THE STORAGE OF FAT IN THE MUSCULAR TISSUE OF THE KING SALMON AND ITS RESORPTION DURING THE FAST OF THE SPAWNING MIGRATION

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CONTENTS.

	Page
Introduction	73
Material and methods of investigation	74
Types of salmon muscular tissue as regards the storage of fat	78
Normal loading of fats in the muscles of the king salmon at the time the spawning migration begins	80
Protocols	84
Variation of the amount of fat in the salmon during the spawning migration	86
Distribution of the fats of the salmon muscle at tide water	87
Analytical determinations of the percentage of fats in salmon from the mouth of the Columbia River	91
Protocols	93
Distribution of the fats at an early intermediate stage of the spawning migration	98
Protocols	102
Distribution of the fats at a late intermediate stage of the spawning migration	108
Protocol	109
Fat in the tissues of salmon from the spawning grounds	111
Analytical determinations of the percentage of fats in the spawning salmon	114
Protocols	115
Significance of the observed changes of the amount of fat	118
Transportation of fats in the fasting salmon	119
Historical	119
Mechanism of fat transference in the salmon body	124
Transference of fat in the pink muscle	125
Transference of fat in the dark muscle	127
Relative abundance of stored fat and its disposal in the organs	129
Salmon lipases	130
Source of the lipases	131
Résumé	136
Explanation of plates	137

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INTRODUCTION.

To the present no study has been made of the distribution of fats in the musculature of the king salmon, *Oncorhynchus tschawytscha*. We have the classic studies of Miescher[a] on the quantitative chemical variation in the fats of the Rhine salmon, *Salmo salar;* also the studies of Noël Paton and his coworkers on the same species from the Scottish rivers. Miescher found, as did Noël Paton after him, that there was a great decrease in the quantity of fats as the fish ascended the rivers and as the spawning season approached.

Miescher also observed fat in the muscle fibers in considerable quantity. This he considered to be a stage of fatty degeneration preliminary to the use of the fats for the building up of the immense store of food materials present in the salmon ova at the time of spawning. Miescher states that the fat granules increase in numbers in midsummer, and are absent after the salmon have spawned. He also states that the fats are practically absent in the fibers of the muscles of the smaller fins and of the head.

Mahalanobis,[b] as a part of Noël Paton's series of studies, made a special histological examination of the fat in the muscles under the subtitle "Microscopic observations on muscle fat in the salmon." There are six pages of text and seven microphotographic figures presented in his paper. Less than two pages are used to present the findings as to the distribution of fats in the muscles. He contrasts the variations in the fat of "a fish fresh from the sea" with fish taken up the rivers at later dates. The greater part of the text is consumed in a discussion tending to disprove the "fatty degeneration" theory which Miescher has assumed, and Miescher is undoubtedly in error and Mahalanobis right in this particular matter. But Mahalanobis has failed in his special comparisons between two extreme types of fish from the simple fact that he based the comparisons on dissimilar muscles. (See p. 121 of this paper.) The studies on the Atlantic salmon have, therefore, left us without adequate description of the normal histological distribution of the muscle fats. As for the variations in the microscopic

[a] Miescher, Friedrich: Statistische und biologische Beiträge zur Kenntniss vom Leben des Rheinlachses im Süsswasser. Schweizerischer Fischerei-Ausstellung in Berlin, s. 154, 1880. Also reprinted in Die histochemischen und physiologischen Arbeiten von Friedrich Miescher, s. 116, Leipzig, 1897.

[b] In: Paton, D. Noël, Report of the Fishery Board for Scotland, 1898, p. 143.

picture as the salmon proceed up the rivers, one may say, with all due respect to the previous work, that such picture has not yet been given for any species.

The king salmon of our Pacific waters is an entirely distinct genus from that of the Atlantic salmon. It is decidedly unique in its biological characteristics, as shown in a number of fundamental ways. These characteristics have been enumerated at various times,[a] but three facts of peculiar importance may again be stated here as most vital to this investigation:

First, *Oncorhynchus tschawytscha* is an anadromous fish.

Second, it fasts completely during its entire journey from the sea through the tidal waters and up the rivers to the spawning grounds.

Third, *Oncorhynchus* always dies within a short time after spawning.[b] It can not, therefore, return to the sea for a second period of feeding.

These facts, taken in connection with my numerous quantitative chemical determinations revealing variations in the percentages of the fats within wide extremes, form the scientific background of interest in this investigation. There are enormous economic interests involved on the Pacific coast in the catching of the king salmon and the conserving of the flesh as food. There is a well-known great variation in the grade of the commercial products derived from this fish. These facts add to the scientific interest in the problem, and emphasize the necessity of a study of the histological distribution and utilization of the fats in the king salmon, the largest and finest of all the members of the family of Salmonidæ.

At the time the king salmon leaves the ocean for the fresh water it has reached the crest of the life cycle as regards the amount of fat deposited in its tissue. In the Columbia River those salmon caught at the lowest point at the mouth of the river are the fattest of all. The salmon do not feed, i. e., they do not absorb new food materials, during their return passage in fresh water. There is no other adequate storage of food material than this fat. One may, therefore, safely assume with Miescher that the fat is the chief source not only of the fats that go to build up the ovaries but also of the energy liberated in muscular contractions during the migration journey. One may also assume as a working hypothesis that the fat will decrease in amount until the spawning is accomplished and the death ensues. I have followed the variation in chemical composition in the king salmon muscles, including the amount of fat at the different stages of the spawning migration, work that is represented by unpublished results obtained from chemical analytical determinations, and have also followed the microscopic distribution and variations of the fat in fat-stained muscular tissues. It is the results of this latter work to which attention is called in this paper.

MATERIAL AND METHODS OF INVESTIGATION.

The facts presented in this paper concerning the salmon fats have been determined by the microscope.[c] The chief reliance has been placed on the special methods for

[a] Greene, C. W.: Physiological studies of the chinook salmon, Bulletin of the U. S. Bureau of Fisheries, vol. XXIV, 1904, p. 429–456.

[b] Evermann, B. W.: A preliminary report upon salmon investigations in Idaho in 1894, Bulletin U. S. Fish Commission, vol. XV, 1895 (1896), p. 253. Also, A report upon salmon investigations in the headwaters of the Columbia River in the State of Idaho in 1895, together with notes upon the fishes observed in that State in 1894 and 1895, ibid., vol. XVI, 1896, p. 184.

[c] I wish to express my deep obligation to Mr. Thomas J. Heldt, instructor in anatomy, University of Missouri, and to Mr. George T. Kline, biological artist, University of Missouri, for their valuable assistance in the taking of samples and in the making of the numerous field observations on fresh materials, on the excellence of which much of the detail of this paper rests.

staining fats for microscopic determination. The particular technique used throughout the series is the Herxheimer method of staining with scarlet red as modified by Bell. This direct histochemical method has been supplemented by histological preparations fixed and sectioned in paraffin and treated with various differential stains.

Selection of salmon types.—The problem of fat variation in the salmon during the migration is twofold. First, there is variation in any one region of the body at different stages in the migration journey, and second, there is variation in the amount of fat present in different parts of the body at any one time. In order to determine the variations of the first class one must, of course, select typical localities representative of different stages in the journey. For this comparison I have used salmon from the Columbia River Basin, selecting four stations, the first one being at the mouth of the river, working from the town of Ilwaco; the second below the cascades, working at Warrendale; the third at Celilo Rapids and Celilo Falls, working from Seuferts; and the fourth at the spawning grounds on the Clackamas River at Cazadero.

There is a great variation in type among the individual fish present at any one station at any given time. It therefore becomes a matter requiring some considerable skill to select consistent types throughout a comparative series. Only after several years of experience can one select these types with some little assurance that he will be following similar salmon through the variations that occur at the different stations or stages in the migration journey. The attempt was made to secure fish just as early in the migration as possible, i. e., the stage at which the feeding ceases and the migration begins. In several years of collecting at Ilwaco at the mouth of the Columbia River, I have found that this very desirable stage can not be had within the fishing limits of the locality; in fact, the cessation of feeding must begin some considerable time before the fish reach the fishing zone. In order to secure the facts as to the normal fat loading, it was necessary to make a study of the feeding salmon at Monterey, where salmon that are known to reenter the Columbia Basin are caught during the feeding stage.

Salmon from the four stations on the Columbia mentioned above were collected during the months of August and September, 1911, 30 salmon in all being studied in detail. The stations were visited in the order in which they are named above. Invaluable aid and cooperation were constantly received from the local fishermen and from the packers at the various stations.[a] There is an undoubted seasonal variation in the condition of salmon on the Columbia River, but by a rapid collection of material from station to station the effects of the seasonal variations on the series ought to be reduced to a minimum. The finest salmon at Ilwaco, for example, as regards the amount of fat, are reported by the cannery men to come earlier in the season, though the fact has not yet been determined by scientific methods of measurement.

As a routine practice, salmon from each station were chosen of only two classes, the choice or best type from the packers' point of view, and the poorest type, as interpreted by the same standards. Only the choice sizes, ranging in length from 80 to 100 centimeters were selected in this series.

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[a] I am particularly indebted to the P. J. McGowan Co. for aid in securing salmon types, and for numerous material favors in the pursuit of the field work at Ilwaco and at Warrendale. The Warren Packing Co. and the Columbia River Packers' Association granted the use of their receiving house on the Ilwaco Dock for a laboratory at that station. Mr. Frank A. Seufert, of The Dalles, granted an unlimited supply of salmon from his Celilo fish wheels and the Tumwater seining grounds at Celilo Falls. The Cazadero observations were made at the station of the U. S. Bureau of Fisheries at that point on the Clackamas River, where the hatchery force rendered liberal and invaluable assistance.

Selection of tissue.—In order to follow the variations of fat in different regions of the body one must, of course, select typical tissues as regards their relations to fat. For this purpose I have chosen primarily the great lateral muscle, because it represents by far the greatest mass of the fat-storing tissue of the salmon. This muscle is divided into two types; the deep or pink muscle and the superficial or dark muscle. Each extends from the head and pectoral girdle to the base of the caudal fin. There is considerable variation in the amount of fat in different parts of these muscles. Two type regions of the lateral muscle have, therefore, been selected for study. The first is the mid region of the side of the body, in the transverse section which cuts the body just at the front margin of the dorsal fin. This with the muscle anterior to the section is the fattest portion of the great lateral muscle mass. Samples from this region are called trunk muscle, dark or pink, respectively, as the case may be. The second region of the great lateral muscle chosen is that portion at the base of the tail opposite the fifth and sixth caudal vertebræ, counting from the posterior end. This region is called the caudal muscle, dark or pink, according to its type. The caudal muscle always has a relatively less amount of fat than the trunk muscle. It is assumed that there is a more or less gradual variation from the caudal region to the mid lateral or trunk region as regards the percentage amount of fat.

While the primary comparisons are with reference to the two regions of the lateral muscle mentioned, still a considerable number of samples were taken from other muscles; namely, the anal fin muscles, the "belly" muscles, the intercostals, and the cheek muscles.

Fixation of tissues.—In view of Bell's[a] experience, it was thought better to make the examination of the fat in the salmon muscle in as fresh condition as possible, and a full Bardeen freezing microtome equipment was therefore taken into the collecting field. Samples of the tissues from the type localities were thus cut, stained, mounted, and studied with the minimum of delay. It was quickly found to be quite impossible, however, to carry through all the tissues in the fresh condition. There was not time enough, with a field force of three, to do the necessary work before disintegration began. It was therefore found necessary to use a fixative, i. e., 10 per cent formalin.

As a matter of routine practice, tissues that were to be held in the prolonged and tedious processes of working up the material were always fixed in 10 per cent formalin. This fixation was not found to be detrimental to the preservation of the fat, but on the other hand, favorable. The frozen sections cut after two hours or more in formalin were firmer, retained their shape better, and therefore were not so much torn and distorted in the process of handling necessary to staining. In short, the formalin-fixed sections enabled one to arrive at a better conception of the relationships of the fat than is to be had from the tissue cut directly from physiological saline. After a series of observations made along these lines it was decided to pass all the tissues through a formalin fixation. It is not claimed that a long immersion of tissues in formalin is wholly without effect on the fats, yet for salmon muscle we are convinced that the change produced is very slight. In certain samples sections of fresh tissues have been compared with sections made after four months' fixation in formalin. The difference in quantity of fat shown is not easily determined, but there is some variation

[a] Bell, E. T.: The staining of fats in epithelium and muscle fibers. Anatomical Record, vol. IV, p. 199. 1910.

in the picture—enough to prevent one from using any but freshly fixed tissues for close comparative estimates.

In taking samples, in all instances where possible the muscles were quickly cut from the salmon selected while the tissue was yet alive. The muscles were cut in thin pieces with a razor and immersed in a large excess of 10 per cent formalin. Other tissues, such as the liver, cæca, stomach, etc., in which fat studies were to be made were also included. Portions of the material not studied on the collecting grounds were preserved for future use, some of which still retains its fat in approximately the normal quantity and relations (after six months).

Portions of the muscles and of certain other tissues were fixed in different standard preservatives for future study and comparison. But as these studies are not presented in this paper the detail of fixation will be omitted.

Fat stain used and its preparation.—Sudan III and scarlet red are the fat stains on which great reliance is placed in the histological identification of the fats. Since the salmon tissues are so filled with fat it was decided to use the scarlet red as giving the sharper differentiation. Bell has made a series of special studies on the micro-chemical staining of fats. It is to him I am indebted for the modifications of the Herx-heimer method which were followed.

The scarlet red was prepared in saturated solution in alkaline alcohol: Scarlet red, 2 grams; sodium hydroxide, 2 grams; 70 per cent alcohol, 100 cubic centimeters. This was ripened in a 4-ounce wide-mouth vaseline bottle in a water bath at about 75° C, for from 20 to 30 minutes. The ripening took place in a closed bottle. But it was found essential not to heat too strongly lest the reactions lead to the production of a die that would stain general protoplasm. This stain is supersaturated while warm and some crystalization will occur on cooling. Bell's recommendation that the stain be filtered just before using was followed, also the precautions recommended by him against evap-oration and sedimentation while staining. This stain retains its properties very well for a very much longer time than one is led to expect from the directions published by Bell.

