THE HISTOLOGY OF VOLUNTARY MUSCLE IN CATTLE

AND

CHANGES WHICH OCCUR DURING FATTENING

by

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Table of Contents.

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I Introduction

The present investigation does not enter into a general consideration of the histology of voluntary striated muscle but is confined to a few phases of the subject. The occurrence of fat in the muscle fiber will be considered in some detail. Some observations bearing on the changes in striated muscle incident to fattening, growth, and inanition will be presented.

The investigation was carried on in the Anatomical Laboratory of the University of Missouri. I am much indebted to Doctor E. T. Bell, under whose supervision the work was done. I am also under many obligations to Professor H. J. Waters, Director of the Missouri State Agricultural Experiment Station, for the material used. The material was made available in connection with some extensive feeding experiments now being carried on at the Experiment Station.

II Material and Methods.

The following table gives a list of the animals from which material was obtained in the course of this investigation. These animals will be referred to by number and the table may be consulted to determine their age and nutritive condition:

Number	Sex	Age at Slaughter	Condition at Slaughter	Feeding during period preceding slaughter
S	Male	3 years	very fat	full fed 2 years
B	Male	3 years	fat	full fed 1 year
J	Male	3 years	fat	full fed 2 years
U	Male	3 years	fat	full fed about 1 year
48	Male	3 years	fat	full fed about 2 years
63	Female	6 years	thin	ordinary feed (milk cow)
591	Male	15 months	very thin	fed to lose .5 pounds per day for 6 months
597	Male	17 months	Moderately thin	fed to keep weight constant for 6 months
593	Male	16 months	fat	fed to gain .5 pounds per day for 6 months
592	Male	21 months	very thin	fed to lose .5 pounds per day for 8 months
595	Male	21 months	thin	fed to keep weight constant for 8 months
504	Male	21 months	fat	full fed

Number	Sex	Age at Slaughter	Condition at Slaughter	Feeding during period preceding slaughter
5970	Male	2 years	fat	full fed
5971	Male	2 years	fat	full fed
527	Male	8 months		
529	Male	8 months		
Fetus I	(?)	7 months, 60 centimeters long		
Fetus II	Female, nearly full term			

Samples for histological purposes were commonly taken twenty four hours after slaughter. From two to twenty samples were taken from various muscles of the head, neck, and extremities of each animal. The muscles of which samples were obtained will be mentioned in connection with data presented later. Animals 527 and 529 were not slaughtered but small samples were taken from the biceps femoris by operation.

The fixative used for animals 504 and S was prepared by adding 4 cc of pure formalin to 100 cc of 70% alcohol. The fixative used in all other cases was made by adding 20 cc of pure formalin to 80 cc of distilled water.

In studying the connective tissue Mallory's aniline blue stain was found to be of little value for material fixed in formalin. Celloidin sections stain weakly with Van Gieson's stain. At the suggestion of Doctor C. McGill, the celloidin sections were soaked for twenty minutes in strong potassium bichromate solution, after which Van Gieson's solution was used. This method gives excellent results. As far as I know this method has not been previously employed.

In studying the fat occurring within the muscle fiber about seventy-five samples were examined, four hundred preparations being made.

Frozen sections and teased preparations were employed. The stains first used were ~~employed were the commonly used~~ saturated solutions of Sudan and of Scarlet red in 70% alcohol. Marchi's osmic acid method was used to a limited extent. As the results obtained by the use of these stains were found to be unsatisfactory Herxheimer's stain was tried. The formula for this stain is as follows:

Absolute alcohol	70 cc.
10% sodium hydrate	20 cc.
Distilled water	10 cc.

Scarlet red to saturation.

This stain was found under certain conditions to stain some fat that remains unstained by the other solutions mentioned. It is found to exert a solvent action on the fat, however, and much of the fat in the fiber is entirely unstained. Because of the unsatisfactory results obtained by the use of these stains an attempt was made to devise a better method. A limited number of testw were made. Solutions of Sudan aned of Scarlet red in alcohol of various strength and in alkaline alcohol were tried. Mixtures of Sudan and Scarlet were also employed and sodium, potassium and ammonia were used as the alkaline reagent. The solution giving the best results of any of these tried was prepared as follows:

100 cc. of 50% alcohol

1 gm. of sodium hydrate

Scarlet red to saturation

Heat mixture for 20 minutes on water bath and allow to stand for 24 hours. Filter immediately before using.

A noticeable feature of preparations stained with this solution is the great number of fine droplets which are to be seen, such as those shown in Fig. 1. This stain should be kept tightly stoppered while in use. If placed in a watch glass a precipitate occurs and the fat droplets are stained black. Solutions of Scarlet red give best results when used in watch glasses, one being used as a cover. The slight evaporation which occurs from the surface of the solution lowers the alcohol percentage and keeps the solution saturated. The modified Herxheimer's stain gave much better results than any other stains used.

III The Occurrence of Fat in the Muscle Fibers

(a) Discussion of Literature.

Many histologists, physiologists and pathologists fail to mention the occurrence of fat in the voluntary striated muscle fiber. That microscopically visible fat may occur in the muscle fiber, under certain conditions, is a generally accepted fact. Chemical analyses of muscle are thought, by some observers, to indicate that much fat is present which can not be demonstrated microscopically. Those who mention invisible fat regard it as a physiological constituent of the fiber. The presence of fat which can be demonstrated microscopically is regarded by some as of pathological significance. Many observers, however, regard it as normal. The amount of fat inside the muscle fibers is usually regarded as independent of the nutritive condition of the individual.

Fat occurring in the muscle fiber under pathological conditions is usually spoken of as a fatty degeneration or as a fatty metamorphasis. Virchow used the term fatty degeneration to indicate a formation of fat, in situ by direct transformation of the proteid constituents of protaplasm. Pettenkafer, Voit, Wentscher, Lindmann, Bauer and others have advocated Virchow's view. At the present time the term fatty degeneration is often used to include all instances in which there is a degenerative change and simultaneous fat formation, without reference to the manner in which the fat may be suppoed to arise. The term fatty metamorphosis is usually employed as synonymous with fatty degeneration, but is sometimes used to indicate a physiological transformation of protaplasm or some of its constituents into fat.

Fatty infiltration is generally recognized as occurring under both pathological and physiological conditions. The term is used to indicate the bringing of fat into the cell from an external source. Among those who think that all intracellular fat is of infiltrative origin, as opposed to Virchow's degeneration idea, may be mentioned Liebdeff, Kraus and Rosenfeldt.

The text books on pathology by Delafield and Prudden, and by Hamilton mention fat as occurring in the voluntary striated muscle fiber in parenchymatous myositis, exhausting disease, various forms of atrophy and in phosphorous poisoning.

Schultzer (1865) and Manassein (1869) observed a granular and fatty degeneration of muscle in starvation, and Folch (1875) observed granular degeneration. Knoll (1880) regards the occurrence of fat droplets in the muscle fibers of birds as normal. Ochathim (1883) observed granular and fatty degeneration of the muscle fibers of starving rabbits. Rindfleisch (1886) is not decided as to whether the fat in the fiber is to be regarded as a degeneration or as an infiltration but in either case he thinks it of pathological significance. Zeigler (1886) thinks that the occurrence of fat droplets in the muscle fiber is due to degenerative atrophy. He makes no mention of fat infiltration of the fiber.

Koelliker (1889) considers the fat which occurs in the muscle fiber to be intimately connected with certain interstitial granules. He describes these granules as lying in the sarcoplasm between the fibrillae and thinks that they are a normal constituent of the fiber. He thinks that the interstitial granules, under certain conditions, are transformed into fat. One of his figures shows a large amount of fat in a human muscle fiber.

