAR CONSTITUENTS AND NITROGEN CONTENT*

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PLATES 37 AND 38

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A review of the literature concerned with the anatomy, histology, and physiology of normal joints reveals that our knowledge and understanding of many details is very meagre, indefinite, and inadequate. Therefore, we have undertaken studies which it is hoped will lead to a better understanding of the physiology of articulations. Further knowledge of the anatomy, histology and physiology of normal joints should have an important bearing on the physiology of pathological joints and the treatment of arthritis. Much of our work will concern the normal and pathological joints of cows, because of our ready access to an unlimited amount of material. This paper is the first of a series of studies of joints of cattle, and is a cytological study of normal synovial fluid.

A clear, viscid liquid known as synovial fluid is found in all normal joints. In the smaller laboratory animals, as well as in man, this fluid is present in such minute amounts that the aspiration of quantities sufficient for careful cellular and chemical study is difficult or impossible. The astragalotibial (hock) joint of normal cattle is a source of a large quantity of easily obtainable synovial fluid. Smaller amounts can be aspirated from the carpometacarpal (front knee) joint.

* The intensive study of arthritis at the Harvard Medical School has been made possible by friends and patients of the late Prof. Robert W. Lovett, who are giving a fund in his memory "to be devoted to the study of the most crippling disease."

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The following observations represent a part of the data obtained in a study of normal bovine synovial fluid.

**Material and Methods**

The synovial fluid was obtained by direct puncture of the astragalotibial and carpometacarpal joints of slaughtered animals, within 30 minutes after death. A lumbar puncture needle, with stylet in place, was inserted into the joint space through the lateral pouch of the capsule and synovial membrane. After the stylet was withdrawn, the fluid was aspirated into a Luer syringe. Most specimens were obtained after the legs had been skinned, although 10 of the astragalotibial joint aspirations were made through the intact hide from 1 to 8 minutes after the animals had been killed. In these 10 instances a special needle sleeve was used to puncture the tough hide overlying the joint. Since no apparent changes in the cellular constituents or nitrogen content of the fluids occurred in the 15 to 30 minutes after death, this procedure was abandoned.

Such specimens of synovial fluid were placed in warm test tubes, which were tightly corked and transferred to an inside shirt pocket at a temperature of approximately 33°C. On reaching the laboratory they were kept at a temperature of 38°C. In this manner the cells were kept viable for several hours.

Total cell counts per cubic millimeter of synovial fluid were made on 126 specimens. In order to distinguish more clearly the red blood corpuscles from the nucleated cells, a film of cresyl blue (dissolved in alcohol) was allowed to dry on the cover slip of the hemocytometer. When the synovial fluid flowed into the counting chamber this dye was dissolved in sufficient concentration to cause nuclear staining.

Differential cell counts were done on 40 synovial fluids by means of the supravital technique described by Sabin (1). The glass slides that were used were allowed to stand for 3 or 4 days in concentrated sulfuric acid to which a few crystals of potassium bichromate had been added. They were afterwards washed in running water, rinsed in three changes of distilled water, allowed to stand over night in distilled water, and then stored in 80 per cent alcohol. When taken from the alcohol, they were dried, flamed, polished with silk, and flooded with a stain which was prepared as follows: 30 drops of a saturated solution of neutral red in absolute alcohol and 7 drops of a saturated solution of janus green B in absolute alcohol were added to 10 cc. of absolute alcohol. This concentration of dye was found by trial to be the most satisfactory for our purposes. The clean glass slides were flooded with this freshly prepared stain and immediately placed in the vertical position to dry. For study, a single large drop of synovial fluid was placed on a prepared slide, covered with a clean rectangular cover-slip and sealed with a vaselin-paraffin mixture. Microscopic observations and cell counts were made in a warm box at 38°C. In addition many preparations were studied which were stained either with neutral red or with janus green B.

The phagocytic action of the various types of nucleated cells was observed in
12 synovial fluids. In these instances a graphite suspension was prepared in the manner described by Drinker and Churchill (2) and diluted with 0.85 per cent sodium chloride solution to obtain the desired concentration. This suspension was found to be superior to India ink because the particles are smaller and do not agglutinate. A few drops of this suspension were added to the synovial fluid either at the time of its withdrawal from the joint or when the fluid was placed on a similarly prepared glass slide. Observations were made as in other supra-vital preparations.

Total nitrogens on 150 fluids were done by the macro Kjeldahl method (3). The non-protein nitrogen was determined by the Folin method for blood (4) on 20 fluids. Total nitrogen determinations, before and after precipitation of mucin* (5), were made in 17 instances.