Technique of sectioning and staining fresh or formalin-fixed material for fats.—Either fresh tissue or tissue fixed in 10 per cent formalin was frozen with carbon dioxide on a Bardeen freezing microtome. The fixed material can be cut as thin as 25–30 μ with comparative ease, but the temperature of the block must be just right. The sec-tions were received from the microtome knife directly into 70 per cent alcohol and stained as quickly as possible.

The sections were handled always with a spatula of proper size. They were stained directly from 70 per cent alcohol. After 5 to 10 minutes in the scarlet red stain the sec-tions were passed as quickly as possible through 70 and 30 per cent alcohol into water. The rinsing was sometimes through the alkaline alcohol of the grade in which the stain was dissolved. This is a safer plan to prevent precipitation of the excess of stain from adhering to the surface of the sections.

When sections were to be counterstained a short washing in acid water, 0.2 per cent hydrochloric acid, preceded the hæmatoxylin counterstain. The acid treatment was finally adopted as a routine procedure for all sections.

The stained sections were mounted directly from the wash water into pure glycerin. The glycerine was of course slightly diluted by the adherent water. The glycerin mounts were sealed with paraffin, or better with paraffin-beeswax cement.

The splendid keeping qualities of many of the scarlet-red preparations made during the investigation is attributed to a thorough neutralization of the alkali in the bath of acid water.

TYPES OF SALMON MUSCULAR TISSUE AS REGARDS THE STORAGE OF FAT.

The musculature of the salmon[a] is relatively simple. The main mass consists of the great lateral muscle of Cuvier. A number of smaller muscles are associated with the various fins and with the structures in the head region. But the storage of fat takes place chiefly in the great lateral muscles.

Great trunk muscles.—The great lateral muscles include the major masses of muscular tissue along the sides of the body from the head and the pectoral girdle to the base of the tail. These masses represent from 60 to 70 per cent of the total weight of the mature fish when in prime condition.

The great lateral muscle of the salmon has lately been described.[b] In *Amiurus*[c] it is described as divided into five longitudinal portions more or less distinct. In the salmon there is a connective tissue septum, the lateral line septum, running the length of the body just under the lateral line. It separates the lateral muscle into a dorsal and a ventral portion, each of approximately the same size. Aside from this there is no further subdivision along the lines designated by McMurrich.

However, the great lateral muscle is divided into two distinct muscles on the basis of the well-marked anatomical and histological differentiations that have taken place. There is a superficial thinner portion which is anatomically distinct and easily identifiable because it is darker in color; and a deeper, wider, and thicker mass which is pink in color. These two divisions are well separated by a thin lamina of fibrous connective tissue bearing an excess of adipose tissue in most of its extent. The lateral line septum separates both the superficial and the deep muscles into dorsal and ventral divisions.

The fin muscles, and to a less extent the head muscles, are the ones in the most constant but slight activity in the daily life of the fish, and, strangely enough, whether for this reason or not, these muscles are not largely loaded with reserve fat.

Musculus lateralis superficialis, or dark lateral muscle.—The superficial dark muscle forms a type of tissue with respect to its loading of fat that has not previous to the preliminary notices of the present writer[d] been described, in so far as I can discover, though it was noted by Miescher.[e] He undoubtedly refers to the superficial lateral muscle when he says: "A thin muscle plate lying along the side of the body just beneath the skin degenerates most strikingly (Hautmuskel)." Miescher makes the reference quoted[e] in the brief discussion of the microscopic picture of the fat in the so-called fatty degeneration.

This muscle is characterized by the following points: (1) Its compact arrangement of fibers; (2) the smallness and uniformity of size of the fibers; (3) the relatively small

[a] It does not seem wise to regard the supracarinales and infracarinales as divisions of the lateral muscle in the ordinary sense of the designation.

[b] Greene, C. W. and Carl H.: The skeletal musculature of the king salmon. Bulletin U. S. Bureau of Fisheries, vol. XXXIII, 1913 (1914), p. 21-60, pl. I-II.

[c] McMurrich, J. P.: The myology of Amiurus catus. Proceedings of Canadian Institute, n. s., vol. II, 1884, p. 328.

[d] Greene, Charles W.; A new type of fat-storing muscle in the salmon, Oncorhynchus tschawytscha. The American Journal of Anatomy, vol. XIII, p. 175, 1912. Also an undescribed longitudinal differentiation of the great lateral muscle of the king salmon. Anatomical Record, vol. VII, p. 99, 1913.

[e] Miescher, op. cit., p. 186 (145).

amount of interstitial connective tissue; and (4) most important of all, by its enormous loading of fat.

Musculus lateralis profundus, the lateral pink muscle.—The profundus, or the deep division of the great lateral muscle, is the pink salmon muscle as it is ordinarily spoken of. It has a totally different appearance from the superficial dark muscle. The fibers of the pink muscle vary enormously in size, from 40 up to 250 μ in diameter in the adult. The average size of the fibers varies somewhat in different regions of the pink muscle even of an adult fish. In the young salmon, 10 to 15 cm. long, there is a greater range of variation than in the adult, as is shown by the outline figure 16, plate X. This is due to the fact that the fibers are undergoing longitudinal cleavage which is very unequal. This cleavage leads to a large number of very slender fibers. It is the method of reproduction of new fibers which leads to the irregular outlines noted in the cross sections of all the fibers of the profundus of the salmon, both young and adult.

The amount of supporting connective tissue in the pink muscle is relatively great. Beside the blood vessels, a large amount of adipose tissue is present. It is the adipose tissue of the pink muscle, which is heavily loaded with fat, that carries the greater part of the fat of the salmon commercial products.

Myocommata of the great lateral muscles.—The myocommata which separate the muscle myomeres are always crowded with fat in the normal adult salmon. These connective tissue partitions are composed of white fibrous connective tissue into which the short longitudinally placed muscle fibers are attached. The tissue is largely filled with adipose cells. Its fat cells are large and relatively uniform in size when filled. They form a considerable mass within the myocommata most thickly studded near its center. There are also large numbers of fat cells on the surface and crowded beneath the ends of the muscle fibers. This fat forms no inconsiderable portion of the storage fat present in the adult salmon.

Supracarinales.—There are longitudinal-paired muscles along the middle of the back of the salmon. These extend from the head to the dorsal fin, the supracarinales, and from the dorsal fin to the adipose fin and to the dorsal lobe of the caudal fin. These paired muscles are cylindrical in shape and about the size of a lead pencil in the thickest part, but spreading out somewhat anteriorly. The muscles are of interest in this connection chiefly because they are imbedded in a relatively thick and prominent mass of adipose tissue. In a prime fish this adipose tissue is crowded with fat.

Infracarinales.—A similar collection of adipose tissue is even more striking along the mid-line of the belly of the salmon. The fat of the belly surrounds the protractor ischii anteriorly and the retractors posteriorly. The mass is from 1 to 2 cm. thick and twice as wide in a prime 80 cm. fish. The cylindrical muscles inclosed will be about 0.8 to 1.5 cm. in diameter, and the rest of the area an almost solid mass of fat cells. In a fish low in fat the fat is taken up, leaving a white fibrous connective tissue mass. These two areas of adipose tissue form a considerable storehouse of salmon fat.

Muscles of the fins.—The storage of fats in the fin muscles has been studied only sufficiently to demonstrate the type. Adequate comparisons have not been made to make the observations complete, except in establishing the normal type. The fin muscles of the pectoral, ventral, dorsal, anal, and caudal fins (the deep caudal muscles) are much alike in general fat loading. Sections of the erectors and of the depressors of the anal fin have been most extensively examined. The two muscles are much alike in general appearance, are a light reddish-brown color—neither pink nor as dark as the superficial lateral muscle. The constituent fibers are loosely attached to the interhæmal septa, and the pair of muscles between adjacent interhæmal spines are incased each in a stout connective tissue sheath. This arrangement is also characteristic of the corresponding muscles of the dorsal fin.

Muscles of the head.—The masseter muscle is the largest of the muscles of the head. Under the name of "cheek muscle," the masseter is highly prized as a delicacy by the fishery folk. It is of good size, has its fibers compactly arranged, and is not colored like the trunk muscle of the salmon. The cheek muscle of feeding salmon has not been examined, but the Ilwaco and Cazadero types, representing the mouth of the Columbia River and the spawning grounds, respectively, are presented in the proper places. Other muscles of the head region have not been examined for fat.

NORMAL LOADING OF FATS IN THE MUSCLES OF THE KING SALMON AT THE TIME THE SPAWNING MIGRATION BEGINS.

It is exceedingly difficult to secure salmon just at the moment when fasting begins. In the first place, it is not easy to determine just when a salmon ceases feeding; that is, whether a given salmon in hand is one that is still feeding or one that has just ceased feeding. A second and more important difficulty is that of catching salmon at this critical stage in the life cycle. There are only limited regions where the king salmon are captured from the feeding grounds. There is no such place near the mouth of the Columbia River. The lowest point at the mouth of the Columbia where fish are caught is between the Canby Lighthouse on the north bank and the end of the Government jetty on the south shore. Salmon from this locality have already ceased feeding, probably some little time earlier.

Monterey Bay and its immediate vicinity is a popular ground for king salmon fishing. When the salmon schools are in this vicinity they are actively feeding and are readily caught with the trawl. During the spring and early summer months they are taken in large numbers. At this time a considerable business is done in the salmon fisheries at the city of Monterey. There is good evidence that the fish caught at Monterey Bay are from schools which ultimately enter both the Sacramento River basin and the Columbia River basin. This is indicated by the fact that on both rivers specimens are occasionally taken which have in their mouths fish hooks of the type used at Monterey.[a]

Monterey Bay is about 100 miles south of Golden Gate, the entrance to the Sacramento Basin. It is about 800 miles south of the mouth of the Columbia River. A well-developed salmon from Monterey would serve very well as a normal type for the Sacramento Basin, provided one could assure himself that the fish were on its way to and would enter the Golden Gate and the Sacramento. The amount of change that could take place due to additional feeding between Monterey Bay and the Golden Gate would be negligible, assuming a reasonably direct journey. If additional fat were stored it would be too slight to change the average materially. But facts tending to verify these assumptions can not readily be obtained.

[a] Mr. George Warren, of the Warren Packing Co. of Portland, Oreg., showed me a number of such salmon hooks taken from salmon that have come into their packing establishments on the Columbia River.

It is out of the question to assume that a Monterey fish destined to migrate as far north as the mouth of the Columbia River is in the condition that will exist at the time of arrival. Such a salmon is in the growing stage. It will certainly increase in size in so long a feeding journey and probably will also somewhat increase its fat content.

The Monterey fish are the only ocean-feeding fish available as examples of mature specimens typical of the Sacramento and to a certain extent of the Columbia Basins. It follows, therefore, that one must use these specimens as best he can for the purpose. The matter resolves itself, somewhat, into a question of the ability of the investigator to select and judge the type that most nearly approaches the mature one.[a] There is a wide extreme of maturity represented among the Monterey fish. The small fish give all evidences of being relatively young and growing specimens. The larger fish are proven to be the older ones by the work of Gilbert, who finds a close correlation between size and age.[b] His determinations indicate that these larger fish have been feeding in the ocean for four, five, or even more years, according to the size of the specimen chosen. It seems reasonable to assume that such fish will not undergo any very great change in the average fat content during the intervening months between the time of the Monterey feeding and the beginning of the spawning migration. The larger Monterey fish may be taken as the best available examples typical of the disposal of the tissue fat in the late stages of the feeding cycle. On this ground observations and protocols are presented on Monterey specimens.

In the chapter on the types of salmon muscular tissue as regards the storage of fat the muscle characteristics are given in sufficient length to enable one to use them in presenting the picture of the fats and fat variations. It remains now to give the detailed picture of the normal fat content of salmon muscle at the time when the feeding ceases. Under this category will be presented the following muscle types:

Normal fat content of the trunk pink muscle.—The pink muscle, which represents the greater proportion of the total mass of muscle of the salmon, contains an enormous total load of fat at the time the salmon cease feeding. Estimating on the basis of various chemical studies made in other connections, I would say that this fat loading varies between 15 and 25 per cent. This great variation represents the normal variation in fat content.

The fats of the pink muscle are distributed in the connective tissue between the muscle fibers—i. e., they are intermuscular. The pink muscle carries a relatively large amount of connective tissue which supports the muscle fibers and the blood vessels, and this connective tissue has a high percentage of adipose tissue. In it are found enormous numbers of fat droplets, which vary within a wide range of size. The smallest droplets are often not more than 1 or 2 μ in diameter, but there are numerous fat globules of this region that are as much as 100 μ in diameter. No figure is presented of this normal material, but figure 8, plate VI, drawn from an Ilwaco specimen (no. 118), represents very well the average appearance of the intermuscular fat of the normal tissue.

[a] The alternative is to figure back from the first available stage in the spawning migration. For the Columbia River this latter method I believe gives a truer picture of the normal condition. Attention will be called to this fact in the discussion of Ilwaco types.

[b] Prof. Chas. H. Gilbert, who is making extensive studies on the salmon migration and the salmon age, observes that there is, within certain limits, a close correspondence between size and age. It follows that the larger fish have a longer ocean-feeding period, a fact for which we have heretofore had no conclusive proof. Also it is evident that salmon mature sexually at greatly varying ages. (Gilbert, C. H.: Age at maturity of the Pacific coast salmon of the genus Oncorhynchus, Bulletin of the U. S. Bureau of Fisheries, Vol. XXXII, 1912, p. 1.)

There is little or no intramuscular fat in the normal pink muscle. The loading of the fat is intermuscular, in contrast with that in the dark muscle, where it is intramuscular. In the normal feeding, growing salmon there is no intramuscular fat, or at most only a trace of fat in the pink muscle. This condition exists up to the time when the salmon cease to feed. This statement is based on the examination of tissues of the smaller salmon in the rivers and also on the examination of different sizes, including the largest adults coming into the market at Monterey, Cal. In the latter there may be an occasional trace of liposomes within the smallest fibers. Monterey fish that will enter the Sacramento River basin can not be assumed to be wholly typical salmon that have ceased feeding. Yet I think it safe to consider these as sufficiently mature adults to serve for comparative purposes.