Schaffer (1893) thinks that fat in the muscle fiber, commonly regarded as pathological, is often physiological. In the human muscle he found a few fat droplets near the nucleus in perhaps 40% of the cases examined. He also found numerous instances of isolated darkly staining fibers (osmic) with many fat granules in rows between the fibrillae. He considers the interstitial granules to be a regular and normal constituent of the fiber. Many differences in color and brightness depend upon the relative number of granules present. These interstitial granules often undergo a physiological metamorphosis forming fat droplets. He draws a sharp distinction between fatty metamorphosis depending upon a chemical change of pre-existing interstitial granules and fatty degeneration, the latter bearing no relation to interstitial granules. In some cases of chronic tuberculosis and infectious fevers he found the interstitial fat granules formed by metamorphosis to an extent regarded as pathological. In the later stages of slow wasting diseases there is an entire absence of interstitial granules. He believes that the interstitial granules, including fat and fat-like substances, are to be regarded as stored food-stuffs.

Hafmann (1894) believes that a fatty metamorphosis of interstitial granules occurs normally in the frog at certain periods of the year. Upon section of the nerve supplying a muscle he observed an increase of fat in the fiber followed by a partial or complete disappearance of fat.

Statkewitch (1894) examined the muscles of forty-four animals at various stages of starvation. The animals used included mammals, birds and amphibia. He used Flemming's fixative and osmic acid. In no case did he find fat within the muscle fiber. He concludes that fatty degeneration of the muscle fiber does not occur in starvation.

Poirier (?) mentions the interstitial granules of Koelliker as identical with the sarcosomes of Retzius. They occur between the muscle columns and in the sarcoplasm surrounding nuclei of the muscle fiber and are to be regarded as a normal constituent of the fiber. The granules are united to each other by a thin protaplasmic network. They are not uniform in number or arrangement. Under normal physiological conditions they are often changed to fat droplets.

Ricker and Ellenbeck (1899) investigated the histological changes following nerve section in the muscles of rabbits. They find fat droplets first appearing 15 to 20 days after operation. At 33 days all the fibers are affected moderately. A slight increase follows and this amount is constant to about 99 days, when the fat begins to disappear. At 125 days it is found in very small amount in a few isolated fibers.

Walbaum (1899) investigated the musculature of 119 cadavers of which were of rachitic children. He finds some fat in a few muscle fibers in about 60% to 80% of the cases investigated. He finds no connection between the occurrence of fat in the fiber and the nutritive condition of the individual. In fevers leading to a fatal termination no connection is observed between the etiology, duration or height of temperature and the amount of fat found. There is no relation between the occurrence of fat and the age of the individual excepting that the amount of fat found in the older fetus and in children under one year of age is small. In many instances the droplets appeared to have no definite relation to the striation of the fiber and such droplets he regards as of infiltrative origin. He sometimes found, however, that the droplets were arranged in longitudinal rows the size of the droplet being dependent upon the width of the striation bands. He says that a droplet occurred to each striation, but it is not clear whether he considers the striation as consisting of one band or of both a light and a dark band. He believes the relation of the fat droplet to the striation to indicate that a fatty metamorphosis of the fibril has occurred. He is not clear as to how he arrives at this conclusion. He finds that neither osmic acid nor sudan stains all the fat to be demonstrated microscopically.

Eycleshymer (1904) finds in the developing musculature of necturus the yolk granules gradually diminishing in size until in the twenty-sixth millimeter stage they lie in rows between the fibrillae and correspond to the transverse markings. He makes no mention of the exact relation of the yolk granules to the striation.

Kainath (1904) investigated the muscles of various animals, including man and the ox in adult and fetal stages. He is unable to verify the observation of Walbaum that the fat droplets sometimes have a relation to the striation. He finds fat droplets arranged in the sarcoplasm around the muscle fiber nuclei in all the animals observed. Droplets were not found around every nucleus, however. In some instances fat was found in the fiber in other situations than around the nucleus, but this seems to have been regarded as pathological. In the embryo of the ox fat was found up to the twenty centimeter stage but was absent from this and subsequent stages. Kainath used a 70% alcoholic Sudan stain and osmic and his preparations were counterstained with haematoxylin. He examined only one muscle sample from the adult ox.

Kemp and Hall (1907) examined the muscles of animals fattened for slaughter. Microscopically visible fat within the muscle fiber was not found in any of the animals studied. "Some specimens of very lean meat yielded a much larger percentage of fat by extraction than could be accounted for by the fat which showed under the microscope". They used osmic, Sudan and Scarlet red as fat stains.

Pappenheimer (1909) in two cases of progressive muscle atrophy finds no fatty degeneration of the muscle fiber, the small amount of fat present being regarded as normal. The data show that fat which failed to stain with scarlet or Sudan was sometimes stained by osmic.

Bell (1909) examined a few of the muscles of the ox embryo from the 3 cm. to the 105 cm. stage. He found fine fat droplets between the fibrillae in some of the fibers from the 7 cm. to the 28 cm. stage. The formation of true adipose tissue begins about the time that fat disappears from the fiber. He thinks, however, that the fat of the muscle fiber in the embryo is not to be regarded merely as stored fat but may be intimately connected with the growth of the fiber. He mentions that I had found fat present in some of the muscle fibers of several very thin oxen.

As is seen from the foregoing, various questions that arise concerning the occurrence of fat in the muscle fibers cannot as yet be answered with certainty. It will appear later that observations here presented concerning the fat of the muscle fiber bear a certain relation to some recent observations concerning the glycogen of the muscle fiber. Brief mention will accordingly be made of this work.

Mascati (1908) has shown by chemical analysis that the glycogen content of muscle differs in different regions of the body and disappears at a somewhat rapid and uniform rate after death.

Arnold (1909) has succeeded, as he believes, in staining the glycogen occurring in the skeletal muscle of the frog. It is seen as very small granules associated with the sarcosomes. These granules occur in the J band and close to the Z line. They are not found in the fibrillae, This is taken as evidence that the sarcoplasm is the nutritive portion of the muscle fiber while the fibril is the contractile element.

III The Occurrence of Fat in the Muscle Fiber.

(b) Observations and Conclusions.

The observations here presented are based on an examination of about one hundred preparations stained with the modified Herxheimer's stain previously mentioned. Fat which can be demonstrated microscopically was found in considerable amount within many of the muscle fibers of the ox. The position of fat droplets in the fiber and their relation to the structure of the fiber will first be indicated. The extent to which fat in the muscle fiber is found in animals of various ages and different conditions of nutrition will next be considered. In this connection observations on two late ox fetuses will be included and brief mention will be made of the occurrence of fat in the muscle fibers of two human subjects.

Distribution of Fat within the Fiber.

In specimens fixed in 20% formalin, and teased into individual fibers all the striation lines can sometimes be made out with great certainty. Hensen's line can be made out usually with difficulty but it sometimes shows clearly when the Zeiss apochromatic is used. The position of the other striation bands is very clear. In the condition of the fiber considered by Rollet, Haycraft and others as the fully extended state the striation lines are most clearly seen, the following description will apply especially to fibers in that condition and with slight modification to fibers in other states of contraction. In referring to the lines of striation the terminalogy of Rollett will be followed and the striation lines and bands will be considered as applying to the whole fiber and not to individual fibrillae.

The first point to be determined in considering the relation of fat droplets to the structure of the muscle fiber is whether they occur in or between the fibrillae. As is seen from the literature most investigators are agreed that fat droplets occur in the sarcoplasm and no mention is made of their having been observed within the fibrillae. Walbaum, however, believed that fat sometimes arose by a fatty metamorphosis of the fibrillae. He was led to this belief by the observation that fat droplets are occasionally arranged in longitudinal rows corresponding to the striation bands.