**Presentation of Data**

Because of constant differences in total cell counts, total nitrogen values, and physical properties of synovial fluid obtained from carpometacarpal and astragalotibial joints, the two types of fluids have been studied separately and tabulated. These variations, together with differences attributed to the age and habitat of the cattle, will be discussed in detail below. Tables I, II, and III represent cell counts and determinations made on synovial fluid from young western beef cattle.**

The average cell count on 63 astragalotibial joint fluids was 112 nucleated cells and 64 red blood corpuscles per cubic millimeter. A series of 15 carpometacarpal joint fluids from some of the same animals yielded an average of 222 nucleated cells and 572 red blood corpuscles per cubic millimeter. When 13 of the 78 specimens in which the red blood corpuscle count exceeded 200 per cubic millimeter were

* By mucin we mean the ropey, stringy substance precipitated by the addition of acetic acid to synovial fluid. The procedure used was: The synovial fluid was diluted with four parts of distilled water. For each cubic centimeter of undiluted synovial fluid 0.13 cc. of 7 normal acetic acid was added and the solution vigorously shaken.

** By beef cattle we mean young steers and heifers approximately 2 to 4 years of age. These animals were largely Herefords, shipped mainly from the western states. The milch cows referred to below were much older (estimated between 8 and 12 years of age) and obtained largely from the New England district. Since these studies were made during the winter months, it seems safe to consider milch cows as stable cattle and beef animals as pasture cattle.
discarded, the average cell count on the remaining 56 astragalotibial joint fluids became 103 nucleated cells and 29 red blood corpuscles per cubic millimeter. The remaining 9 carpometacarpal joint fluids then gave an average of 165 nucleated cells and 59 red blood corpuscles per cubic millimeter of fluid.

The average total nitrogen of 56 astragalotibial joint fluids was found to be 161 mg. per 100 cc. Total nitrogen determinations on 18 carpometacarpal joint fluids from the same group of animals yielded an average of 270 mg. per 100 cc.

In a separate series of 17 astragalotibial joint fluids an average total nitrogen value of 169 mg. per 100 cc. was found. After precipitation of mucin an average total nitrogen value of 132 mg. per 100 cc. was obtained. Non-protein nitrogen determinations on 14 fluids gave an average of 23.9 mg. per 100 cc. The above averages indicate that 37 mg. of the total nitrogen of synovial fluid is taken out by this method of mucin precipitation. When the 37 mg. thus accounted for and the 23.9 mg. of non-protein nitrogen were deducted from the average total nitrogen (169 mg. per 100 cc.), 108.1 mg. of nitrogen per 100 cc. remained. Multiplying 108.1 by the factor 6.25, one obtains 680 mg. or 0.68 per cent, which we believe is the approximate total protein content for normal astragalotibial joint fluid of cattle.

The total nitrogen was found to be constantly higher in the car-
Comparison of Carpometacarpal and Astragalotibial Joints

Gross differences were noted immediately when fluids obtained from the carpometacarpal and astragalotibial joints of the same or different animals were examined. The quantity of synovia obtainable from the carpometacarpal joints was smaller than the amount that could be aspirated from the astragalotibial joints. In young beef cattle an average of 3 to 7 cc. of synovial fluid was obtained from the carpometacarpal joints, whereas 15 to 40 cc. of fluid could be aspirated from the astragalotibial joints. The fluids obtained from the carpometacarpal joints were viscid and occasionally clotted. Astragalotibial joint synovia was much less viscid, being about the consistency of 6 per cent gum acacia solution. Coagulation never occurred in any of the astragalotibial joint specimens. As shown above, the total nucleated cell counts, as well as the total nitrogen content, were constantly higher in the carpometacarpal joint fluids than in the fluids from the astragalotibial articulations.

A probable explanation of these differences was found when the two joints were examined macroscopically and microscopically. Inspection of the opened carpometacarpal joints revealed constant defects in the articular cartilage. These defects varied in size. They were always situated on the medial side of the metacarpal bone and the opposing articular surface of the adjacent carpal bone (see Fig. 1). In gross these erosions were irregularly shaped depressed areas in the articular cartilage, apparently going down to the subchondral bone. The margins were sharp and in some instances slightly undermined. The bases frequently appeared to be covered by fibrin. Histological study revealed a sharply defined and abrupt area of cartilage degeneration, in some places extending down to bony trabeculae (Fig. 2). In other places one or two rows of distorted cartilage cells remained. At the base of these defects small blood vessels and capillaries nearly reached the surface. In some of the sections a moderate infiltration of small round cells and mononuclear phagocytes was seen. Because
of these constant defects in practically all cattle 2 years of age or older. The synovial fluid from these joints was considered pathological. The increased viscosity and higher nitrogen values of these fluids, together with their tendency to clot, seemed best explained on the assumption that there was altered capillary permeability in the abnormal joints and a possibility of filtration of fibrin into the joint spaces.*