In the quite young salmon, from 7 to 16 cm. long, there is no fat in the pink muscle, either between the fibers or in the fibers along the lateral portion of the body. In the ventral or "belly" muscle there is some intermuscular fat at this stage of development. Salmon of this size are still feeding in fresh water. Of the sizes that one obtains at Monterey, which of course are feeding in salt water, fat is beginning to be deposited in the connective tissue between the fibers. This fat is relatively low in amount in the smaller fish. There is great variation in its amount in different individual fishes at Monterey, and while the number of fishes studied is very limited one can say that these indicate that the fat increases in quantity with the size of the fish.

An exception to the above description is found in a narrow zone of pink fibers lying on the surface of the pink muscle. This zone is immediately covered by the deeper layer of the dark muscle fibers. In these pink fibers there is always a slight amount of intracellular fat. This is a special case, the significance of which will be discussed in the chapter on the mechanism of fat transference in the salmon body. (Page 127.)

Normal fat content of the trunk dark muscle.—The trunk dark muscle is described on page 78 as characterized by an enormous loading of fat.

The storage fat is both inter- and intramuscular. It is present between the fibers in a relatively small number of medium-sized drops. These drops vary in size in the adult salmon from 5 to 20 μ in diameter, and are sometimes larger. This fat is most abundant in the immediate neighborhood of blood vessels. In longitudinal preparations it is seen not to be uniformly distributed along the length of the fibers.

The peculiar characteristic of the superficial lateral or dark muscle is its storage of enormous quantities of intramuscular fat. The fat is distributed within the fiber in two general relations. First, in the region between the sarcolemma and the substance of the muscle fiber proper, especially in the young fish (fig. 7, pl. v). It often happens that there is almost a complete ring of fat droplets surrounding the fiber and pushing the sarcolemma out and away from the fiber wall. In a paraffin preparation there will be a series of vacuoles under the sarcolemma, where the fat is extracted. Sometimes these fat drops have grown so large that they have fused or run together into larger masses of fat, but usually the droplets are smaller and remain separated. In the maximum loading these droplets are from 4 to 6 μ in diameter. This sarcolemmal fat is not uniformly present in all regions of the muscle, and in regions where it is absent the sarcolemma is in close approximation to the outer wall of the muscle fiber as usual.

Second, the intramuscular fat is present in large quantities buried within the substance of the sarcoplasm. Especially favorable points for deposit are the angles formed by

Cohnheim's areas. In these locations very large drops, comparatively speaking, are present. They are usually quite uniformly distributed over the surface of the fiber as seen in a cross section. The intracellular droplets vary in diameter from 3 to 6 μ. Beside the larger droplets there are always numerous smaller ones of varying sizes down to a fraction of a micron. Medium to small droplets may be present in close relation to the larger, all more or less evenly distributed among the larger droplets. The smallest droplets are of liposomic size and are deposited in shorter or longer chains between the fibrillæ or groups of fibrillæ. There is evidence that these liposomes[a] are arranged with reference to the striations of the fibrillæ, and it is suggested that such relation is of significance in reference to the function of the fats in the muscle.

Occasionally I have found an enormous fat drop filling up the whole central portion of the dark muscle fiber, the protoplasm of the fiber forming a band-like ring around the drop (fig. 14, pl. IX). Even in these cases the protoplasmic ring is closely studded with smaller fat droplets, in one case as many as 19 droplets 2 to 4 μ in diameter being crowded within the circumference of this protoplasmic ring.

The superficial lateral muscle begins receiving its excess of fat early in the development of the fish, at least as early as the fingerling stage. In this respect the muscle is in marked contrast to the deep lateral muscle, in which there is little or no deposit of intermuscular fat until a considerably later stage in the development of the salmon and no intramuscular fat until maturity.

The main points which characterize the fat disposal in the normal adult trunk muscles may be summarized as follows:

Summary of fat disposal in the normal muscular tissues.—1. The fat is most heavily stored in the superficial lateral muscle, where it is present in enormous quantity both between the muscle fibers and within the fibers. This tissue is heavily loaded with fat at a very early stage, at least by the 7-centimeter stage, and is always found loaded in the feeding fish.

2. The great pink muscle contains little or no fat between the fibers in the fingerling stage, but it has a small amount of such fat in the small Monterey Bay fish. The amount of this intercellular fat increases to its maximum at the time when feeding ceases. The intermuscular fat observed at Ilwaco is relatively high. While it is probably less than at the time of cessation of feeding, it is certainly more than at Monterey Bay.

3. There is no intracellular fat in the pink muscle during the feeding stage, or, at most, a trace of liposomes in the smallest pink fibers. The liposomic fat makes its appearance after the fast begins. An exception is found in the superficial zone next the dark muscle.

4. The fat in the fin and the head muscles is relatively insignificant in amount. It is both inter- and intracellular in its relations to the muscle fibers.

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[a] Various terms have been used to designate the microscopic fat droplets or fat-like droplets. They were first described by Kölliker as interstitial granules. This was before their fatty nature was sufficiently well known. In fact, Kölliker thought they were not true fat droplets. The term liposome was introduced by Albrecht to describe those interstitial granules of muscle which are demonstrated by the scarlet red stain. Bell has used the term "interstitial granules," but he considers the granules that take the scarlet red stain as used in his paper as fat bodies to which the term "liposomes" is applicable. (For historical discussion of the subject see Bell, Internationale Monatsschrift für Anatomie und Physiologie, bd. XXVIII, p. 297; also Anatomical Record, vol. 4, p. 199.) The term liposome is used in the present report to indicate the microscopic fatty bodies staining with scarlet red and of small size, usually under 3 μ, that take the characteristic scarlet red stain. It is not intended to carry any meaning suggestive of the chemical character as regards the specific kind of fat, though it is the opinion of the writer that neutral fats are the ones dealt with in the salmon tissue described in this paper.

5. There is a considerable store of adipose fat in the myocommata, in the adipose tissue around the small longitudinal muscles in the mid-dorsal and mid-ventral lines, and in the connective tissue of the skin. A slight amount of fat in the viscera should be mentioned.

PROTOCOLS.

Male salmon (no. 97), length 25.7 cm., taken at Baird, Cal., July 18, 1911.

This young salmon was caught with hook and line with salmon-egg bait from the deep pool opposite the fish hatchery at Baird, Cal., July 18, 1911. It was a relatively large summer fish derived from the last fall's hatch, as shown by the scale marks kindly identified for me by Prof. Charles H. Gilbert. It was kept alive in a fish can for seven days, after which it was killed and examined for fat. On examination it was found that the testes were well developed, almost mature, and white in appearance. Specimens of the alimentary tract and of the musculature were fixed in formalin for fat staining. Also samples were preserved for paraffin sectioning.

Microscopic examination of the trunk muscle for stainable fat.—Samples of the lateral muscle preserved in 10 per cent formalin were prepared after five months. Freezing microtome sections were stained for fat with alkaline alcoholic scarlet red and counterstained with hæmatoxylin. The fat was present in the trunk dark muscle in large amounts and had not been extracted in any appreciable amount by the long immersion in formalin. As the glycerin mounts were beginning to clear there was a stage of very sharp and distinct differentiation. The fat droplets in the body of the muscle were surrounded or at least partially surrounded with rings of fibrillæ.[a] The clear, brilliant scarlet red of the fat droplets contrasted sharply with the palisade-like bands or rings of fibrillæ. The sarcolemma at this stage of clearing made a clearly marked line inclosing fat droplets between it and the fibrillar areas. These latter fat drops are distinctly outside the areas of fibrillæ, yet some of them press slightly into the interfibrillar spaces. There is not much of this displacement of fibrillæ for the reason that the superficial area of the salmon muscle fiber is bounded by a continuous band of fibrillæ.

The fibrillæ are strap-shaped, i. e., their outlines in cross section are rod-shaped. The fibrillæ are set with their flat sides approximating each other and their narrow edges, therefore, bordering on the surface of the fiber in the case of the superficial area. It is this that gives the palisade-like arrangement in the superficial coat of the muscle. The continuity of the superficial band is occasionally slightly interrupted, since the rows of fibrillæ as seen in cross section here and there turn in toward the central portion of the fiber. Where such turns come there is a slightly greater quantity of sarcoplasm present.

Those dark muscle fibers nearest the skin seem more loaded with fat, although the whole layer is rather uniform in its loading. The striking thing about the material from this fish is the amount of fat which is under the sarcolemma. In general, the fat droplets in this region are fairly uniform in size and are spherical. But often a mass of fat seems compressed and spreads somewhat around the surface of the fiber. Numerous instances are seen in which such masses of fat extend around one-sixth to one-third the circumference of the fiber. If one gets a view of such a fiber isolated from the mass this type of fat droplet or group of droplets stands out like a great blister on the side of the fiber. These droplets are evidently compressed by the pressure of the sarcolemma. They no doubt exist within that sheath under a certain amount of tension.

The fat droplets within the substance of the fiber vary extremely in size and shape; the average of the larger drops is about 4.5 to 6 μ.

Through a typical section four striking variations from the general picture appear. In each of these an enormous fat drop has formed in the center of the muscle fiber. One of these fat drops measures 18 μ in diameter, while the fiber containing it measures 33 μ in diameter. The thinnest part of the muscular ring is 4 μ and the thickest 8 μ. Evidently in this instance an enormous fat drop has formed in the center of the fiber and crowded out the muscular substance into a superficial ring of protoplasm. In this case the ring of protoplasm is filled as full of fat drops as plums in a pudding. There are thirteen such droplets from 2 to 4 μ in thickness. There is not so much fat as usual between the sarcolemma and the muscle substance. Four such fat drops are to be counted in one locality. In another region of the section a fat cavity in the fiber measures 24 μ in thickness. The ring of protoplasm around it is not so thick as in the preceding instance, and the fat drop itself has been pushed to one side, though it is still adherent to the section. Smaller drops of fat are present in the ring of protoplasm. In yet another

[a] Greene, C. W.: A new type of fat-storing muscle in the salmon, Oncorhynchus tschawytscha. American Journal of Anatomy, vol. 13, 1912, fig. 1, pl. 1.

region are two adjacent fibers, each containing an extra large fat drop in the center of the substance of the fiber. One of these drops measures 15 μ in diameter, the other 20 μ in diameter. Here, also, the surrounding muscle substance is loaded down with the usual type of small fat droplets. One can not assume that the large drops of fat arise at the expense of formation of the smaller. Rather is it indicated that these drops are the result of a most active fat storing in this fish at the time it was collected.

The trunk pink muscle is free of fat in the main body of the muscle. There was neither fat between the fibers nor within the fibers. This statement does not hold for a thin layer of pink muscle lying just under the dark muscle. In this intermediate zone the pink muscles show a certain amount of intracellular fat. These fat droplets are never as great in size as in the dark muscle, but are largest in those fibers lying near the dark muscle layer. Passing from fiber to fiber in the direction away from the dark muscle, the amount of intramuscular fat rapidly decreases. This zone is, on an average, only five or six fibers thick. It underlies the whole extent of the thicker portion of the dark muscle.

Microscopic examination of paraffin sections.—These transverse sections were especially fine as giving a negative picture of the fat in the musculature. The sections show a thin membrane or sheath around the dark fibers, the sarcolemma. The interest attaches to the location and relations of this sarcolemma with reference to the substance of the fiber. The sarcolemma is in contact with the sarcoplasm for a portion of its extent round the fibers, but is distinctly separated from it in most of its circumference. The picture is as if the membrane were pushed out and away from the fiber. The space between the sarcolemma and the proper substance of the muscle is subdivided by very delicate strands extending across the intervening space and continuous with the interfibrillar substance. The form of the spaces, their size, and arrangement, all strongly support the interpretation that these spaces are filled with fat in the fresh condition. They are the cavities left when the fat drops are dissolved out, the fat that in the frozen section is so much more difficult to determine as regards its exact relation to the sarcolemmal sheath (fig. 7, pl. v).

The central portion of the muscle fiber presents numerous clear areas around which the fibrillæ are arranged in irregular circles. Where such a group of fibrillæ is unbroken, they usually stand with their broad dimension radial to the center of the clear area. However, there is no particular uniformity about the matter. This arrangement is best shown in figure 7, which is a camera-lucida outline under an oil-immersion lens. The larger angles formed in these whirls of fibrillæ are more or less filled with irregularly arranged and smaller fibrillæ. Between the fibers and forming a slight border along the rows of fibrillæ is the sarcoplasm. In most instances this sarcoplasm is sufficient in quantity to form a very thin sheet surrounding the clear areas already mentioned. The sarcoplasm can usually be distinguished as an extremely thin sheet around the most superficial fibrillæ. It is connected by delicate strands here and there with the sarcolemma.

The pink trunk muscle of these sections exhibits the great variation in size of fibers noted in the frozen sections. The ends of the fibrillæ are very distinct and clear. They are not broad and strap-shaped, as in the dark muscle, but are more thread-like and smaller. In the deeper portion of the pink muscle there is no evidence of interfibrillar spaces. In the intermediate zone of pink fibers, located just under the dark muscle, the fibers are more or less marked by clear spaces. These areas are relatively large and more numerous in the pink fibers lying nearest the dark and decrease in number and size in those fibers further away. Some of the pink fibers show irregular groups of small spaces just under the sarcolemma. In the smaller pink fibers the spaces are more numerous in the center of the fiber. The arrangement of the transparent spaces within the fibers and between the fibrillar portion of the muscle and the sarcolemma corresponds with the distribution of the fat droplets in the fibers of the intermediate zone, as shown by the scarlet-red staining.

Salmon (no. 75 and no. 76) collected at Monterey July 24, 1911, length 100 cm. (estimated).

Microscopic observation of the trunk pink muscle transverse section, oil-immersion lens.—The material was studied after three days' fixation in formalin. The striking picture is that of the intermuscular fat, which is present in large quantity. The fat drops vary in size from 3 μ up to 45 μ in diameter, the smaller drops being very numerous. The fat is far greater in amount than in the young specimen (no. 97) from Baird, on the McCloud River.