Fig. 1, made with a camera lucida from a teased preparation, shows a portion of a muscle fiber in which fat occurs in very small droplets: The droplets are to be seen in the J band on either side of the Z line. They are arranged in longitudinal rows between the fibrillae, the alighment sometimes being almost perfect (as at a a). According to the views of Rollett, Haycraft, Meigs and others, the point between the fibrillae at which there is the greatest accumulation of sarcoplasm is situated in the J band and close to the Z line, and it is at this point that the small droplets are seen. Rows of droplets such as that at "a a" can often be seen at a single focus. It will be noticed that none of the droplets are situated on the Z line.

On the supposition that some of these droplets are situated within the fibrillae it is difficult to account for their arrangement in longitudinal and transverse rows. If they are between the fibrillae, however, at the place of the greatest accumulation of sarcoplasm, and therefore of least resistance, their position is at once accounted for. An examination of cross sections indicates that the droplets do not occur in the fibrillae. Droplets of the size seen in Fig. 1 are sufficiently small to allow several to be placed in the cross section of a single fibril and if they occurred within the fibril they should be easily seen.

Fig. 2 shows a portion of a muscle fiber in which the amount of fat is much greater than that shown in Fig. 1. The drawing was made from a teased preparation of a human muscle fiber but as similar conditions are found in some of the preparations made from the ox, it may be used in this connection. Hensen's line did not show in the specimen but its position is indicated by a dotted line. The position of the small droplets is seen to be similar to those in Fig. 1. It will be noticed that <u>none</u> of the droplets shown in the figure extend across the Z line. At B two droplets are seen to be in close contact with the Z line on opposite side and in the same longitudinal line but altho somewhat flattened by being pressed against the line they do not flow together to form one droplet. While small droplets are seen to accumulate at the Z line they are almost entirely absent from the region on either side of Hensen's line (H). Very few droplets are intersected by Hensen's line excepting those which are sufficiently large to fill up the entire distance between the Z line and Hensen's line, H. The larger droplets shown are seen to extend across Hensen's line but do not extend across the Z line. At certain points the Z line seems to be shoved slightly out of position by a fat droplet. Droplets in close contact on opposite sides of Hensen's line are not to be seen altho they frequently occur at the Z line. Altho two droplets in the same longitudinal line and between adjacent Z lines as at AA are of frequent occurrence three or more droplets have not been observed in that position in adult animals. When droplets are of a larger size than the largest shown in the figure they are found to extend across the Z line. Such a large droplet may sometimes be seen lying squarely across the Z line. There is a tendency for large droplets to accumulate in rows and not to be uniformly distributed thruout the fiber. An examination of cross sections indicates that these large droplets occur in the sarcoplasm between Cohnheim's areas this fact explaining their large size as compared with droplets in other situations. It is observed, however, that small droplets occur at or near Hensen's line in the sarcoplasm between the muscle columns with some frequency, while small droplets in such a position between the fibrillae are of infrequent occurrence.

The sarcolemma cannot be seen in the preparations used. Its probable position, however, can be inferred from the position of the fibrillae occurring peripherally. Under such conditions it is often very difficult to be perfectly sure as to the position of a fat droplet. Fat droplets very frequently seem to occur just beneath the sarcolemma.

In this situation small droplets assume a position near the Z line, the condition being similar to that shown in Fig. 1. Small droplets are often observed just beneath the sarcolemma or near the periphery of the fiber when none can be seen near the central axis.

This fact may indicate that droplets placed deeply in the fiber are frequently unstained. It may indicate that well stained droplets are difficult to see when occurring near the central axis of the fiber. This cause may be especially operative when the fiber is slightly contracted, the striation bands in this condition of the fiber making optical conditions unfavorable. Finally, the appearance may indicate the actual distribution of the fat present. A very careful examination of well stained teased preparation and cross sections will be necessary before this point can be settled with certainty.

Fat droplets frequently occur in the sarcoplasm surrounding the muscle fiber nuclei. They are sometimes so small as to be seen with difficulty. At other times they are two or three microns in diameter. At times they almost completely obscure the nucleus. They are scattered in the sarcoplasm at various distances from the nucleus and seem to have no definite arrangement. Fat droplets were often seen in the Miescher's tubules which are sometimes found in the muscle fibers.

The nearly full term fetus examined shows that fat droplets do not often ~~occur~~ across the Z line, but that small droplets in the Q line are of more frequent occurrence than in the fiber of the adult. An accumulation of droplets on either side of the Z line is very plainly to be seen however. Droplets are also seen just beneath the sarcolemma and in the sarcoplasm around the nuclei.

The relation described between the position of the fat droplets and the structure of the muscle fiber has been observed in many fibers of several animals of different ages and of different stages of nutrition. (The details as to the animals examined will be given later). These relations are found both in the ox and in the human muscle fibers examined. No instance of marked variation from the conditions described has been observed excepting where obvious degenerative change was to be seen in the fiber.

The observations just described afford strong evidence in support of the opinion held by some investigators that Krause's membrane (Z line) forms a complete transverse septum between the fibrillae and between the muscle columns and is attached to the sarcolemma. They make it seem probable that Hensen's line does not extend between the fibrillae. They afford some evidence in support of Haycraft and Meigs view that the fibril has a somewhat beaded form, with the widest transverse diameter at Hensen's line.

Walbaum's idea that fat droplets within the fiber are sometimes formed by fatty metamorphosis of the fibrillae, and therefore, correspond to the striation bands is not in accord with the view here expressed. According to recent observations made by Arnold, the glycogen which he found associated with the sarcosomes in some of the muscle fibers of the frog assumes a position in the J band between the fibrillae and close to the Z line. This may be taken as affording some confirmatory evidence in support of the observations here presented concerning the relation between fat droplets and the structure of muscle fibers. So far as is to be seen from the literature this relation has not been previously observed. This is probably accounted for by the fact that small fat droplets are left unstained by the staining solutions in common use.

Occurrence of Fat in the various Animals Studied

The extent to which fat is found to occur in the muscle fibers of the various animals studied will now be considered. For a more detailed statement concerning the nutritive condition of the various animals studied reference may be made to the table of material previously presented. All preparations described are of teased fibers unless otherwise stated.

Animal No. 48. Fat steer, three years old, full fed two years:

<u>Diaphragm</u>, 3 preparations. Droplets which can be seen without the use of the oil immersion lens occur between the fibrillae in about 5 to 10 per cent of the fibers. The droplets are present in considerable number in each fiber in which they occur. They occur most frequently in the J band. Droplets are found in the sarcoplasm around the nuclei in some of the fibers. Most of the fibers seem to be free from fat and where it occurs around the nuclei it is absent from around many nuclei of the fibers. No degenerative change is to be seen in any of the fibers. An examination of cross sections is confirmatory of the description given for teased preparations. No droplets are to be seen within the fibrillae.

<u>Vastus Internus</u>, 1 preparation. Fat droplets are to be seen without the oil immersion in a few fibers. Examined under 1-16 inch oil immersion lens very fine fat droplets are seen to occur in the J band. They are of most frequent occurrence near the sarcolemma but are often placed near the central axis. They are not evenly distributed along the length of the fiber but occur in places in large numbers and are

entirely absent from other situations. Most fibers seem to be entirely free from fat droplets. They are occasionally seen around the nuclei. Fibers showing the presence of fat droplets are not seen to differ in other respects from fibers apparently free from them. Fig. 1 was made from this specimen.

Inferior oblique of eye, 1 preparation, 1-12 oil immersion lens used. Fat droplets are of very infrequent occurrence. They are seen just beneath the sarcolemma and around the nuclei in a few fibers. The preparation seems not to be well stained.

Animal No. S, very fat steer, 3 years old. Full fed two years.