Because of the fact that the carcasses are hung by the tendo-Achilles, direct inspection of the astragalotibial joints was impossible. In beef cattle, 2 to 4 years of age, incision into such joint spaces and palpation of the articular cartilages of the joints from which the fluids were withdrawn did not reveal palpable defects in any instance. Direct inspection of 6 astragalotibial joints of older stable cattle, that had been condemned, revealed 4 joints showing areas of beginning marginal degeneration of the articular cartilages. For these reasons it was assumed that the astragalotibial joint synovia of young beef cattle was normal, but that the synovial fluid of the carpometacarpal joints of animals over 2 years of age must be considered pathological.

The quantity of synovial fluid obtainable from the joints of stable cattle (milch cows) is strikingly low as compared with young beef cattle. In the former an average of 2 to 3 cc. of fluid could be aspirated from the carpometacarpal joints and only 5 to 10 cc. were obtainable from the astragalotibial joints. Von Holst (6) noted that synovial fluid was obtained from a single joint of slaughtered cows in quantities varying from almost none to 15 or 20 cc. He also confirmed Frerichs’ (7) earlier observation that much more fluid could be obtained from the joints of young or stall fed cattle than from the joints of grazing animals. From a single ox 65 cc. of fluid were withdrawn from the astragalotibial joint.

**Types of Cells**

There was no striking difference in the type of cells found in the synovial fluids from young beef or stable cattle. More cellular fragments, solid particles and unidentified debris were found in the fluids from stable cattle than in those of young beef cattle.

* A more detailed study of these articular cartilage lesions and the age at which they appear is being made.
Table II gives in detail the numbers and types of cells seen in the carpometacarpal and astragalotibial joint fluids obtained from the same animals. The cells have been divided into groups according to the classifications of Sabin, Doan, and Cunningham (8), (9), (10), and Key (11), (12). We have found that the nucleated cells of cows’ synovial fluid correspond quite accurately to the cell types described by Key (11) in his study of normal rabbits’ synovial fluid. It has been our purpose, however, to classify the nucleated cells as to their phagocytic properties without reference to their origin. We have limited ourselves to brief descriptions of the major groups of cells present.

Ninety to ninety-five per cent of all nucleated cells present were phagocytic for particulate matter and, as seen in a number of instances, for cells and fragments of cells. Fourteen macrophages were found in which 1 to 6 recognizable phagocyted cells were present. Cells of the monocyte series formed 85.5 per cent of all the nucleated cells, clasmatocytes 4.1 per cent, while 3.3 per cent consisted of other types of phagocytic cells.

The monocytes were as a rule easily recognized, though they varied greatly in size and degree of stimulation. They were almost invariably spherical or oval in shape and contained eccentrically placed nuclei. The nuclei were usually kidney-shaped, although sometimes oval, and contained a considerable amount of chromatin. In almost all instances there was an accumulation of neutral red granules and phagocyted particles in the cytoplasm near the nuclei on the indented side and often rosettes were seen at this point. Mitochondria in the form of dark green or blue rods and dots could usually be seen best at the poles of the nuclei.

The clasmatocytes were larger than the monocytes. They were almost always oval in shape although sometimes irregular in outline. The nuclei of these cells were relatively smaller and appeared more glassy. The granules and vacuoles which were stained by neutral red varied more in size and were more widely distributed than in the monocytes. Mitochondria when seen were in the form of small dots and rods, well scattered. Frequently in the double stained preparation mitochondria could not be seen.

Both of the above types of cells were actively phagocytic for graphite particles (Cells 9 to 13, Fig. 3).

Polymorphonuclear leucocytes were actively ameboid and phagocytic for particles of graphite. There was a varying number of phagocytic cells without sufficiently definite morphology to be classified in any special group. These cells have been called unclassified phagocytic cells.

Small round cells with relatively large, round, or slightly indented nuclei were occasionally seen. The cytoplasm of these cells was scant in amount and clear.
### Table II

A Comparison of Cells and Total Nitrogen Values of Synovial Fluids Obtained from the Carpometacarpal and Astragalo Tibial Joints of the Same Animals*

<table>
<thead>
<tr>
<th>Animal number</th>
<th>Joints</th>
<th>Phagocytic cells Percentage</th>
<th>Non-phagocytic cells Percentage</th>
<th>Totals</th>
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<td>*<strong>Phagocytic monocytes</strong></td>
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<th>Non-phagocytic cells Percentage</th>
<th>Totals</th>
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<tr>
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<td>1</td>
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<tr>
<td>Min.</td>
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<td>Aver.</td>
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* All specimens were taken from normal young beef cattle within 30 minutes after death. Corresponding animal numbers indicate the pairs of fluids.