This section is well fixed by its three days' immersion in formalin. It shows a splendid picture of the fibrillar structure. The muscle fibers are without intracellular fat, or, at best, have only a trace. The large and most of the medium-sized fibers are perfectly free of fat. There are a few of the smaller fibers and occasionally a medium-sized one which show a trace of fat around the superficial ring of protoplasm. Such fibers are surrounded with fat droplets massed on the surface of the fibers in the connective tissue. Some droplets are undoubtedly under the sarcolemma. It is this fat which gives the show of color at the superficial coat of fibrillæ. In the smallest fibers of the section some scattered liposomes of minute size are present between the fibrillæ of the surface of the fiber.

A longitudinal section of pink-trunk muscle (slide H81) shows numerous fat droplets of comparatively small size adherent to the surface of the fibers. The sarcoplasm shows the striations in splendid contrast, but no liposomes were to be found within the fiber.

The intermediate zone of pink fibers.—The line of separation between the pink and the dark trunk muscle is marked by a connective tissue septum. Occasionally a microscopic group of small fibers may be found on the dark-muscle side of the septum (sec. H82). These intermediate pink-muscle fibers have in their protoplasm a few liposomes, which are limited to the small fibers. There is not so broad a zone of intermediate fibers as was noted in the young muscle—for example, protocol no. 97. Well out in the field of pink fibers of section H82 there is an abundance of intercellular fat, but no evidence of intracellular fat.

Notwithstanding these exceptions, the general picture is that of muscle free from intracellular fat.

Microscopic examination of the trunk dark muscle of fish no. 75 (section H70).—Observation with one-twelfth oil immersion. The section shows an abundance of fat both between the fibers and within the fibers. The fat between the fibers is in droplets from 6 to 10 μ in diameter. The muscle fibers themselves are only from 25 to 50 μ in diameter and somewhat irregular in outline. The fat droplets are rather uniformly distributed among these fibers, though not so great in amount as in the same type of muscle from Ilwaco.

The intramuscular fat is present in large amount and very uniformly distributed through the protoplasm of the fibers. The droplets, strictly within the fiber, vary around 4 μ in diameter.

It is difficult to determine whether the fat droplets around the superficial zone are under the sarcolemma, and therefore intracellular, or lie outside this membrane. Certainly in a number of cases the former is the fact. In comparison with the dark muscle of the younger fish it is noted that the intracellular droplets of the Monterey muscle are more uniformly distributed through the protoplasm and have a more uniform size.

The intermuscular fat of the dark muscle of fish no. 76 (sec. H73) is in relatively large drops, 30 μ on an average in diameter; but there is only a small proportion of the intercellular fat present in the finer droplets. The intracellular fat is rather uniformly distributed through the sarcoplasm; but the droplets are smaller than in fish no. 75, 2 μ, or slightly larger, in diameter.

VARIATION OF THE AMOUNT OF FAT IN THE SALMON DURING THE SPAWNING MIGRATION.

It is to be expected that the amount of fat present in different portions of the musculature of the salmon will vary sharply at different times during the migration. Whether this variation will be directly proportional to the time since the migration began remains to be discovered. The attempt in this paper is to present the normal distribution of fats at the end of the feeding period—i. e., the beginning of the migration phase—and to follow the variations through four typical regions of the Columbia River Basin. The regions chosen represent (1) the tidewater stage of the migration, (2) an early intermediate stage in the migration, (3) a later intermediate stage, and (4) the condition at the spawning ground and at the time of death.

As representing an early stage I have chosen a station at Ilwaco on the Washington side of the mouth of the Columbia River. At this point P. J. McGowan & Sons have a canning establishment, the lowest on the river. It is in the midst of the Bakers Bay field of traps and is the most accessible point to the lowest channel fishing done on the Columbia River.

For the second stage Warrendale, Oreg., about 6 miles below the cascades in the canyon of the Columbia, was chosen. The upriver cannery of P. J. McGowan & Sons is located here. The region is accessible to fisheries which depend upon the catch of salmon below the cascades of the Columbia. The samples of this series were chosen from the fisheries at the seining grounds on the Washington side about 1½ miles below Warrendale.

The third stage was chosen at the Frank A. Seufert's fishery at The Dalles, on the Columbia. The fish wheels and seining grounds along the course of the Columbia below the Celilo Falls furnish splendid opportunity for salmon which have run the lower mountain course of the Columbia River through the cascades and through the lower portion of the rapids of The Dalles.

The spawning-ground stage was that on the Clackamas River, Cazadero, Oreg. A United States fishery is located here. This is the most accessible, in fact, the only point where spawning salmon can be had during the time of the year in which the field work was done.

DISTRIBUTION OF THE FATS OF THE SALMON MUSCLE AT TIDEWATER.

At the mouth of the Columbia River the salmon have already ceased feeding and the muscles have begun to show the first stages of change in the amount and distribution of the fats. This change is readily detected in the pink muscle, though not so in the dark muscle. In the dark muscle the amount of fat is so great that one has no adequate microscopic comparisons for showing the variations. But it is easy to convince one on general comparisons that the storage of fat is even as great in amount as when the salmon first cease to feed, as they do at some considerable time before this locality is reached in the migration journey.

Trunk pink muscle.—In the trunk pink muscle the most striking change consists in the fact of the appearance of intramuscular fat not noted previous to this stage. This seems to be one of the first histological evidences of the cessation of feeding. At this time the central core of the pink muscle fibers, and especially of the smaller fibers, is dotted through with extremely small fat droplets. These fat droplets are rarely as much as 2 μ in diameter, usually not more than 1 μ, and from this size down to droplets so small as to be scarcely visible by the 1/12 oil immersion. All evidence that I have points to the fact that this microscopic salmon fat reacts uniformly to the Herxheimer stain whether the droplets be large or small. The pink muscle fat at this stage is quite evenly distributed through the cross section of a fiber except in the outer circle of fibrillæ. In this circle there is no intramuscular fat. This gives the fibers the appearance of having a clear surface border as distinguished from the inner portion of the fiber, which is of course slightly pink from the presence of stained fat. At this stage I can distinguish a few small and scattered fat droplets between the sarcolemma and the muscle substance. The intramuscular liposomes are largest in the smallest pink fibers, usually from two to three times greater in diameter on the average than in the very large fibers.

The trunk pink fibers show the details of liposome arrangement best in teased preparations. The liposomes are in short chains consisting of a few individual droplets in each. At this stage the liposomes in the middle of the chain are largest and they decrease quite uniformly from the middle toward each end. These chains are loaded in the interfibrillar spaces. They are present only in certain, not all, spaces between groups of fibrillæ. The number of such spaces occupied by the chains of liposomes, therefore the relative number of liposomes, varies in preparations from different individual salmon. The amount of fat in the pink muscle fibers is measured therefore by two microscopic factors; first, the number of chains in a given mass of fiber; second, the size of the individual liposomes in the chains.

The pink muscle fibers vary within a wide range of size of fiber, from 25 to 250 μ in diameter. This variation is illustrated in the figure 17, plate X. In the larger fibers of the Ilwaco fish (notably no. 111 and no. 118, the latter of which is figured in figs. 8 and 9, pl. VI) the chains of liposomes are quite evenly distributed throughout the mass of the fiber. However, they are characterized by the relatively small number of liposomes in the chains and the comparatively small size of the liposomes. In the large fibers of no. 118 the largest liposomes in the centers of the chains are about 0.5 μ in diameter and the smallest ones which form the ends of the chains are just identifiable with the oil immersion. In the smaller fibers of this same fish the liposomic chains are somewhat larger, the largest liposomes in the chains about double the diameter of the largest in the large fibers. The liposomes in the small fibers are more thickly distributed around the central core of the fiber. This variation is not noted in the relatively large mass of the fibers whose diameters run over 200 μ.

Fish no. 117 seems an exception to the group from the Ilwaco station. It is certainly very far below the average of the other specimens as regards the amount of fat revealed by the microscope. Reference to the protocol will show that this fish came from a trap some little distance up Bakers Bay. The whole appearance of the salmon, both its gross appearance and the microscopic appearance, suggests the type of fish characterized by a certain degree of retrogression. The weight is much below the standard for the length, as much below the average as certain farther advanced salmon taken from stations higher up the river. These comparisons lead to the deduction that salmon no. 117 has been in fresh water some time. Although it has not gone up the river, the probability is that it has undergone as much migratory change in fats as specimens that have gone farther up the river. The chemical quantitative determination of the fats abundantly confirms the above deductions. (See page 92.)

In this salmon the amount of intermuscular fat in the trunk pink muscle is very much reduced. The number of fat drops is less and the size smaller. The intramuscular fat is present in all of the trunk pink, but the number of liposomic chains and the size of the liposomes themselves is reduced. In the very largest fibers there is almost no liposomic fat. Another point associated with the amount of fat is the decrease in the intermuscular spaces, so that the fibers themselves seem more compact in arrangement.

Caudal pink muscle.—The pink muscle from the caudal peduncle in each Ilwaco specimen examined has a strikingly smaller quantity of intermuscular fat than the muscle from the middle of the body of the same animal. It would seem from the Ilwaco fish that the intermuscular fat is never laid down in the caudal region in as great quantity as in the lateral or trunk region of the body. The fat drops are relatively smaller and, in general, fewer in number than from the fatter region of the body. The intramuscular fat of the caudal pink muscle in the specimens from the Ilwaco station is less than in the trunk pink muscle. Those conditions which at the beginning of the migration lead to an infiltration of fat into the muscle cells do not result in as great a deposit in the caudal pink muscle as in the trunk fibers.

In the fattest fish the caudal pink muscle is characterized by the smaller size of the liposomes; also by a smaller number of liposomes in the center of the fiber. This gives the impression of a somewhat greater quantity of fats around the superficial layer of the fiber.

In those Ilwaco fish which have less fat than the average, for example, no. 117, the amount of fat in the caudal pink muscle is very strikingly less than in the middle of the body. In this particular fish the fat between the fibers is very noticeably less in quantity; in fact, it is practically absent except in those areas which have a relatively large amount of connective tissue. The caudal intramuscular fat of no. 117 has almost disappeared, or at least is present in extremely small amount.

The caudal pink muscle also shows that fish no. 117 has already passed well into the retrogressive stage which comes with the migration fast, an indication noted in connection with the discussion of the trunk pink muscle. This is apparent from the character and arrangement, particularly the arrangement, of the fat in the connective tissue, as well as in the spaces between the muscle fibers. It is certainly true that the amount of fat present sharply increases as one proceeds through successive segments from the caudal peduncle to the mid-lateral region of the body. Light on the significance of the above observations is had by considering the condition of the fat in the dark muscle of the two regions.

Trunk dark muscle.—The dark muscle forms a distinct type of muscle, as previously announced. In this case the fat has been loaded into the muscle in enormous quantities, both intermuscular and intramuscular. At the Ilwaco station the amount of fat in the dark muscle is enormous, as illustrated by fishes no. 111, 113, 115, 116, and 118, in all of which the fat deposited in the dark muscle has reached its maximum.

The intermuscular fat is relatively much less than in the pink muscle. This is due among other things to a structural factor. The muscle fibers are very compactly arranged, forming a much denser mass than is formed by the pink fibers. The interstitial connective tissue is correspondingly reduced in mass, hence there is not so much fat carried. On the other hand, the muscle substance has received so great a deposit of intramuscular fat that one must regard this muscle as a definite fat depot. Attention has already been called to the fact that deposit in this muscle begins in embryonic life. It increases in amount up to the time of the cessation of feeding and, we assume, has not appreciably changed when the good-conditioned fish reach Ilwaco. The trunk dark muscle contains so much fat in the muscle substance that one can not make adequate comparisons showing slight variations.

When this tissue is examined in teased preparations, so that a side view is had of an individual fiber, it is found that the fat droplets are so large and so numerous that the fibers are difficult to distinguish as individuals. This is shown in figure 1, plate III, where a transparency is figured of a fiber from fish no. 115. Often in the examination of these teased fibers one notes elongated fat drops or rods. These have formed in the interfibrillar spaces owing to the fact that the droplets have increased so much in size that adjacent ones have run together, thus fusing into the mass noted.

Caudal dark muscle.—The superficial or dark muscle from the caudal region in all these Ilwaco specimens has a very considerably less amount of fat than the corresponding muscle from the lateral region. Even fish no. 113, which is as fat as any in the series, presents a sharp contrast as regards the comparison of the amount of fat in the caudal dark and in the trunk dark muscle. The size of the intermuscular fat droplets has sharply decreased, though the number of droplets is as great or even greater than in the lateral region.

The sharpest contrast lies in the intramuscular fat. In the caudal region this fat is very markedly less, especially in the size of the larger droplets. Even in the fatter fish the larger droplets seem to be congregated around the superficial border of the fibers. The superficial fat droplets are under the sarcolemma, though this fact is often very difficult to determine. There is a large supply of the finer fat droplets and liposomes scattered through the protoplasm of the caudal muscle substance. The contrast between this and the fatter regions is not so much a matter of the number of the liposomes as in the size and arrangement, especially of the larger droplets. The largest droplets in the caudal muscle will not average more than one-half as great in diameter as in the trunk muscle.

In a salmon like no. 117, which is poor in the general amount of fat of the body, the caudal dark muscle presents the sharpest contrast in comparison with the standard of this station. Under the low magnification, sections of the caudal dark show that in the regions bordering along the blood vessels there are areas which by contrast with other portions of the section are relatively free of fat. These areas are faded. This is a condition undoubtedly indicative of the removal of stored fat. The retrogressive process has already gone so far that one can distinguish the regions in which the active process of fat resorption is going on with most vigor. This is the first clear-cut picture showing the process of fat resorption. The appearance of the section is exactly the reverse of that shown for the dark muscle in the growing stage, also of that in certain pathological processes wherein fat is being very rapidly laid down.[a] In discussing later stages it is argued that these contrasts are due to the irregularity of resorption of the fat from the tissue. In other words, the fat is being taken up from the tissues and transported to other parts of the body, to be utilized by the body in the construction of new tissues (egg yolk, etc.) or in the production of energy. This movement is best facilitated in the neighborhood of the small blood vessels, and is expressed microscopically by these contrasts in fat content. These facts are in further confirmation of the deduction that fish no. 117 has been for some time on the migration phase of its life cycle.