Diaphragm, 10 preparations. The amount of fat in the fibers is very large. Most of the fibers show fat droplets along their whole length. Many fibers are loaded with large fat droplets. Some droplets are so large that they extend across several striation bands, while others are very small. A few fibers show very little fat but nearly all show some. [redacted] Degenerative changes of the nuclei seem to be present in a few of the fibers. A definite relation of the droplets to the striation exists. A cross section from this sample is show in Fig. 3.

Psoas, 2 preparations, 1-12 oil immersion lens. The amount of fat is very small. Small droplets are to be seen around a few nuclei. About 95 per cent of the fibers show no fat droplets.

Intercostal muscle, 1 preparation. A few droplets are to be seen around some of the nuclei. Examined with No. 7 objective. About 70 per cent of the fibers show no fat droplets.

<u>Superficial muscles of back of neck</u>: 1-12 oil immersion lens. A few small droplets are seen around some of the nuclei and between the fibrillae in a few fibers. About 70 per cent of the fibers show no fat droplets.

Animal No. J, fat ~~ox~~ steer, 3 years old:

<u>Diaphragm</u>, 1 preparation, oil immersion: Fine droplets are seen between the fibrillae and around the nuclei of a few fibers. A very small number of fibers show droplets as large as two or three microns. A very few fibers show thickenings in places and such fibers contain the largest fat droplets.

<u>Rectus abdominis</u>, cross section: 1-12 oil immersion lens. Small droplets are seen around the nuclei in a few fibers. Occasionally fine droplets are found between the fibrillae.

Animal No. U, fat steer, 3 years old.

<u>Diaphragm</u>, 3 preparations, 1-12 oil immersion lens. The number of fat droplets is large in some fibers; others show none at all. Some of the droplets have a diameter of three or four microns. Many of the fibers showing no distinct droplets have a redish color.

<u>Erector spinae</u>, 2 preparations, No. 7 objective. Many of the fibers are of a redish color. The number of fat droplets is very small. Some droplets are seen around the nucleus.

Animal No. B, fat steer, 3 years old:

Diaphragm, No. 7 objective, 1 preparation: Fat droplets are to be seen in only a few of the fibers. The droplets are usually small but a few fibers show large droplets arranged in rows for short distances along their length. A few droplets are found around the nuclei.

Animal No. 63, thin milk cow, 6 years old:

Diaphragm, 2 preparations, 1-16 oil immersion lens: A few fine droplets are to be seen in about one-third of the fibers present. Droplets are frequently seen around the nuclei when none are present in the fibers elsewhere. A few fibers show the presence of a large number of droplets from one to two microns in diameter. The droplets in these fibers accumulate near the Z line . No degenerative changes are seen.

Psoas, 1 preparation, 1-16 oil immersion: A few droplets, sometimes 3 microns in diameter, are to be found around some of the nuclei and between the fibrillae in a very few fibers. Between the fibrillae the smaller droplets are near the Z line when this line is to be seen. Small droplets are found near the sarcolemma in some fibers where none are seen at the central axis.

Animal 591, very thin steer, 15 months old:

Adductor, 6 preparations, 1-16 oil immersion lens: About 20 per cent of the fibers show thickenings along their length. The droplets in all such fibers are usually very large, some of them being 10 to 15 microns in diameter. The larget droplets are seen just beneath the sarcolemma. The fibers show degenerative changes in the nuclei and in the fibrillae. The fibers showing no thickenings along their length also contain fat in a large amount. It occurs in droplets from

2 microns to 3 microns in diameter. The droplets are distributed along the entire length of the fiber with great uniformity and are in almost every fiber present. The small droplets accumulate at the Z line. No degenerative change is to be seen excepting in the fibers with thickened nodes along their length. In cross section these fibers show a rounded outline, The amount of fat in this sample is much greater than in any of those yet described, with the exception of S, diaphragm.

Erector spinae, 2 preparations, 1-12 oil immersion lens. The condition in these preparations is closely similar to that noted in Adductor 591, with the exception that fibers showing thickenings are only about one-half as numerous as those in the sample just mentioned. The amount of fat in the other fibers is also much less. Cross sections show rounded fibers.

Latissimus Dorsi, 1 preparation, 1-12 oil immersion lens. The number of fibers with thickenings along their length is very great and there is a large amount of fat in nearly all the fibers. The condition in these fibers is closely similar to that in the fibers of the Adductor of 591.

Animal 597, moderately thin steer, 17 months old:

Adductor of thigh, 1 preparation, Zeiss apochromatic: The amount of fat in the fibers is small. Droplets are seen around the nuclei and between the filbrillae. The droplets are small and are most numerous just beneath the sarcolemma. They are close to the Z line. No degenerative change is to be seen. Droplets are not evenly distributed along the entire length of the fibers

Erector, 1 preparation, Zeiss apochromatic: This preparation is similar to the one just described. The amount of fat is small. The droplets are very small.

Animal 593, fat steer, 16 months old:

Adductor, 2 preparationa; Psoas, 2 preparations, Zeiss apochromatic: The amount of fat in the fibers of these samples is very small. Most of the fibers seem to be free from fat. The droplets are small and occur around the nuclei abd between the fibrillae at the Z line. The amount of fat in the adductor is slightly greater than that in the psoas.

Animal 592, very thin ox, 21 months old:

Diaphragm, 2 preparations, 1-12 oil immersion lens: A large number, perhaps 30 per cent of fibers, are thickened along a considerable part of their length. The amount of fat in such fibers is large but very much less than was observed in the same kind of fibers in 591. The thickened or rounded fibers often show degeneration changes. The amount of fat in the other fibers of the preparation is small.

Rectus abdominis, 1 preparation, 1-12 oil immersion lens: This preparation is similar to the one last described. The number of rounded fibers and the amount of fat is somewhat less, however.

Biceps femoris, 1 preparation, 1-16 oil immersion. A few fine droplets are seen in a small percentage of the fibers. They are placed around the nuclei and near the Z line.

Masseter, 1 preparation, cross section, 1-12 oil immersion lens: A very few fine droplets are scattered irregularly in a few fibers. The amount of fat is very small.

Animal No. 595, thin steer, 21 months old:

<u>Diaphragm</u>, 2 preparations, 1-16 oil immersion lens: There are many fibers with thickenings along part of their course. The amount of fat in such fibers is greater than that to be seen in 592 but less than that found in 591. The other fibers contain few fat droplets.

<u>Rectus abdominis</u>, 3 preparations, 1 cross section, 1-12 oil immersion lens. These preparations are similar to those of the diaphragm of the same animal.

<u>Psoas</u>, 2 preparations, 1-12 oil immersion lens: There is a considerable amount of fat in a few fibers. Small droplets are seen around the nuclei and near the Z line.

<u>Gastrocnemius</u>, 1 preparation, cross section, 1-12 oil immersion lens. A few fine droplets are just under the sarcolemma and near the nuclei. Occasional droplets are near the longitudinal axis of the fiber.

Animal 504, fat steer, 21 months old.

<u>Diaphragm</u>, 1 preparation, 1-12 oil immersion lens: Poorly stained droplets are found in a few fibers. They seem to be absent from most of the fibers.

<u>Pectoralis Anticus</u>, 1 preparation, No. 7 objective. Fat droplets are to be seen around a few nuclei. None are found in other situations.

<u>Psoas</u>, 1 preparation, Erector spinae, 2 preparations, 1-16 oil immersion lens: A few poorly stained droplets seem to be placed just beneath the sarcolemma but their identification is doubtful.

Animal No. 5970, fat steer, 2 years old.

Erector spinae, 1 preparation, Zeiss apochromatic: A few small droplets are found around some of the nuclei and between the fibrillae.

Animal No. 5971, fat steer, 2 years old.

Erector spinae, 1 preparation, Zeiss apochromatic: The condition is similar to that of the specimen last described.

Ox Fetus No. 1. 7 months old, 60 centimeters long.