** Small phagocytic cells classified as monocytes because of nuclear characteristics and accumulation of neutral red granules in the Hof of nucleus.

*** Monocytes which had ingested recognizable cells.

**** Large stimulated cells which were phagocytic and resembled monocytes more than clasmatocytes.
The mitochondria, although sometimes scattered, were usually seen in one small area. These cells have been classified as lymphocytes.

A few cells interpreted as being synovial cells were found in most specimens. These were non-motile, varied greatly in shape and size, and contained relatively small oval nuclei, which were usually clear. The cytoplasm was always finely vacuolated and contained numerous finely distributed dark granules interspersed with the vacuoles. Granules stained with neutral red were never observed in these cells. In most instances these cells appeared to be degenerating.

**COMMENT**

Relatively few studies of the cytology of normal synovial fluid have been made.

Key, by means of supravital staining, was able to show that the synovial fluids of normal rabbits contained an average of 58 per cent monocytes, 15 per cent clasmocytes, 14 per cent indeterminate macrophages, 5 per cent leucocytes, 1 per cent primitive cells and 3 per cent synovial lining cells. In all instances 85 per cent or more of the cells were macrophages. He found from 175 to 225 living cells per cubic millimeter. He further believed that red blood corpuscles were normally present in slight excess of the nucleated cells. In 1926 Key (14) attributed to the macrophages and leucocytes the function of removal of waste and foreign matter from the joint cavity. Hammer (15) washed out supposedly normal joints with Mueller's fluid and examined the sediment from the washings. He described the following: (a) Large round or irregular, vacuolated cells with one to three nuclei, which he believed to be desquamated synovial cells; (b) small round cells with little protoplasm; (c) nuclei of degenerating encapsulated cells; (d) cell rests, free nuclei and small granular masses of protoplasm; (e) thin membranes staining with carmine and showing fine striations; (f) threads with a homogenous or striated structure which had an affinity for hematoxylin and were devoid of cells; (g) elastic fibers; (h) large tissue masses and broken off villi of various kinds; (i) fat droplets; (j) red blood corpuscles.

Our studies show that the astragalotibial joint fluid of young beef cattle contains an average of 103 to 112 nucleated cells per cubic millimeter (see Table I), whereas the carpometacarpal joint fluid contains an average of 165 to 222 cells per cubic millimeter. We do not believe, however, that the carpometacarpal joint fluid can be considered normal. Of all the nucleated cells present 90 to 95 per cent have been shown to be phagocytic for particulate matter. We cannot agree with Key (11 and 13) that red blood corpuscles are normally present in numbers slightly in excess of the total number of nucleated cells. We have had three fluids in which no red blood corpuscles were seen in
### TABLE III

**Total Cell Counts and Cell Types Contained in Normal Synovial Fluid Obtained from the Astragalotibial Joints of Twenty-One Young Western Beef Cattle**

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<th>Phagocytic monocytes</th>
<th>Large stimulated phagocytic cells</th>
<th>Eosinophils</th>
<th>Lymphocytes</th>
<th>Synovial cells</th>
<th>Cells unclassified</th>
<th>Phagocytic cells</th>
<th>Non-phagocytic cells</th>
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<th>Erythrocytes seen in counting 100 nucleated cells</th>
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Max. | 88 | 17 | 2 | 21 | 11 | 7 | 3 | 6 | 7 | 4 | 99 | 12 | 140 | 292
Min. | 55 | 4  | 0 | 1  | 0  | 0 | 0 | 0 | 0 | 0 | 88 | 1  | 55  | 1
Aver. | 67.3 | 9.8 | 0.5 | 9.4 | 3.7 | 2.4 | 1  | 1.3 | 2.6 | 2.0 | 94.5 | 5.9 | 90  | (*20.6 |