Fat in the fin muscles.—A few examinations were made of the small muscles of the fins at the Ilwaco station. The samples selected were the pairs of erector and depressor muscles located in a single interspace between two interhemal spines. These muscles are made up of fibers rather loosely bound together. There is a small amount of interfibrous connective tissue with a tolerably thick sheath around each muscle slip. The fibers themselves are of a type somewhat like the cheek muscle of the head.

The intermuscular fat is present in droplets of good size, but not in very large numbers. In the connective tissue sheaths around the muscles the amount of fat corresponds more nearly to that of the myocommata of the lateral muscle. In general, the amount of intermuscular fat is considerably lower than that of the pink lateral muscle of the same salmon.

[a] My colleague, Dr. W. J. Calvert, tells me that in his unpublished work on the plague he often noted a striking deposit of fat in the parenchyma along the immediate border of the blood vessels of the liver. This deposit in the early stages of the disease extends out only a short distance into the parenchyma of the liver. The course of the smaller blood vessels is easily followed through the parenchymatous tissue by the bordering deposit of fat. This is, of course, the reverse picture of that described above. In the salmon the fat is in process of removal; in the plague liver the fat is in process of rapid deposit, but in each case the histological picture is that of the early, therefore differential, stage in the process.

The intramuscular fat is relatively small in amount. Many of the fibers show liposomes of extremely fine size, often so small that one can trace them with difficulty. There are no rings of fat droplets under the sarcolemma such as characterize muscle that is beginning to show fat exhaustion. There are certain groups of fibers in these sections which show a relatively larger amount of intracellular fat. In such fibers the liposomes will average as much as 2 μ in diameter. The liposomes are quite evenly distributed through the cross section of the fiber and are occasionally quite numerous under the sarcolemma. This latter type of fiber is suggestive of the dark muscle type. Not enough comparative work has been done in studying these muscles to determine whether or not the dark muscle fibers are present in portions of these muscles. There is some indication that the superficial muscle of the anal fin, the inclinator analis, contains fibers of the dark type, whereas the erector and depressor muscles are more nearly of the pink type. If the inclinator contains fibers of the dark type it would suggest that that muscle is more nearly homologous with the superficalis lateralis, a homology that needs further investigation.

Fat of the adductor mandibulæ or cheek muscle.—The fibers of the muscle are more compactly arranged and different in appearance from the other portions of the salmon musculature. They are, however, most like the great lateral pink muscle. At Ilwaco the intermuscular fat is distributed in scattered but relatively large fat droplets, 60 to 70 μ in diameter. There is also a comparatively large number of small droplets not over 20 μ in diameter.

The intracellular fat is always present. The large fibers in the muscle carry a few scattered chains of extremely minute liposomes. On the other hand, the smallest fibers have liposomes about 0.6 μ in diameter.

Considering the muscle as a whole at the Ilwaco station the fat distribution is most nearly like that of the great lateral pink muscle, though both the intermuscular and intramuscular fat is very much less in quantity. This muscle, like the fin muscles, carries a relatively small amount of intramuscular fat. This fat is more than adequate for the uses of the muscle, but the striking fact shown by the sections is that there is never an excessive accumulation of the fat.

ANALYTICAL DETERMINATIONS OF THE PERCENTAGE OF FATS IN SALMON FROM THE MOUTH OF THE COLUMBIA RIVER.

When this study was projected it was planned to take a full set of samples of the muscles studied and make fat determinations by accurate chemical methods. Such a full set of determinations would have been very valuable in itself but of inestimable value as corroborative evidence in connection with the microscopic comparisions. It turned out to be impossible to carry through the full program of the work and the sacrifice fell on the chemical series. Chemical samples were taken, however, whenever it could be done, though the analyses were reserved to be made not in the field but in the home laboratories. The few samples secured were not analysed until after the microscopic work was completed and the results sent off for publication.

The fat determinations secured on samples from Ilwaco are inserted at this point. Considering the fact that the eight Ilwaco fishes were chosen to represent the entire range of types present in the lower Columbia at the time of the expedition, this showing of fat percentages is most significant. The salmon were taken, first, from the main channel as far out toward the end of the jetty as the fishermen go (111, 112); second, from the main channel south of Sand Island (113, 114, 115, 116); third, from the north channel leading out of Bakers Bay at a point near Fort Canby (118); and fourth, from Bakers Bay at the Whitcomb trap (117).

The two chief types of muscle described, the pink muscle and the dark muscle, were the only ones selected for analyses. The samples were taken in the mid-lateral region just in the plane that cuts the front of the dorsal fin, the same region from which histological samples came.

The greater amount of fat in the lateral dark muscle as compared with the pink was revealed by the microscope. But this fact is even more strikingly shown by the quantitative percentages given in the table below. A glance shows that the percentage of fat in the dark muscle is roughly twice as great as in the pink. There is no law to be deduced about it from so few samples. The fattest salmon have relatively the highest quantity in the pink muscle. The intermediate salmon from this station have a greater reduction in the fat of the pink than in the dark.

Particular attention is directed to the two females, no. 112 and 117. The former is from the channel of the Columbia from the farthest point out toward sea. The latter is from Bakers Bay, quite out of the main channel of the river. Undoubtedly the great difference is due to the fact that no. 112 was just coming in from the sea. No. 117 had undoubtedly lost most of its fat and is quite comparable with the salmon in better condition from the spawning grounds of the Clackamas River at Cazadero.

TABLE I.—ANALYTICAL DETERMINATIONS OF FAT IN THE TISSUES OF CERTAIN SALMON OF THE 1911 SERIES.

Date.	No. and sex of fish.	Muscle fats in per cent of wet weight.		Caught in Columbia River, Ilwaco, Wash.
		Pink muscle.	Dark muscle.	
1911.				
Aug. 3	111♂	15.680	28.018	Outer channel opposite the end of the jetty.
Aug. 3	112♀	19.655	30.813	Do.
Aug. 4	113♀	14.662	....	Mid-channel, off the upper end of Sand Island.
Aug. 4	114♀	20.179	29.080	Do.
Aug. 7	115♂	10.062	27.420	Do.
Aug. 8	116♀	5.395	23.248	Do.
Aug. 10	117♀	2.727	14.324	Bakers Bay, Whitcomb trap.
Aug. 11	118♂	10.507	....	North channel (from Bakers Bay), McGowan's trap near Fort Canby.

Significance of the fat in Ilwaco salmon with reference to the normal quantity of fat at the beginning of the migration.—In discussing the normal salmon type, the type at the beginning of the migration deduced from the study of feeding salmon secured at Monterey Bay, it was suggested that one might arrive at a better conception of the normal type by figuring back from the first migration station. But Ilwaco fish show a number of signs of physiological change presumably due to the cessation of feeding. Among these changes the most striking are to be had by the examination of the alimentary tract, where, in the stomach, and especially in the intestine and cæca, one finds extensive evidences of degeneration. These changes are almost wholly in the direction of retrogression of structure.

In the muscular tissue there are two factors mentioned above which are interpreted as changes that have come on in the amount and distribution of fat in the muscle since the beginning of the migration fast. These are, first, the abundance of intracellular fat laid down in the pink-muscle fibers; second, the evidence in the dark-muscle fibers of removal of fat. Interpreting these two phenomena broadly one may assume that there has occurred already in the best conditioned channel fish a using up of a certain amount of fat. Considered from the standpoint of percentages this amount has not reduced the total storage enough to be readily measured by the microscope except in the second case. In the poorer fish it is quite obvious that fat is disappearing, undoubtedly due to the prolonged fast. This is especially shown in the Bakers Bay type illustrated by no. 117. This interpretation is supported by the quantitative chemical determinations of fat.

There is absolutely no microscopic evidence which can be legitimately interpreted as meaning a fatty production from the disintegration of protoplasm. If, therefore, one could follow back the physiological condition of an Ilwaco salmon to that point in its history where it first ceased to feed, and would examine its tissues for the loading of fat, he would find that this time represented the maximum amount of fat present in the animal. In other words, this stage of the beginning of the fast, i. e., end of the feeding period, represents the climax of fat storage during the salmon's history. The microscopic picture obtained by a study of the Ilwaco specimens is therefore applicable to this normal stage, provided, first, that the intramuscular fat of the pink muscle be omitted, and, second, that all the areas of dark muscle which appear to be losing fat be considered as uniformly filled with fat. Figure 8, plate VI, representing the fat in a cross section of pink muscle of salmon no. 118 from Ilwaco, would, if the intramuscular fat were eliminated and the intermuscular fat increased in quantity, represent very well my conception of the quantity of fat in this tissue when the fast begins. So also in the dark muscle, figure 3, plate I, would serve as a type for the dark muscle at the beginning of the fast. These figures fail in the fact that they have too little intermuscular fat to represent the normal, but the percentage difference is one which can not easily be estimated by the microscope. Salmon no. 114 has nearly twice the amount of fat in the pink muscle shown by no. 118. This fat is wholly intermuscular and would show in the microscope in the form of larger drops rather than in a greater number. Judging wholly by the microscopic comparisons, one would never judge that the difference is as great as that revealed by the chemical determinations of the fat percentages.

PROTOCOLS.

Male salmon (no. 111) length 950 mm., weight 13,776 grams, taken between the jetty and the black buoy at the mouth of the Columbia River, August 3, 1911.

This was a clean, bright, silvery salmon of the short, deep type. It is a perfect looking specimen of the sea type. It was caught with a gill net by Mr. Cliff Sweeney. This salmon had all the appearance of a first-class, very fat specimen. Its flesh looked oily and there was a thick layer of cutaneous fat.

Microscopic examination of trunk pink muscle, teased (slide J6).—These isolated fibers of pink muscle are simply crowded with liposomic fat. The fat is arranged in longitudinal rows or chains of liposomes between the fibrillæ which bear relation to the striations. The liposomes are from 0.2 μ or less to 2 μ

in diameter, rarely larger, as observed in a certain large fiber under examination. This fiber is 200 μ in diameter. The liposomic chains are comparatively uniform in their disposition throughout the mass of the fiber. The liposomes themselves are not uniform in diameter in the rows. Adjacent droplets may alternate between small and large sizes, though in some of the rows the droplets are fused, thus making an oval droplet extending across the intervening striation membrane. In some of the smaller muscle fibers the fusions are much more extensive, extending over four or five striations. In the smaller fibers the fat droplets are relatively larger, averaging between 1.5 and 2 μ in diameter. Over the surface of the fibers and under the sarcolemma there is a sprinkling of small fat droplets from 2 to 5 μ in diameter. These are irregularly placed.

The caudal pink muscle was not prepared in this fish.

Trunk dark muscle (longitudinal section, J1).—The preparation is so filled with fat that the structure is obscured. The intermuscular fat is in the largest drops observed for the trunk dark muscle, the average diameter being about 30 μ. These drops are often compressed into oval outlines by the pressure of the fibers. The muscle fibers themselves are only about 40 μ in diameter.

There are large quantities of intramuscular fat, the droplets being simply crowded throughout the whole structure. The larger intramuscular droplets are from 15 to 20 μ in diameter. These large droplets often appear in rows along the course of the fiber, giving the appearance of splitting the fiber. However, they only press the fibrillæ apart. There are relatively few of the smallest liposomes present, though the bundles of fibrillæ all show a certain number of these small liposomes. The quantity of fat in this preparation is the greatest for any dark muscle noted in the whole season's work. The droplets are larger, relatively more numerous, and have so distorted the relations of the fibrillæ as to break up the regularity of the structure.

Another section (J33), fixed 18 hours in formalin, gives a much better view of the outlines of the fibers. The fibers are crowded thickly with relatively large intracellular droplets. They are so numerous as to form almost a continuous layer of drops. A section (J35) stained in sudan shows the same crowding of fat as those stained with scarlet red. The contrasts are less sharp.

The myocommata of the trunk dark muscle are filled with adipose cells which are crowded with fat. Many of the cells have ruptured and the fat has run together, but the fat drops of those cells still intact measure from 50 to 70 μ in diameter.

Caudal dark muscle (transverse section, J20).—The dark muscle of the caudal peduncle is very fat, but not so fat as in the trunk muscle. The drops are relatively smaller. Those between the fibers are from 6 to 15 μ in diameter. The fat within the fibers varies extremely in different parts of the section. I notice one region in which the fibers are almost free of large drops of fat; only smaller liposomes are present. In the near neighborhood of this group the fat is gathered around the surface of the fibers, apparently just under the sarcolemma, where the drops vary from 5 μ down. The centers of these fibers have liposomes averaging only about 1 μ in diameter. In that portion of the section which is fattest the central portion of the fibers has larger droplets, not averaging more than 3 μ, however. From this section it seems that the caudal dark muscle must have a greatly reduced amount of fat in comparison with the trunk region. Slide J21 shows relatively more fat than slide J20. The fibers are cut somewhat obliquely, and this brings out the fact that the drops are oval in shape, as in the lateral line region.

Intercostal muscle (longitudinal section, J7).—This section has a large amount of intermuscular fat. There are numerous large drops averaging 30 μ. The connective tissue of the whole section is jotted full of very fine fat droplets, from 1 to 10 μ. There is a trace only of intramuscular fat, nothing comparable to that in the teased trunk muscle. This fat is in extremely fine liposomes, averaging only a fraction of a micron in diameter. It seems quite uniformly distributed throughout the substance of the fibers.

Muscles of the anal fin (transverse section, J23 and 31).—This section was across the group of muscles between the interhemal spines and should therefore be of the erector and depressor muscles. There is a small group of fibers on the outer margin of the section different from the main body, which probably belongs to the inclinator muscle of the fin.

There is a very small quantity of intermuscular fat. The drops are scattered but relatively large. The main portion of the muscle has only traces of liposomic fat in extremely fine granules. There are no rings of fat droplets under the sarcolemma of the type which characterizes fat-exhausted muscle.

The group of fibers on the outer margin of the section has a uniform distribution of intracellular fat in comparatively large liposomes. These liposomes average 2 μ in diameter. They are quite evenly distributed throughout the substance of the fiber and under the sarcolemma, where they are somewhat more numerous. The liposomes appear as rows running between the fibrillæ which the oil immersion lens shows have an arrangement with reference to the cross striations. In one fiber six striation segments have a length of 8 μ, an average of 1.3 μ per striation. The liposomes that are spaced with reference to these particular striations are from 0.4 to 0.6 μ in diameter. The arrangement of fat in this group of fibers is very like the arrangement in the dark muscle of the lateralis superficialis.