Diaphragm, 1 preparation, Psoas, 1 preparation; Erector spinae, 1 preparation; Zeiss apochromatic: Isolated droplets of small size are to be seen near the Z line, and a few occur in the Q band. Most of the droplets are situated just under the sarcolemma, Their occurrence in other situations is very rare. Droplets are found in the sarcoplasm near two or three nuclei. The amount of fat in these preparations is less than that in any of the preparations of the adult with the exception of Steer No. 504.

Ox Fetus No. 2, nearly full term.

Diaphragm, 2 preparations; Rectus abdominis, 1 preparation; Psoas, 2 preparations: Most of the fibers in these preparations are similar to the fibers of fetus No. 1. About 5 per cent of the fibers show a very large amount of fat evenly distributed along their length. The droplets in these fibers occur near the central axis with as great frequency as they do peripherally. Small droplets are most numerous near the Z line but frequently occur in the Q band. Droplets are found around a few nuclei.

Autopsy No 90, human female, very thin, age 60 years.

Diaphragm, 1 preparation; Psoas, 1 preparation, 1-16 oil immersion lens. About 10 per cent of the fibers show large droplets arranged in rows. These droplets are usually large and take up the entire distance between adjacent Z lines. The rows are short and are distributed irregularly over the surface of the fiber. A few droplets are seen near the central axis. Degenerative changes are seen in many nuclei.

Autopsy No. 91, human female, age 60 years.

Diaphragm, 6 specimens, 1-12 oil immersion lens, and Zeiss apochromatic: In a few fibers very little fat is seen. The fibers usually show fat along their entire length. The droplets are large, occur in rows, and are limited by adjacent Z lines. Many medium sized and small droplets are found near the Z line. Droplets occurring near the central axis are as numerous as those occurring peripherally. The amount of fat in this sample is greater than that of any sample examined.

Rectus abdominis, and Psoas, 2 preparations, 1-16 oil immersion: The fibers in these preparations are similar to that of the fibers in the diaphragm. The amount of fat is much less, however.

As is seen from the data given above, there is a wide variation in the amount and position of the fat within the muscle fibers. The variation exists in individuals, in muscles of the same individual, in fibers of the same muscle, and in parts of the same fiber. It is difficult, therefore, to draw conclusions from the data obtainable.

Animals 591, 597 and 593 are growing animals at approximately the same age and are on different nutritive planes. Animals 592, 595 and 504 form a similar group, the individuals of which are about six months older than those of the first group. The amount of fat in the fibers of the thin animals of each group is greater than that in the fibers of the fat animals. This is due largely to the amount of fat in fibers with thickening along their length. These fibers show a rounded outline in cross sections. The rounded fibers are of much more frequent occurrence in the thin animals than in the fat animals. Such fibers are numerous in progressive muscular atrophy and are regarded by Schaffer, Pappenheimer and others as of pathological significance. Fibers showing a rounded outline, however, are often the result of contraction which sometimes occurs when a fresh sample is put into the fixative. With the exception just discussed the amount and position of fat in the muscle fibers of growing animals seems to be independent of the nutritive condition of the animal. In animal 591 there is a large amount of fat in fibers not showing a rounded outline in cross section. The amount of fat in these fibers is no greater, however, than that found in the fibers of some of the adult fat animals.

Animal 63 is the only thin adult examined. This animal was not as thin as 591 and 592. Rounded fibers were found very infrequently in samples from this animal. The muscle fibers did not differ in the amount of fat from those of several of the adult fat animals. The fibers of the diaphragm of the very fat 3-year old steer S, show an amount of fat that is probably to be regarded as pathological and as somewhat similar to a condition of obesity existing in the human individual. Fig. 3 was drawn under low power from a frozen section made from this sample. Other samples have a very small amount of fat in the fibers. Isolated fibers showing fat in a very large amount were also found in several of the other animals.

The above facts are, with the exception of the large amount of fat found in rounded fibers of thin animals, in accord with the observations of Walbaum that there is no relation between the amount of fat in the muscle fibers and the age or nutritive condition of the animal.

The amount of fat found in the fibers of the diaphragm is equal to, or greater than, that found in other muscles. This is in accord with the observation of Walbaum that the fibers of active muscles contain more fat than is found in the fibers of less active muscles.

The finding of fat droplets within some of the muscle fibers of each of the fourteen animals from which samples were taken is not in accord with the observations of Kemp and Hall; who found no fat which could be microscopically demonstrated within the muscle fibers of cattle. It is probable that the results obtained by these investigators are to be accounted for by the fact that their preparations were stained with the commonly used solutions of osmic acid, Sudan, and Scarlet red.

The occurrence of fat within some of the muscle fibers of a seven months ox fetus and of a full term ox fetus is not in accord with the observations of Kaineth and of Bell. The former investigator found no fat within the muscle fibers of the twenty centimeter fetus and subsequent stages; the latter found no fat in the muscle fibers after the twenty-six centimeter stage.

In conclusion it may be said that fat which could be microscopically demonstrated was found in considerable amounts within many of the muscle fibers of each of the fourteen cattle examined. The occurrence of fat in small droplets in fibers showing no degenerative chage is probably of little or no pathological significance. With the exception that fibers regarded as pathological and containing large fat droplets occur with greater frequency in thin animals, no simple relation is found between the amount of fat in the muscle fibers and the age or nutritive condition of the animal.

IV Changes Which Occur During Fattening, Growth, and Inanition

Histological changes occurring in inanition are much more easily controlled in the laboratory than are changes due to growth and fattening. This probably accounts for the fact that while the former changes have been the subject of numerous investigations, the latter have received comparatively little attention. McCallum (1898) counted the fibers found in the sartorius muscle of the human fetus at various stages of development. He found no increase in number after the 170 mm. stage. The work of this observer has met with general acceptance and most authors believe that the number of muscle fibers is, under normal conditions, approximately constant after birth.

According to the observations of Schaffer, the fibrillae of the muscle fibers of the late fetus correspond to the muscle columns of the adult. Pappenheimer observed no marked decrease in the size of the fibrillae in muscular atrophy. The relation existing between the number of fibrillae and the amount of sarcoplasm was observed to be constant. He thought it probable that the number of fibrillae often changed during adult life. An examination of the fibers of a late ox fetus (No. 2) make it seem probable that the number of fibrillae is much greater in the adult animal than in the fetus.

The size of the fibers in the very thin animals 591 and 592 is much less than that of the fibers of the moderately fat and of the fat individuals of the same age. A corresponding difference in the size of the fibrillae is not found, however. No difference in the size of the fibrillae due to inanition are to be observed with certainty.

The following table gives a few measurements of muscle fibers and of intra-muscular fat cells. The figures for the muscle fibers of 591, 597 and 593 are from an average of one hundred measurements. All other measurements were made by selecting under low power what seemed to be averaged sized muscle fiber or fat cell. The cell or fiber so selected was measured, a number 7 objective and number 2 micrometer eye piece being used. The measurements were taken from cross sections made with the freezing microtome. For details as to the age and condition of the individual animals the table of material may be consulted;

Diameter of Fat Cells and Muscle Fibers (microns)

		Erector Spinae		Psoas		Diaphragm		Adductor Femoris		Biceps	
		Fat Cells	Muscle Fibers	Fat Cells	Muscle Fibers	Fat Cells	Muscle Fibers	Fat Cells	Muscle Fibers	Fat Cells	Muscle Fibers
592 Very thin 21 months old	Max.	25	50	35	50	37	73			30	60
	Av.	12	36	25	27	18	35			15	44
595 Moderately thin 21 months old	Max	64	65	90	65	100	70			75	90
	Av.	25	43	53	37	65	48			40	75
504 Fat 21 months old	Max.	95	60	90	52	110	70			110	85
	Av.	70	38	60	32	70	50			70	63
48 Fat 3 years old	Max.	100	82	100		130	90				
	Av.	70	50	62		79	67				
Jerry Fat 3 years old	Max.	98	75	110	70	110	80				
	Av.	60	46	70	48	72	53				
63 Thin 6 years old	Max.	95	73	92	62	95	90				
	Av.	56	43	55	40	68	50				
591 Thin 16 months old	Max.	76	56					75	76		
	Av.	37	35					37	39		
597 Moderately thin 16 months old	Max.	87	72					88	75		
	Av.	46	49					46	43		
593 Fat 16 months old	Max.	95	69					63	72		
	Av.	52	48					55	48		