* These fluids were obtained in from 5 to 30 minutes post mortem.
** Small phagocytic cells classified as monocytes because of nuclear characteristics and accumulation of neutral red granules in the hook of nucleus.
*** Monocytes which had ingested recognizable cells.
**** Large stimulated cells which were phagocytic and resembled monocytes more than clasmocytes.
(*) By not including the two high counts (292 and 120) the erythrocyte average was 20.6. Including these abnormally high counts the average was 38.3 erythrocytes to every 100 nucleated cells seen.
the entire ruled field of the counting chamber. Many fluids contained only 1 to 5 erythrocytes per cubic millimeter and in only 15 out of 78 specimens counted did the number equal or exceed the total number of nucleated cells. From our observations we believe that the number of red blood corpuscles present are in direct proportion to the trauma to which the joint is subjected. Key (11) obtained fluid by passing a capillary glass pipette through the shoulder muscles and joint capsule of a rabbit's shoulder joint and then flexed the limb so that increased intra-articular pressure forced the fluid to rise into the tube. We know from perfusion experiments that the synovial membrane through which a needle or pipette must pass to enter the joint cavity is extremely vascular. The rupture of a single capillary of the synovial membrane would be enough to give the number of red blood corpuscles reported by Key, to say nothing of the blood that would probably enter the unprotected end of the glass pipette in its passage through the shoulder muscles.

In addition to the recognizable cells present in synovial fluid, we have occasionally noted fragments of cells, rare curved fibrils and oval bodies. Some of these bodies, because of concentric lamina, suggested degenerating cartilaginous fragments. In old stable or milch cows the amount of cellular detritus and unidentified solid material was much greater than in the young beef animals whose habitat is pasture-lands rather than barn stalls. Occasional bloody or yellow fluid and in a few instances turbid fluids were obtained. These were omitted from our studies.

SUMMARY

1. The astragalotibial (hock) joints of normal young beef cattle contain large and uniform quantities of synovial fluid which is easily accessible for study.

2. Nucleated cells found in such synovial fluid are similar in numbers and types to those described previously in normal rabbits' synovia.

3. The fact that 90 to 95 per cent of all nucleated cells present are actively phagocytic implies that the function of these cells is the removal of the products of wear and tear from the articular cartilages and synovial membranes.
4. Red blood corpuscles seem to be present in numbers directly proportional to the trauma to which the synovial membrane is subjected.

5. The astragalotibial joints of young beef cattle contain from 3 to 5 times more fluid than do the corresponding joints of stable cattle. More débris and unidentified solid material is found in the latter group of animals.

6. The total nitrogen content of this synovial fluid was found to be 169 mg. per 100 cc. The approximate total protein content was calculated to be 680 mg. or 0.68 per cent per 100 cc.

7. No correlation between the total protein level and the total cell counts of isolated specimens of synovial fluid is possible from these data.

8. Further studies of synovial fluid and of its chemical similarity to blood serum of the same animals are in progress.

9. Articular cartilage defects occurring in the articular cartilages of the carpometacarpal joints are described. (A more detailed study of these articular cartilage lesions, the age at which they appear, possible causative factors, etc., is now being made.)

We wish to thank the New England Dressed Meat and Wool Company for their cooperation and generosity, without which this work could not have been possible.

BIBLIOGRAPHY

NORMAL SYNOVIAL FLUID OF CATTLE. I


EXPLANATION OF PLATES

PLATE 37

Fig. 1. A photograph of the opened carpometacarpal joint (about ½ natural size), showing the opposing areas of medial articular cartilage degeneration. A cartilage laceration, indicated by an arrow, is the result of a knife cut. Specimen obtained from a young western beef steer (C-3).

Fig. 2. Photomicrograph (low power) showing the margin of an articular cartilage defect illustrated in Fig. 1. Note the total absence of the articular cartilage and that the base of the defect is formed by subchondral bone.

PLATE 38

Fig. 3. Camera lucida drawings (× 1800) illustrating the cells seen in synovial fluid of normal cattle. The drawings were made from supravital preparations in which both neutral red and janus green B were used as the vital stains. The black particles illustrated in Cells 9–13 represent the graphite particles which had been ingested by the cells before they were supravitaly stained. Entirely clear portions of cells represent those areas which remained unstained, neutral red staining is indicated by various shades of grey, while mitochondria are best seen in Cells 2 and 8 as minute dark rods.

Cells 1 to 4 are monocytes; 5, a phagocytic monocyte with ingested dead cell; 6 and 7 are varying sized clasmatocytes; 8, a lymphocyte showing a small clump of mitochondria but no neutral red staining; 9, clasmatocyte with ingested graphite particles scattered throughout the cytoplasm between neutral red stained vacuoles; 10 to 13 varying sized monocytes showing ingested graphite. Note the accumulation of neutral red stained vacuoles and graphite particles in the hof of the nuclei; 14 to 17, desquamated synovial cells with unstained vacuoles. When graphite was added to the fluids these cells showed no phagocytic properties.
Fig. 1

Fig. 2

(Bauer et al.: Normal synovial fluid of cattle. 1)
Fig. 3

(Bauer et al.: Normal synovial fluid of cattle. 1)