Masseter or cheek muscle (transverse section, J9).—The fibers of the cheek muscle vary considerably in diameter, running from 30 up to 110 μ. This transverse section has a medium amount of intermuscular fat distributed in large droplets from 60 to 70 μ in diameter. There are also numerous fat drops from 15 to 20 μ in diameter.

The muscle fibers themselves are much split up by ice crystals in sectioning, yet it is clear that the fibers contain intracellular fat. This fat is greatest in amount in the smallest fibers, where the droplets are about 0.6 μ in diameter. The larger fibers also contain intramuscular fat, but the droplets are smaller.

Male salmon (no. 115), length 940 mm., weight 12,225 grams, caught in the Columbia River channel just above Sand Island, August 7, 1911.

This salmon was a clean fish, free of sea lice; testes slightly developed and dark venous red in appearance; stomach relaxed, 5 cm. in diameter with mucous content. Dark muscle teased immediately in physiological saline and figured.

Microscopic examination of trunk pink muscle teased in physiological saline.—These muscle fibers show extremely fine liposomes within the fiber. In larger fibers the liposomes are difficult to distinguish, but are readily seen in the small ones. The amount of intramuscular fat is not so great as in the trunk pink muscle of no. 113. Section J99, stained with hæmatoxylin only, differentiates the interfibrillar sarcoplasm in such a way as greatly to emphasize the outlines of transparent liposomes. The liposomes themselves appear highly refractive and have not taken more than a trace of the stain (1/12 oil immersion).

Microscopic examination of trunk dark muscle teased in physiological saline (J46).—A drawing of a fiber from this slide is presented (fig. 1) showing the fat throughout the dark muscle. Practically all the fibers in this slide are loaded down with fat. There is an enormous quantity of fat present, more than one can adequately represent by any graphic method. The fat drops within the fiber are relatively large and are so numerous that they push out the sarcolemma, making its outlines irregular. The drops are somewhat oval in shape, measuring 8 by 13, 5 by 6, 7 by 16 μ, and smaller. The diameters of some of the fibers are 35, 36, 40, and 45 μ. The fat droplets are in rows. They are relatively large in almost all parts of the field. This is due to the fact that the liposomes have grown in size until adjacent ones have fused, a condition throughout the fiber.

With the fusion of droplets the resultant is an oval mass with the long axis with the interfibrillar space. As the fat mass has grown the fibrils have been forced out of their normal relations. Where the drops lie outside the sarcoplasm and under the sarcolemma this membrane is seen to be pushed out in numerous irregular protuberances. This section is unusually clear and transparent, probably because it was not subjected to formalin.

Female salmon (no. 116), length 975 mm., weight 14,530 grams, caught in the channel of the Columbia River opposite the lower end of Sand Island, August 8, 1911.

A bright silvery fish, no sea lice, stomach small and contracted with thick walls; intestine one-half as large as in no. 115; ovaries relatively large, weighing 965 grams.

Microscopic examination of the trunk dark muscle (slides J77–91).—The trunk dark muscle of this fish has less fat than either no. 111 or no. 115. The larger fat drops are between the fibers. They measure 12 to 14 μ, but the average is not much over 7 μ.

The intracellular fat is in smaller droplets, from a fraction of a micron to 3 and 4 μ in diameter. There is a massing of the fat droplets around the surface of the fibers.

A series of teased preparations were treated in various ways to test the method The fresh muscle teased in physiological saline is more transparent than the other preparations and the fat gives the appearance of a greater quantity, largely because it is more clearly distinguished. Fibers teased in formalin were very opaque. Those teased in alcohol were somewhat intermediate in character between the saline and the formalin preparations.

Female salmon (no. 117), length 940 mm., weight 8,245 grams, taken from the Whitcomb trap located in the bend of Bakers Bay.

This salmon was more slender than no. 111, was a clean fish, but did not appear in as prime condition. The ovaries weighed 510 grams. The flesh appeared less oily and was very pale in color, especially in the caudal peduncle.

Microscopic examination of the trunk pink muscle (transverse section, K31).—This section is taken ventral to the lateral-line septum. There is very little intermuscular fat. Even along the myocommata there are only a few droplets and these are of small size, from 1 to 10 μ in diameter. The very largest drop seen was only 15 μ in diameter.

The substance of the muscle fiber contains a supply of liposomes arranged in chains throughout the mass of the fiber. These liposomes run from 0.3 to 0.5 μ in diameter. The section is cut obliquely, making it difficult to interpret the point, yet it is obvious that the liposomes are in greater numbers along the superficial region of the fibers while the deeper portion of the fibers is relatively poor in liposomes. This gives the section as a whole a mosaic-like appearance. The point is not absolutely certain, yet I am convinced that this increased quantity of liposomic fat is under the sarcolemma and between the more superficial layer of fibrils.

A section taken from the dorsal division of the deep lateral muscle has a less amount of fat than that from the ventral. The fibers of this section are very compactly arranged and the outlines are correspondingly sharp and angular, similar to that shown in later stages. (See salmon nos. 125 and 126.) The diameters of the fibers themselves vary between 30 and 150 μ, rather smaller than the average pink muscle fibers.

There is a very small quantity of very fine liposomes within the substance of the fibers. In many fibers no liposomes are to be distinguished. In the smallest fibers shown in the field, those 30 μ in diameter, the liposomes are present in the middle of the fiber. In the medium-sized fibers the liposomes are largely around the superficial area of the fiber and are from 0.2 to 0.3 μ in diameter, rarely larger (1/12 oil immersion). In the middle of these medium-sized fibers and in most of the body of the large fibers, liposomes are much fewer and exceedingly small, scarcely discernible.

The smallest fibers often have rings of very small fat droplets, the droplets running from 2 to 3 μ in diameter. These droplets are just under the sarcolemma. In areas where they are more numerous there are occasional fat drops 15 μ in diameter located between the muscle fibers.

Microscopic examination of the caudal pink muscle (transverse sections, K19 and 20).—These sections of caudal muscle show a markedly less amount of fat than from the dark muscle. The type of arrangement is that of the trunk muscle except that there is less intermuscular fat.

The intramuscular fat is also conspicuously less in quantity. The liposomes are practically absent from the larger fibers and are very minute and few in numbers in the smaller fibers.

Microscopic examination of the trunk dark muscle (transverse section, K7).—The fat droplets are relatively small in this preparation, notably smaller than in salmon no. 115. The intracellular fat is evenly scattered through the substance of the dark fibers. The liposomes vary from 2 to 5 μ in diameter (tissue cut fresh). In the regions which have the smallest amount of fat the number of droplets of the larger size which are so prominent in fish no. 111 are greatly reduced in size, running from 1 to 5 μ in diameter. In certain areas of the section lying near the connective tissue partitions there are small groups of dark fibers which apparently have their fat reduced greatly below the average. Such fibers will contain irregularly placed liposomes from 1 μ down to a just visible size, 0.2 to 0.3 μ, while adjacent fibers will have a more prominent loading of fat in which the droplets average from 3 to 5 μ in diameter. Also the number of droplets in the latter fibers is greater than in the former. This picture suggests the thought that the fat of the dark muscle is being removed along the course of the larger blood vessels.

Microscopic examination of the caudal dark muscle.—Insufficient study was made of the caudal dark muscle, but the rather poor sections bring out one point, namely, that the fat is reduced much below the average and that the fat droplets are massed around the surface of the fiber.

Male salmon (no. 118), length 940 mm., weight 12,470 grams, taken in McGowan & Co.'s trap at the mouth of Bakers Bay near the Fort Canby Dock.

This fish was a deep smooth specimen, skin bright and clean, no sea lice, head shaped like the female, medium depth, a splendid specimen apparently comparable to no. 111. The testes were quite small and immature.

Microscopic examination of the trunk pink muscle (sections K38, 45, K55–58).—The intermuscular fat is crowded in every angle formed by groups of fibers. The drops vary in size from small ones to as high as 100 μ in diameter. The muscle fibers themselves vary greatly in size, from 50 to 300 μ in diameter. In cross section the muscle fibers are oval to round in outline, the round contour of the individual fibers being in sharp contrast to the polygonal shaped outlines of fibers of salmon no. 117.

The two sections on slide K45 were made free-hand and thick in order to show the relations of the intermuscular fat. The section includes a tendinous myocomma. Where the muscle fibers are very close together a single row of large fat drops extends down the length of the fiber from the myocomma. In two or three regions the intermuscular space is filled up with two or even more rows of such fat drops. These fat drops are from 50 to 60 μ in diameter. They are somewhat compressed, having their long axis in the longitudinal axis of the fibers. The myocomma itself is crowded with fat.

The intramuscular fat is present in all the fibers. It consists of extremely fine liposomes, being most minute in the large muscle fibers and greatest in amount in the small fibers. They are uniformly distributed throughout all of the body of the muscle fiber, with the exception of the narrow ring of band-shaped fibrils which forms the surface layer.

The chains of liposomes are rather evenly distributed throughout the substance of the large fibers, but consist of very small liposomes from those just identifiable up to 0.3 μ. In the medium-sized fibers the liposomes are somewhat larger and in the smaller fibers considerably larger than in the ones just described. In the latter the liposomes reach the diameter of 1.5 μ, though the average is less than 1 μ. In one fiber 56 μ in diameter the liposomes were in unusually long chains and large in size, similar to the arrangement in dark muscle at a late stage in the resorption. Several liposomes in this muscle were measured which were 2 μ in diameter, but the average was from 1 to 1.2 μ. Figure 8, plate VI, shows the distribution of fat in the trunk pink of salmon no. 118.

Pink muscle from the belly shows an even greater amount of intermuscular fat; also minute liposomes in chains throughout the substance of the fibers.

Microscopic examination of the trunk dark muscle (K41 and 42, transverse sections).—There is a large amount of intramuscular fat in the lateral dark muscle of fish no. 118. The size of the fat droplets in this region is from 9 to 12 μ in diameter. In a certain interseptal region the fat drops are large, running as much as 60 μ in diameter. This fat belongs to the adipose tissue proper. A noticeable difference in the staining character is present between it and the fat in general; i. e., the large fats are less red. The muscle fibers of this section are so compact that it is often difficult to determine whether a given fat drop is within the sarcolemma or without. It is judged that a rather large proportion of the fat which is massed around the surface of the fiber is under the sarcolemma. The section throughout its whole extent shows an enormous quantity of fat massed along the lines which separate the fibers.

The teased dark muscle (slide K52) shows numbers of relatively large fat droplets along the sides of the fiber wall and adherent to the protoplasm. These droplets are smaller on the average than those of the cross section which were judged to be intermuscular.

Within the dark muscle fibers of this teased material the fat is present in masses—no other word seems adequately to express the condition. There are numerous fibers, in fact nearly all of them, in which many chains of liposomes are displaced by long masses or rods of fat. Undoubtedly, these rods of fat have been produced by the fusion of liposomes in the loading of the fiber with a higher percentage of fat than is found when liposomes are typically present, as, for example, in salmon no. 132. In the present section there are four such rods in one microscopic field. In another similar field there are six. In a fiber 40 μ in diameter these rods continue unbroken for as much as 126 μ. They are located in the areas between the bundles of fibrillæ, where one finds in the ordinary loading either chains of liposomes or, at most, short oblong droplets.

There are fibers in this teased material that have a somewhat less quantity of fat than that described in the last paragraph. In one such typical case the smallest liposomes observed measure 1.5 to 2.5 μ. In close proximity to this last chain of liposomes there is a chain of fused liposomes, i. e., a rod, which is continuous for 120 μ. This rod has, however, several partial constrictions which undoubtedly represent points where in the earlier stage of fat deposit the rod is discontinuous.

In the transverse section (1/12 oil immersion) the fat is crowded into the fiber in a way comparable only to no. 111. The whole surface of the field is taken up with fat droplets almost as thick as they can stand. There are relatively few liposomes that measure less than 1.8 μ in diameter and the size varies up to 5 μ. There are chains of these smaller liposomes throughout the protoplasm, even in fibers obviously distorted by the long rods of fat.

DISTRIBUTION OF THE FATS AT AN EARLY INTERMEDIATE STAGE OF THE SPAWNING MIGRATION.

The first station above Ilwaco where salmon were collected was at Warrendale, Oreg. This station is about 6 miles below Cascade Locks and is in the midst of an extensive fishing field. Salmon taken here have not yet passed the swifter runs of the river, but have already made a run of about 135 miles above the mouth of the river. The station was located at the cannery of P. J. McGowan & Sons, and I am particularly indebted to the superintendent, Mr. Charles A. McGowan, for many special favors. This company has a seining ground on the sand bar on the Washington side about 1½ miles below Warrendale. Our specimens were chiefly taken from this point, as the fish captured there were fine conditioned channel fish.

Fish nos. 120, 121, 122, 125, and 126 were taken at this station during the month of August. In August one secures salmon which clearly show stages of the removal of fat from storage localities. There is at this time of the year considerable variation in the grade of fish at this point. The fatter salmon, for example, no. 120, have their tissues well loaded with a reserve of fat. The poorer salmon, no. 126, show marked stages indicative of retrogression as regards the loading of fat.

Trunk pink muscle.—There is wide variation in the microscopic appearance of the fats in the trunk pink muscle of the fishes at this station. The fattest observed was no. 120 and the poorest no. 126.

The intermuscular fat is disposed in the muscle according to the same general plan as in salmon from Ilwaco. However, there is a very great diminution in the amount of this fat. This is indicated by the decrease in the size of the larger droplets, and to a less degree in a decrease in the number of droplets. A striking fact in comparison is that in these Warrendale fish the fat is very much less uniformly distributed among the fibers than in either the Ilwaco or in the normal tissue. The comparison between two stations is difficult to make. One can not microscopically measure the number of fat droplets and compute their diameters and thus the volume of material from the two stations. Rather he is limited to impressions made by placing the slides side by side. It is largely on this type of evidence that the above comparisons are made. However, as regards the intermuscular fat drops, a comparison of the diameters of the largest drops is illuminating. At Warrendale these largest drops seldom measure over 50 μ in diameter (see the protocol for salmon no. 121), whereas at Ilwaco they often measure 100 μ and more in diameter. In observations made at the time the material was collected and sectioned on the grounds at Warrendale it was judged that the amount of intermuscular fat in fish no. 120 was about one-half to two-thirds as great as in fish no. 118 from Ilwaco. Each of these fishes is among the best represented at its station. In those fishes which were relatively poor in fat, as no. 126, the amount of intermuscular fat is very greatly reduced. The amount of this reduction is best shown by comparing figures 8 and 10 of plates VI and VII. Judging from the comparison of a large number of preparations, I would say that the intermuscular fat of this poorest salmon could not be above 25 to 30 per cent of that of the normal type.