On account of the great variability in size all estimates of the diameter of muscle fibers, based on measurements of single fibers, are thought by many observers to be of no value whatever. Such measurements cannot indeed be regarded as very accurate, but the figures given in the table point to certain conclusions with some probability. The diameter of fibers of the diaphragm is greater than that of fibers of the Psoas. The fibers of the erector spinae occupy an intermediate position. The fat and the moderately thin individuals do not differ appreciably in the size of their muscle fibers. The muscle fibers of the very thin animals are markedly decreased in size. The fat cells of moderately thin individuals are considerably smaller than those of fat animals and the diameter is much less in the very thin individuals. The following date show the effect of inanition on the size of the muscle fibers, and the rapidity with which the fibers are restored to their normal size when the individual again receives proper nourishment.

Animal No. 529, age 5 months, weight 208.2 lbs. was kept at constant weight for 7 months. At the end of this period a small sample of the biceps muscle was taken by operation. Fig. 4 shows a drawing under camera lucida of the fibers of this sample. The average diameter of the fibers is 20 microns. The animal was full fed for fourteen weeks, after which a sample of the biceps muscle was again taken. The fibers of this sample are shown in Fig. 5. They have an average diameter of 50 microns.

Animal No. 527, full fed, age one year. A sample of the biceps muscle of this animal was taken by operation. The diameter.

of the fibers is 50 microns. The fibers are shown in Fig. 6. A comparison of the measurements and figures shows that the fibers of the thin animal returned to normal size when proper nourishment was received.

The table shows that fat cells in moderately thin animals are of less diameter than those in fat individuals of the same age. The muscle fibers, however, show no decrease in diameter excepting in the very thin animals. The fact is well established by physiologists and pathologists that in inanition the connective tissue fat of the body is called into use sooner and to a greater extent than the protaplasm. The above observations are in accord with this fact. It seems probable that the muscle fibers of thin animals quickly return to their normal size in the first stages of fattening. The amount of intramuscular fat probably continues to increase for some time after the fibers have attained their normal size.

V Conclusions.

The principal conclusions drawn in this paper may be summarized as follows:

I Fat which can be demonstrated microscopically is found within many of the voluntary muscle fibers of cattle. Small fat droplets in muscle fibers showing no degenerative change are probably not pathological.

II From the distribution of the fat droplets within the fiber, the following inferences are made concerning the structure. Krause's membrane forms a complete transverse septum across the fiber, but Hensen's line probably does not. The fibril probably has a somewhat beaded form, there being more sarcoplasm in the neighborhood of Krause's membrane.

III Under normal conditions, there is no simple relation between the amount of fat in the muscle fibers and the nutritive condition of the animal. Muscle fibers of thin animals are reduced in size, but quickly regain their normal dimensions when the animal receives proper nourishment.

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VII Explanation of Figures.

Figure 1 was made with a 1-16 inch oil immersion lens and No. 4 ocular. A few fibrillae from a muscle fiber of the vastus internus of animal 48 are shown extending longitudinally in the direction a.a. The striation bands are lettered according to Rollett's terminology. The fat droplets are stained red with the modified Herxheimer's stain, formalin fixation.

Figure 2 was made with a 1-16 inch oil immersion lens and No. 4 ocular. It was taken from a muscle fiber of the diaphragm of autopsy 91 (human). The striation bands are lettered as in Fig. 1. The position of Hensen's line is indicated by a dotted line (H). The fat droplets are stained as in Fig. 1. They are most numerous near the Z line, and least numerous near Hensen's line H. The large droplets are intersected by the line H but no droplets are intersected by the line Z.

Figure 3 was made with a No. 3 objective and No. 4 ocular. It shows a cross section of a few muscle fibers from the diaphragm of animal S. Fixed in alcohol-formalin, and stained as in Figs. 1 and 2. The pink color of the fibers indicates that they contain many fine fat droplets which can be seen only with a higher magnification.

Figure 4 shows a cross section of muscle fibers taken by operation from the biceps femoris muscle of a one year old steer which had been kept at constant weight for eight months. Formalin fixation. Magnification 475 diameters.

Figure 5 was made from fibers of the same muscle as those in Fig. 4 after the animal had been full fed for 14 weeks. Formalin fixation, Magnification 475 diameters.

Figure 6 shows a cross section of muscle fibers taken by operation from the biceps muscle of a one year old steer that had been full fed from birth. Formalin fixation, magnified 475 diameters.

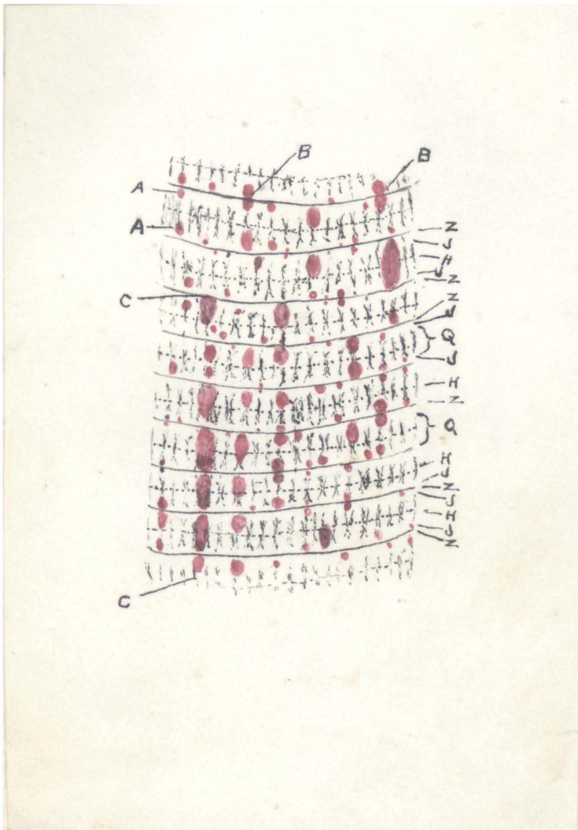

Fig. 2.

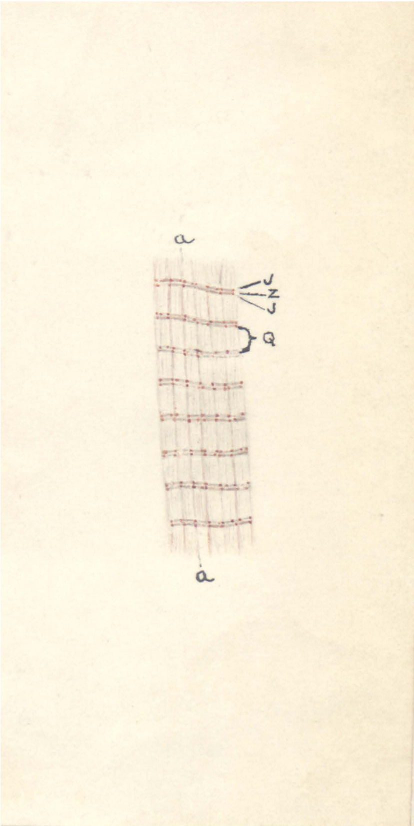

Fig. 1.