The intramuscular fat is abundant in all of the fibers of the pink muscle from the trunk region. The smaller fibers as usual are more heavily loaded with fat than are the larger. This is shown chiefly by the larger size of the liposomes in these small fibers.

In the fatter specimen the largest fibers are relatively thickly filled with numerous chains of liposomes. These chains are more numerous than in fish no. 115 and no. 118 from Ilwaco, and the size of the liposomes in the chains is, if anything, comparatively greater. When the poorest fish are examined, it is found that the large fibers are strikingly low in fat, for example, no. 126, fig. 10, pl. VII. In fact, it is difficult to distinguish liposomes in the largest trunk pink fibers of this fish. In numerous instances observed there were tiny groups of very small liposomes ranged near the surface of the cell, chiefly under the sarcolemma. If liposomes were present in the body of the large fibers at all, they were too small to be distinguished with the 1/12 oil immersion. In many sections of this poor fish the intermediate-sized fibers had their liposomes chiefly at the surface, whereas the central portion of the fiber was comparatively free of liposomes. Disappearance of fat is not accompanied by any signs of degeneration at this stage. The structural detail is clearer and very sharp and distinct, as shown in figure 13, plate VIII.

In teased preparations where one has a view of a fiber for some considerable length it appears that the Warrendale pink muscle is relatively rich in liposomic fat. In the best salmon there is even a greater amount of intracellular fat in the pink muscle than at the Ilwaco station. The chains of liposomes are more continuous and the size of the individual liposomes relatively greater. In the small fibers particularly this comparison holds. In fact, it often happens that in the smallest fibers the liposomes have reached a size at which adjacent ones coalesce, a phenomenon the significance of which is discussed in another connection.

While the above comparison is true and striking it is also true that at this station the range of variation in the amount of liposomic fat in the pink muscle is far greater than at Ilwaco. The fattest muscles have a greater amount of intracellular fat, the poorer muscles have a much smaller amount of intracellular fat.

Caudal pink muscle.—The caudal pink muscle of salmon from the Warrendale station shows the sharpest contrast as regards the amount and arrangement of the fat. In salmon no. 120 the intermuscular fat is all gone except along the connective tissue septa where it is present in scattered but medium-sized drops. In the poorer salmon the amount of intermuscular fat in the caudal pink is practically nil. Here and there in the thicker septa between bundles of fibers one will find an individual droplet or a group of three or four droplets not more than 4 or 5 μ in diameter.

The intramuscular fat of the caudal pink muscle is very slight indeed even in the fattest fish. The smallest fibers are fairly well supplied with liposomes which run in chains comparatively evenly distributed throughout the sarcoplasm. In these instances, however, there are distinct groups of liposomes under the sarcolemma, but at the surface of the sarcoplasm. There is a distinct difference in size between the surface liposomes and the deep ones. The former range from 1 to 1.5 μ in diameter, while the latter are only from 0.2 to 0.4 μ in diameter in fish no. 120. In salmon no. 126 the liposomes are still present in the small fibers, having much the same arrangement as that just described and averaging about 0.4 μ in diameter.

In the intermediate and in the larger sized fibers the amount of intracellular fat is very small. In the larger fibers only an occasional group of very tiny liposomes at the surface of the fiber can be seen. In the intermediate fibers there are now and then fibers which have a comparatively even sprinkling of tiniest liposomes throughout the mass of the protoplasm with somewhat larger liposomes at the surface of the fibers. On an average for the station, however, one must say that the presence of liposomes is very greatly reduced, both in size and number for all the intermediate fibers, while for the larger fibers it is present only in traces.

Trunk dark muscle.—In the dark muscle of salmon from the Warrendale station there is even wider variation as regards the loading of fat than in the pink muscle. In fish nos. 120 and 121 the amount of fat in the trunk dark muscle is very great, while in no. 125 it is low. In the fatter salmon the loading of fat is almost as great as in the specimens from Ilwaco, with the exception of Ilwaco specimen no. 111 which was an extraordinarily fat fish. On the other hand, in the poorer specimens the amount of dark muscle fat is only a small percentage of that at the Ilwaco station.

The intermuscular fat is comparatively plentiful, is located in the connective tissue septa and in the myocommata. However, the fat droplets average much smaller in size than in the Ilwaco specimens. Oftentimes the number of these fat droplets, especially of the smaller ones, seems relatively greater. In Warrendale fish the individual fibers are usually somewhat more definitely separated and this fact makes it easier to determine the relation of the intermuscular fat. In fish no. 125 the amount of this intermuscular fat is very low, but occasionally individual drops are as large in this fish as in those that have more fat. The amount of intermuscular fat varies in different regions of one and the same muscle. This variation undoubtedly is associated with a process of fat erosion which was first observed in certain Ilwaco specimens. In Warrendale fish the erosion process has gone much further and is more readily followed. In areas in which the fat has been most fully eliminated the intermuscular fat is reduced to tiny droplets.

The intramuscular fat of the dark trunk muscle is abundant in all of the fibers of the fatter fish. In no. 120 the cross sections and the teased preparations show that the fibers are especially richly supplied with liposomes in their sarcoplasm. The liposomes are of large size and in relatively long chains. There is considerable fusion of adjacent liposomes. Especially in fish no. 121 the liposomes are so large that one might better describe them as droplets. The diameters run from 1 to as much as 4μ. Certain of the fibers in this fish and also in fish no. 122 show fusion of liposomes into long rods of fat. These slender rods usually appear more or less constricted at points corresponding to the striations of fibrillæ.

The most striking thing about the fat in the trunk dark muscle of fish from the Warrendale station is its great irregularity in different microscopic areas. This has been spoken of in connection with the very fat fish no. 120, but it is an appearance that marks every fish examined. If the specimen is one of low grade, as in no. 125, then these irregularities are most prominent. Certain groups of dark muscle fibers will appear richly loaded with fat while other areas will be almost free, certainly will not contain more than from 30 to 50 per cent as much as in the fatter areas. In these clear areas the reduction in fat is due to two factors: First, the great reduction in the average size of the liposomes; and second, the great decrease in the number of liposomes. In numerous areas where muscle fibers are in close contact with small blood vessels the fat is very low in amount. This condition is described in the protocol of fish no. 125. The characteristic picture presented where a group of fibers lies along the blood vessel is as follows: First, that portion of the fiber next the blood vessel will have no intermuscular fat; second, the intramuscular fat will be either absent or greatly reduced in the corresponding area; third, the portion of the fiber opposite the blood vessel will have a relatively high content of fat; fourth, as a rule the intermuscular fat in contact with the opposite outer border of the fiber will still be present.

The teased fibers of this dark muscle show instructive variations. Different lengths of one and the same fiber show wide variation in the loading of fat. This is expressed especially through the variation in size of the liposomes. But if the fat is very light in amount there will also be a variation in the number of liposomes. Careful focusing will always bring out the fact that the richer portions of the fiber will have a relatively large amount of fat under the sarcolemma. In many instances the liposomes in the chains will have fused, forming slender fat rods showing constrictions at the point of fusion. The poorer areas in the fiber will show a small amount of fat under the sarcolemma, smaller liposomes in the chains and little or no fusion. Where the fat is almost completely eroded the number of liposomes will be obviously reduced. In this case the reduction takes place more completely near the center of the fiber as compared with its superficial area.

In numerous instances at the other stations, as well as at Warrendale, I have noticed that while the fat is being eroded there will appear variations in number and arrangement of fat droplets under the sarcolemma. In a teased fiber a rather definite pattern will often be noted in this subsarcolemmal fat, a pattern which coincides with the blood vessels, the pattern being marked by rows of very small droplets along what would correspond to the border of the capillaries. Also small rings of droplets will appear at various points, sometimes in groups. These rings of droplets are arranged around a clear center. They are interpreted as part of the process of erosion of large intermuscular fat drops. As lipolysis goes on, the fat that is dissolved away from the làrge fat drop will often be redisposed in small droplets within the sarcolemma around the area which is being compressed by the large drop.

Caudal dark muscle.—The caudal dark muscle of the Warrendale fish varies through even a wider range of fat content than the corresponding muscle from the trunk region. There is always considerable fat in the myocommata, but the amount of intermuscular and intramuscular fat varies exceedingly.

In the fatter fish the intermuscular fat is reduced in the number of droplets present, but particularly in their size. In no. 125 there is practically no fat in the caudal dark muscle.

In this salmon certain of the dark fibers are absolutely clear of fat within the fibers, and the fattest fibers observed contained only a sprinkling of liposomes around the superficial areas with a trace in the center of the fiber. The whole muscle is as nearly fat free as any dark muscle examined. It is noticed here also, as in the trunk muscle, that the fibers freest of fat lie in the neighborhood of small blood vessels.

In a few scattered fibers in the caudal dark muscle of no. 125 an appearance is noted fo? the first time that is suggestive of a disintegrative process. We have not been able to convince ouyselves that these fibers are actively breaking down, but they certainly do show appearances suggestive of the initial stages of water absorption characteristic, for example, of cloudy swelling. The fibers stain lightly in a way which characterizes an early stage of muscle degeneration. These fibers also contain small transparent, highly refractive and lightly stained granules. The stain does not have the usual appearance of fat stain—that is, the color is not the brick red of the scarlet red dye. Rather it is a more brilliant and dark appearing neutral red. The amount of stain taken is only slight. These granules do not contain any pigment, as was noted in degenerating cheek muscle of fish no. 140, to be described later.

PROTOCOLS.

Male, salmon (no. 120), length 937 mm., weight 11,480 grams, Warrendale, August 16, 1911.

This fish was taken from the McGowan seining grounds, 1½ miles below Warrendale. It was a fine fish, in splendid condition; the nose slightly hooked, no large teeth, the testes two-thirds developed, color normal, but a trace darker than fish at the mouth of the river; back darker but not rusty; fins perfect.

The muscles were pink and oily. The fish was received fresh from the seining grounds, and the fin muscles were still alive when samples were taken.

Microscopic examination of the trunk pink muscle (K87, 88, and 90).—The intermuscular fat is about one-half to two-thirds as great as in no. 118 from Ilwaco. Its disposal between the fibers is similar to the fish taken from the mouth of the Columbia. There is less intermuscular fat from the middle of the dorsal portion of the great lateral muscle.

The intramuscular fat is abundantly present in all of the fibers. The smaller fibers are more deeply stained, showing the greatest amount of fat. The small fibers of the teased preparation are filled with chains of liposomes, the individual liposomes being larger than in the large fibers. In the fibers of large size the chains of liposomes are not quite so numerous, and the liposomes themselves are relatively small. Two fibers, side by side, one large and the other small, are in sharp contrast.

Microscopic examination of the caudal pink muscle (transverse section, K91).—In this section the intermuscular fat is all gone except along the connective tissue septa, where it is present in scattered but medium large drops (1/12 oil immersion). The substance of these fibers is well fixed in formalin, and the fibrillar outlines show clearly. In the large fibers of the section there is no fat stain in the body of the fibers. Occasionally at the very surface there are tiny groups of liposomes. In the smallest fibers there are in the body of the fibers between the fibrillæ numerous extremely small liposomes. There are distinct masses of liposomes on the surface of the sarcoplasm and under the sarcolemma. The liposomes within the fiber are from 0.2 to 0.4 μ in diameter, those on the surface from 1 to 1.5 μ in diameter. The intermediate-sized muscle fibers have a few scattered groups of liposomes immediately under the sarcolemma, but none in the body of the fiber. These observations are confirmed on fragments of fibers in which the fibrillæ are turned in a horizontal position.

Microscopic examination of the trunk dark muscle (sections K72–76).—The muscle fibers in this material, both in the transverse sections and in the teased preparations, are especially richly supplied with fat. The fat is crowded, both between the fibers and throughout the sarcoplasm of the fibers. The intermuscular fat droplets are numerous, of medium size, but not so numerous nor so large as in fish no. 111 from the Ilwaco station.

Certain areas in the transverse section have an appreciably smaller quantity of fat. These areas are associated with connective tissue septa carrying blood vessels, and are similar to those noted in salmon no. 117 and no. 118, from the mouth of the Columbia. This appearance is undoubtedly due to the beginning of fat erosion from this type of muscle, and is greater in this section than in the two Ilwaco fish referred to. The erosion areas have a much less quantity of fat than in fish no. 118. The fat droplets are not so numerous and are smaller.

On the whole, the amount of fat is somewhat less than in fish no. 118, though the comparison is difficult to make. In the transverse section of one fiber 100 μ in diameter, 46 droplets were counted. They were from 3 to 6 μ in diameter. In the spaces around the particular fiber and in the same focal field were 12 droplets oval in shape, 20 μ long, but from 4 to 6 μ thick.

The intramuscular fat is remarkably uniform in its distribution through the muscle fiber, the larger droplets averaging from 4 to 6 μ in diameter. The disposal of the fat is similar in character to that noted in previous fish and is shown in figure 3, plate III.

Microscopic examination of the caudal dark muscle.—The muscles in this section have very much less fat than the trunk dark fibers. The intermuscular fat is smaller, 6 to 10 μ in diameter, but the droplets are numerous.

The largest intramuscular liposomes average 3 μ in diameter, but there are many smaller liposomes. There are rings of small droplets around the border of the muscle, these averaging 4 μ in diameter. This superficial fat sharply marks the boundaries of transparent cross sections forming a definite mosaic under the low magnification. It is almost wholly intramuscular fat lying under the sarcolemma.

Male salmon (no. 121), length 950 mm., weight not given.

A first-class fish from the McGowan seining grounds, 1½ miles below Warrendale on the Columbia River. The testes two-fifths developed.