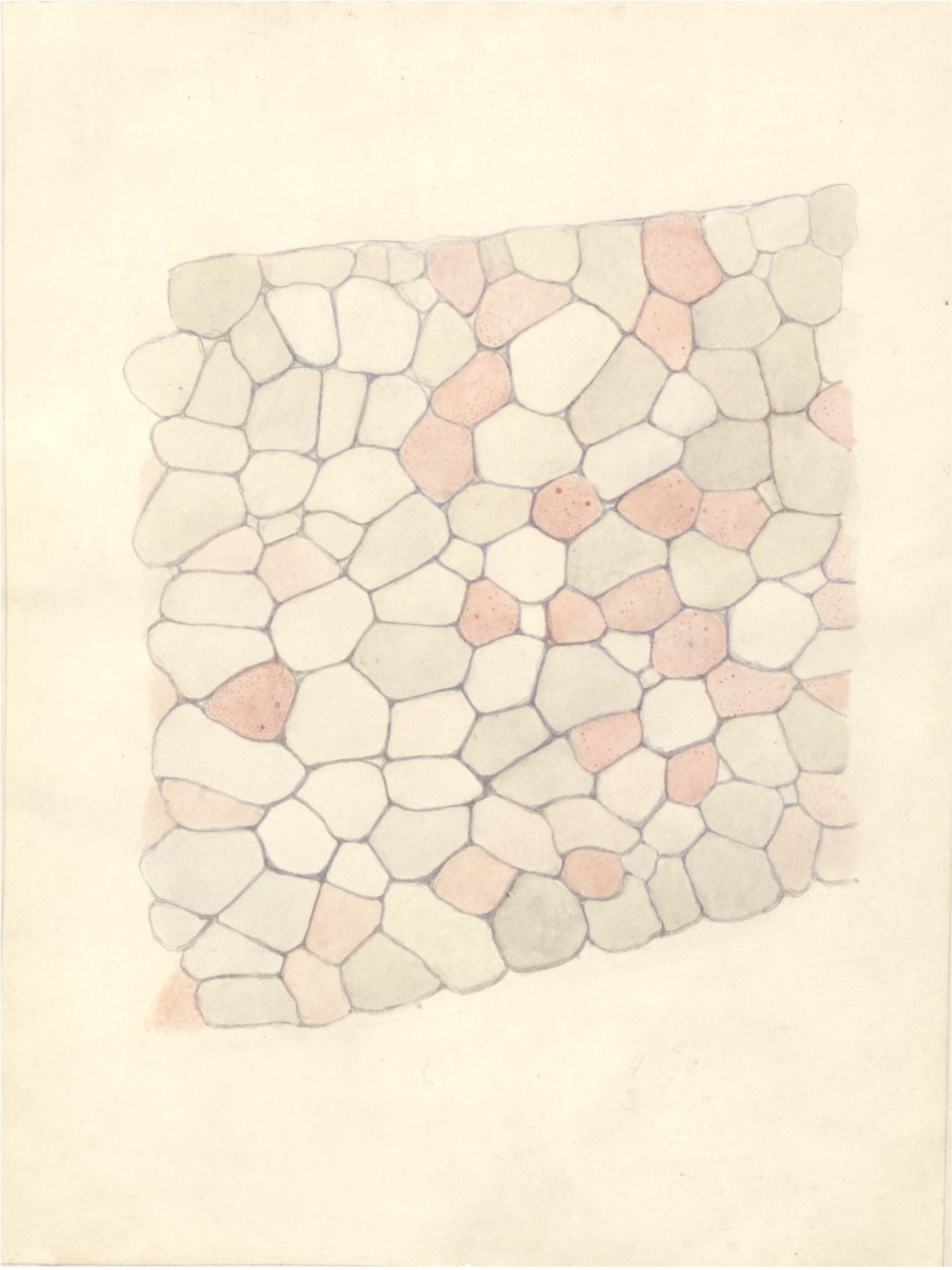

Fig. 3.

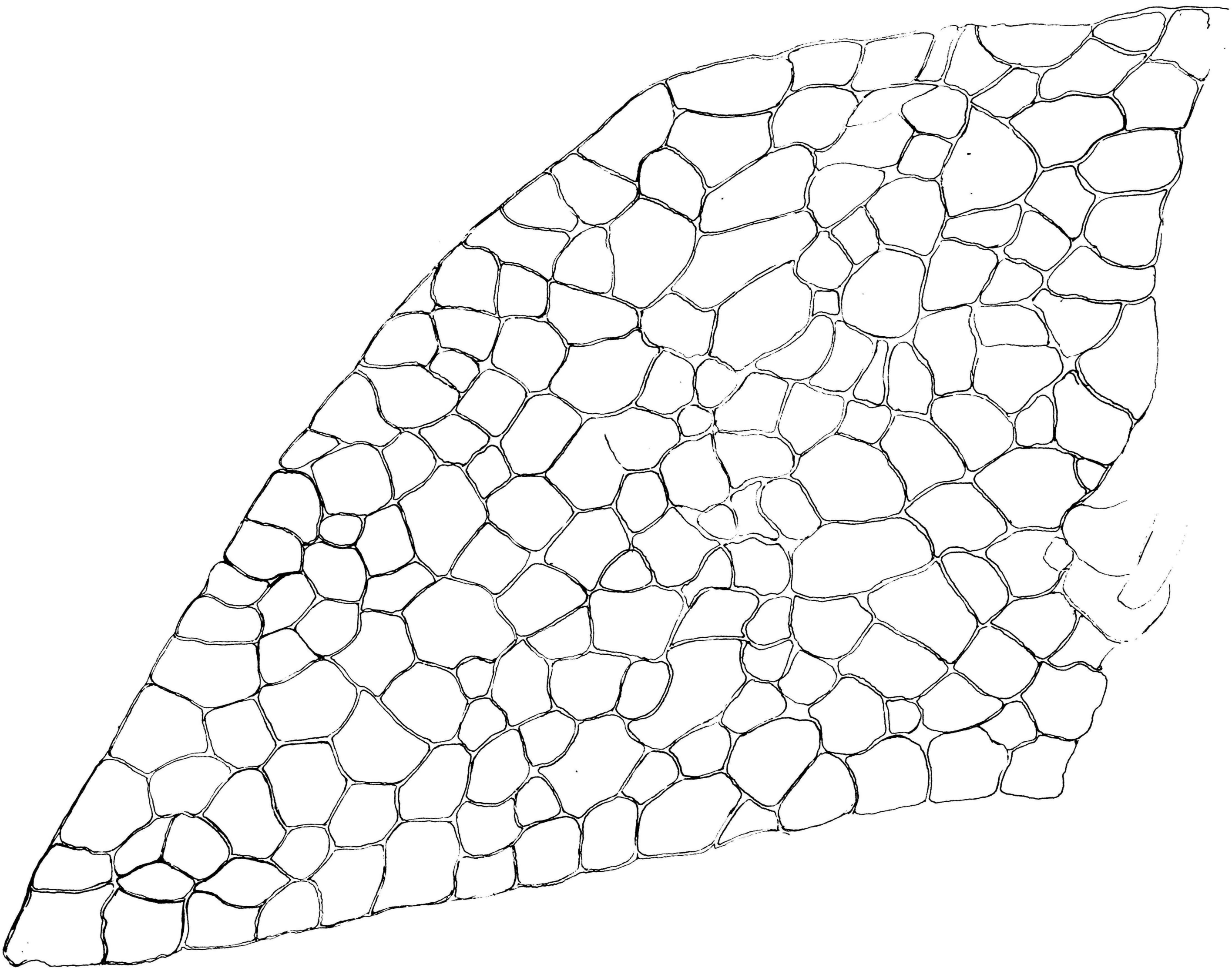

Fig. 4.

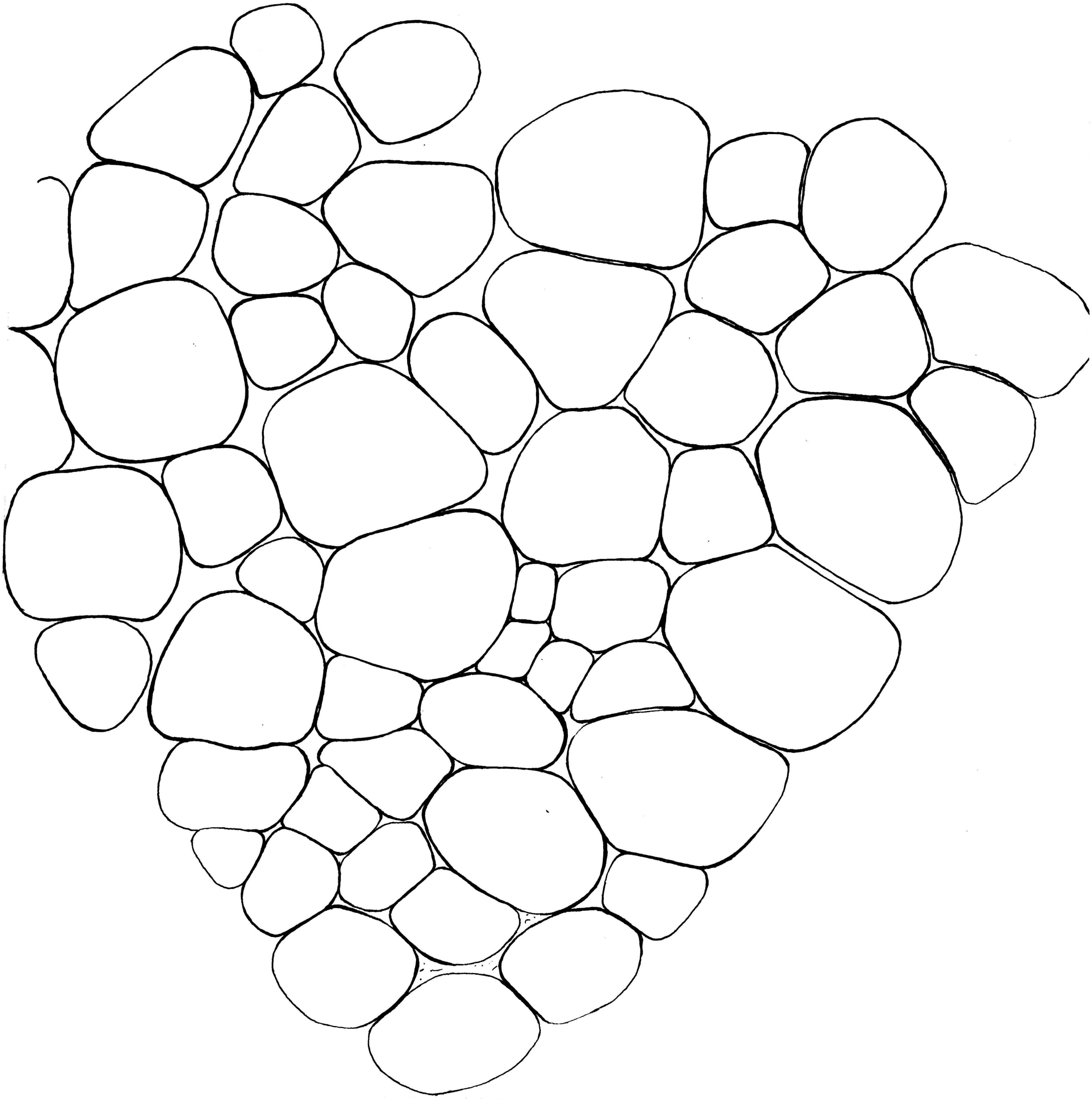

Fig. 5.

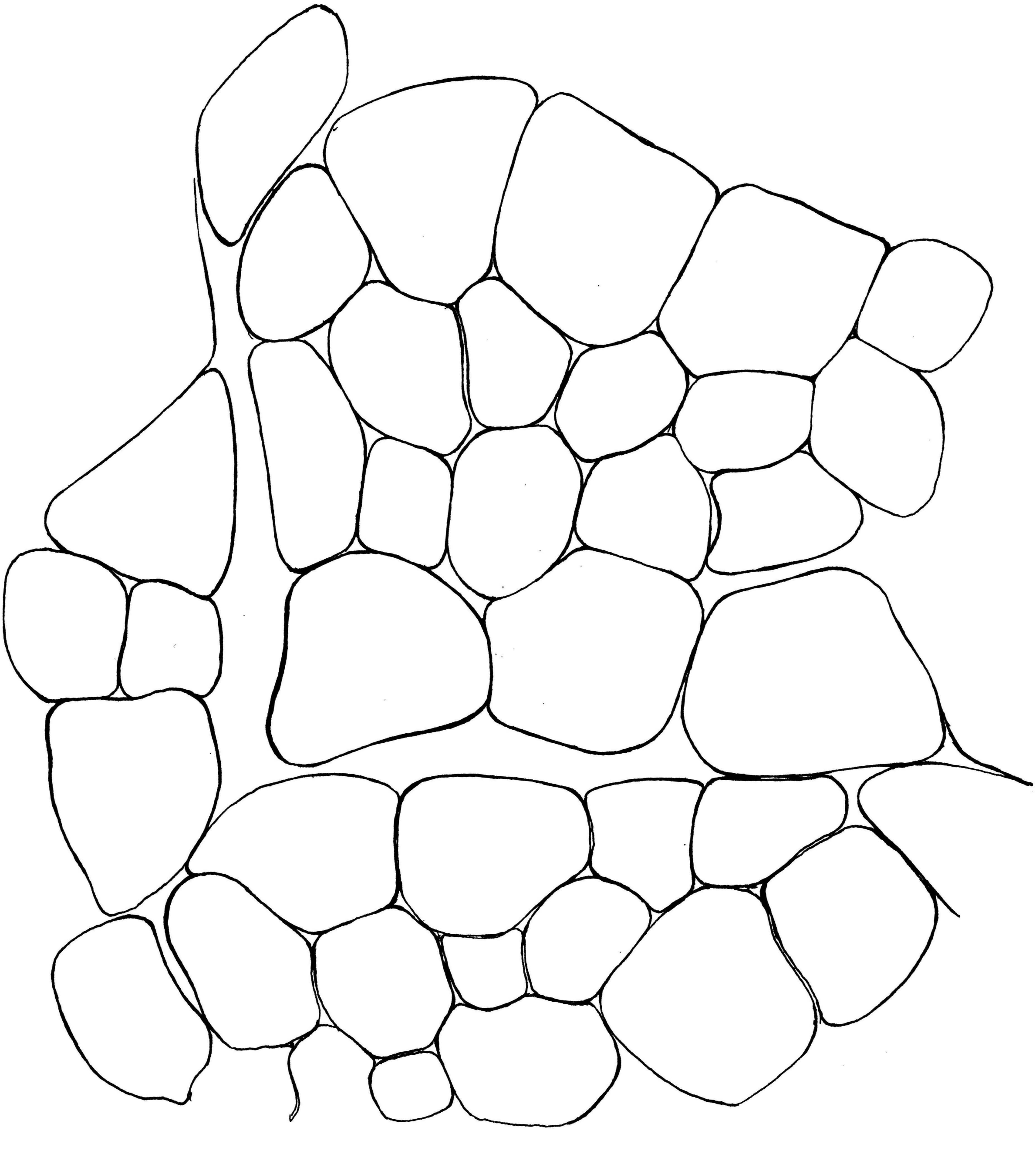

Fig. 6.

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