Microscopic examination of the trunk pink muscle (transverse section, L1).—The amount of intermuscular fat is intermediate between salmon no. 115 and no. 117 from Ilwaco. The fat droplets between the muscles are many of them relatively large but not so numerous, and do not average so large as in no. 118. The largest drops are from 45 to 55 μ in diameter. The fibers themselves are somewhat more compact in arrangement, but the outlines of the fibers in cross section are less smooth and circular than in no. 115, but not so angular, and the fibers do not seem so much compressed as in fish no. 117.

The surprising fact is the great amount of intramuscular fat. This fat is most thickly deposited through the small fibers, where the liposomes have a size from 1 to 2 μ in diameter. These liposomes are quite uniformly distributed through the substance of the small fibers. An occasional fiber will have its liposomes more thickly set around the superficial border. In certain of the smallest fibers, an example 75 μ in diameter, also in other regions of the section, there is fat in relatively small droplets just outside the surface of the sarcoplasm and under the sarcolemma.

Liposomes are present in the largest fibers also, but are exceedingly small and not so plentiful in the body of the fiber. In these very large fibers many liposomes are found between the fibrillæ near the surface of the fiber. They appear as if the liposomes were formed just under the sarcolemma and between the fibrillæ of the most superficial or band-shaped layers. In the inner borders of the band-shaped fibrils there is a second zone where the liposomes are present in relatively greater numbers. The liposomes are not larger but more numerous in this zone.

Microscopic examination of the caudal pink muscle (L9, 10, and 11).—Sections were preserved for 18 hours in formalin. The fibrillæ show well indeed. The surface layer of band-shaped fibrillæ are in contrast to the smaller fibrillæ of the body of the muscle. The fibers are compact in arrangement, but retain a certain amount of round contour which characterizes muscular tissue in prime condition. The following points characterize the tissue: (*a*) There is very little, almost no intermuscular fat in the section. Here and there a small droplet is found in the angles between the fibers. The largest one observed is only 18 μ in diameter. (*b*) The outlines of the fibers of the caudal pink muscle are definitely marked by very small fat droplets, measuring from 1 to 2.5 μ in diameter, many of them even smaller. The point is difficult to determine, but the droplets seem to be within the sarcolemma. (*c*) The caudal pink muscle fibers are relatively low in liposomes. The smallest fibers contain only a few liposomes. The fibers measuring from 50 to 100 μ in diameter have easily identified liposomes, but the larger fibers are free of liposomes in all but the extreme superficial part of the fiber (1/12 oil immersion.) The liposomes in the smaller fibers are chiefly around the outer third of the muscle. In the central portion of the fiber there is not more than one-fourth as much stainable fat as in this superficial rim.

Microscopic examination of the trunk dark muscle (transverse section, L5 and 6).—The lateral dark muscle shows an amount of fat greater than in no. 117, but not so great as in no. 111. The fibers are compactly arranged everywhere in the dark, and under the low magnification their outlines are marked by the excess of fat in that zone, the fat droplets averaging from 4 to 6 μ.

In some of the angles between fibers and in certain regions where the connective tissue is greater there is unquestioned intermuscular fat. The size of these drops runs from 10 to 15 μ in diameter. Along one exceptionally thick septum this intermuscular fat is absent. The section has the appearance which indicates the process of resorption of fat (under the 1/12 oil immersion).

The sarcoplasm of these fibers is full of small fat droplets almost as large as those in no. 115. The droplets are too large to be called liposomes, though every gradation exists between liposomes 1 μ in diameter up to these larger droplets which average 3.6 to 4 μ in diameter. There are certain fields of the section which contain relatively less fat in the sarcoplasm, the fat droplets being almost gone and the liposomes relatively smaller but thickly distributed. In these fields there is also relatively less fat around the border of the fiber, i. e., less fat under the sarcolemma.

Microscopic examination of the caudal dark muscle (L14 and 15).—This transverse section of the dark muscle from the tail is quite well supplied with fat. I do not see fat droplets that are unquestionably between the fibers, but groups of droplets that lie just under the sarcolemma were noticed, the largest of which were 7 μ in diameter.

The intramuscular fat is greatly less than that in the dark muscle of the portion of the body where the largest liposomes measure 3.6 μ in diameter, but this larger size is rare. The largest of the liposomes run about 2 μ in diameter. The smaller are from this size down to 0.3 μ and less in diameter. Judging from the intensity of the stain, one would say that the caudal dark does not contain more than one-fourth, possibly one-third, as much fat as the trunk dark.

There are areas, especially along certain septa, which have a strikingly less quantity of fat. This appearance is associated with the more vascular areas.

Female salmon (no. 122), length 890 mm., weight 8,980 grams, taken at Warrendale, August 17, 1911.

This was a good conditioned fish, taken at McGowan's seining grounds, 1½ miles below Warrendale. The weight of the ovaries was 680 grams, stomach quite small, appearing one-half degenerated.

Microscopic examination of the trunk pink muscle (transverse section, L38).—This section shows a relatively large quantity of fat between the fibers, not so much, however, as in no. 115 and no. 118 (1/12 oil immersion). The larger drops measure about 20 μ in diameter. The distribution is similar to that in the fish just mentioned. In this section the smallest fibers, 40 μ in diameter, are thickly set with liposomes distributed rather uniformly through the fiber. These liposomes vary in size from 0.6 to 1.3 μ in diameter. In the medium fibers, 75 to 100 μ in diameter, the number of liposomes diminishes in the center of the fiber; also there is a marked diminution of the size of those present. In one fiber, 100 μ in diameter, the liposomes in the middle of the cross section measure from 0.1 to 0.4 μ in diameter.

In the larger fibers of the section, those above 100 μ in diameter, there is a marked diminution of liposomes. This diminution is most apparent in the main body of the fiber, i. e., exclusive of the superficial area. This contrast in amount of liposomes between the deep and superficial part of the fiber is sharp, giving the fiber the appearance in cross section of having a superficial ring of fat. In certain of the larger fibers, the central liposomes are absent, or, at any rate, one can not distinguish them with the oil immersion. In these same fibers liposomes around the superficial border will vary greatly in size, measuring from scarcely identifiable liposomes up to as much as 1.4 μ in diameter.

An examination of the longitudinal sections (L33), brings out the fact that there is a relatively high content of fat near the surface of the fiber, both external to the fiber and just under the surface. The external fat is in the connective tissue, the endomysium, therefore, intermuscular.

Microscopic examination of the caudal pink muscle (section L51).—The fibers are almost free of fat. There is no intermuscular fat hanging to them, but the connective tissue, myocommata, of the caudal pink muscle (slide L54) is crowded with adipose fat.

Scattered over the surface of the fiber, all apparently under the sarcolemma, is a good deal of fat in droplets, from 2 to 3 μ in diameter, not uniform in size. The small and intermediate sized fibers have tolerably evenly distributed chains of smallest liposomes. In the larger fibers the central portion is relatively free of chains of liposomes, which, in many instances less than 0.2 μ in diameter, are so small they are difficult to identify. Certain of these teased fibers show the striations clearly. In one such example there are 13 striations in 36 μ (slide L54, 15 striation to 36 μ). The diameter of the fiber showing these striations is 63 μ. The chains of finest liposomes in the largest fibers are not always perfect. The irregularity is due to the dropping out of individual liposomes. In some chains there are more liposomes than fibrillæ. This is due to the presence of two liposomes in the space opposite certain striations. There is not always perfect correspondence in the number of striations and liposomes in the chains in these pink fibers.

This slide gives examples of the cone-like ends of the fibers. These ends are supplied with liposomes just as in the body of the fiber. All the teased material of the caudal pink fibers shows an increased quantity of fat at the surface of the fiber. This fat is in tiny droplets varying from the smaller liposomes 0.2 μ in diameter up to 2.5 and even 3 μ. Many of the droplets are in regular rows, but not so regular as those deep in the muscle fiber. Undoubtedly this fat is just under the sarcolemma, a fact confirmed by the appearance when the optical section cuts the middle of the fiber.

Microscopic examination of the trunk dark muscle (sections L18–25).—These transverse sections show a relatively large quantity of intermuscular fat. The fibers are more widely separated than is usual for dark muscle, and it is correspondingly easy to determine whether the fat is free or under the sarcolemma. The longitudinal section (L25) gives a fine confirmation of the cross section.

The intermuscular fat is present in large quantity, the larger drops averaging from 12 to 15 μ in diameter. There are many smaller droplets interspersed among the larger.

The fat under the sarcolemma shows well both in the cross sections and in teased preparations. It is seen best when the focal plane cuts the surface of the fiber. The drops are irregularly placed over the surface, being held in position by the delicate sarcolemma. When the focal plane cuts the center of the fiber one can see that the sarcolemma incloses the fat drops.

The intramuscular fat in the body of the fiber is uniform in its distribution, as viewed in cross section. The droplets average from 2 to 2.5 μ in diameter, many of them smaller, but some larger. Rarely does one see a droplet greater than 3 μ in diameter.

In the longitudinal sections the chains of liposomes are more numerous and longer than in any tissue examined. In some fibers these chains extend across a whole microscopic field. Several fibers show chains in which the individual liposomes have fused. In such case the fat is in long slender rods, showing constrictions corresponding to the fibrillæ (slide L46). In comparison with salmon no. 111 and no. 115 from Ilwaco, this fish has as many, even more, intramuscular fat droplets, but the droplets are relatively smaller. The larger droplets, which in the Ilwaco salmon measure as much as 6 μ in diameter, are absent here.

Microscopic examination of the caudal dark muscle, teased (L58).—These teased caudal dark fibers show considerable variation in the amount of loading of the fat in the different fibers present (1/12 oil immersion). One fiber contains a relatively large amount of fat on the surface of the fiber, interpreted as under the sarcolemma. The larger fibers show but little fat in this subsarcolemmal region. If present at all, it is relatively small, running 4 to 5 μ.

The fat within the substance of the fibers is greatly reduced in amount in comparison with the fibers from the middle of the body. The liposomic chains are not so numerous and the average size of the liposomes not so great. In these caudal fibers one often finds a chain of liposomes which has become fused, as in the trunk dark. In the muscle fibers least filled with fat the number of chains of liposomes is very much less and the size of the liposomes does not average over 1.2 μ.

A large salmon (no. 125), length and weight not recorded, taken at Warrendale, Oreg.

Microscopic examination of the trunk pink muscle (transverse section, 1/12 oil immersion).—The amount of intermuscular fat is relatively small, the drops are often large, as much as 50 μ in diameter, but they are few in number—not more than 1 to every 3 or 4 fibers.

The amount of fat within the fibers is obviously greater in the smaller fibers than in the larger. In the small fibers, 50 μ in diameter and less, the liposomes are fairly uniformly distributed in size, ranging from 0.6 to 2.5 μ in diameter, but averaging about 1 μ in diameter. The intermediate fibers have the fat collected around a superficial zone about 8 to 10 μ beneath the surface of the fiber. Some of the fat droplets in this region are relatively large, 4 μ in diameter, though these are comparatively rare. In the center of the fibers the liposomes are smaller, the larger ones averaging 1 μ. In a fiber 100 μ in diameter the liposomes are quite uniformly distributed over the surface of the cross section, but run only 0.2 to 0.6 μ in diameter. The liposomes in the largest fibers are of practically the same diameter, but not so numerous. In the largest fibers examined and in those most free of liposomes there is a noticeably greater number of liposomes near the surface of the fiber. In certain of the fibers these liposomes are just under the sarcolemma, between the fibrillæ of the surface layer and in the zone at the inner border of the superficial or palisade fibrillæ.

Microscopic examination of the caudal pink muscle.—The intermuscular fat is limited to the myocommata and to the thick connective tissue septa.

The intramuscular fat is present as liposomes in the small and intermediate fibers and just under the sarcolemma of most of the large fibers. Liposomes are distinguished with difficulty in the central body of the large fibers.

Microscopic examination of the trunk dark muscle.—The intermuscular fat is not so great in amount as in no. 122, though the fat drops run up to 20 μ in diameter. There are areas over the section which have practically all the intermuscular fat as well as much of the intramuscular fat removed.

The fibers are thickly studded with chains of liposomes 0.6 to 2 μ in diameter. Only occasionally are adjacent liposomes fused as in no. 121 and no. 122. The longitudinal section shows a greater quantity of fat along the borders than in the bodies of the fibers.

The fat under the sarcolemma is in drops measuring from 4 to 5 μ in diameter, occasionally 6 μ. The number of these fat droplets around any given fiber varies greatly, due to the fact that the fat is being removed in the neighborhood. Choosing an area containing the most fat, the intramuscular fat is in liposomes from 0.4 μ in diameter up to as much as 4.3 μ. The number of the largest droplets is relatively small, but when present they are evenly distributed through the fiber. There is great variation in the size of the liposomes in different portions of the length of one and the same teased fiber.

In the areas referred to above the intramuscular fat is reduced in amount. The size of liposomes is affected more than the number of chains. There are several fields through which small blood vessels go in which that portion of the muscle in contact with the blood vessel is strikingly free of fat. In the neighborhood of a blood vessel where the fat is most removed the droplets lying under the sarcolemma are reduced to a few liposomes lying on the side farther from the vascular area. The largest of these liposomes measure 1.6 μ in diameter. Through the body of the same fiber in the half opposite the blood vessels the liposomes are fewer in number and relatively smaller in diameter (0.4 to 1 μ) than in portions of the fiber not in contact with blood vessels. In the third of the muscle lying next to the vascular area the liposomes are still present, but small—too small to measure accurately. There are numerous areas in this section (L74) showing contrast as regards the degree of removal of fat.

Microscopic examination of the caudal dark muscle.—There is quite a little fat in the myocommata. Through the body of the dark muscle, however, there is no intermuscular fat.

Under the oil immersion certain fibers of this section are absolutely clear of fat within the fiber. In other fibers there are traces of liposomes too small to measure. These traces are confirmed by fibers which have been turned to a horizontal position in the handling. In still other fibers there are scattered and irregularly placed groups of liposomes at points near the surface, but none deep down in the substance of the sarcoplasm. The fattest fibers observed contained a fairly uniform sprinkling of liposomes around the superficial area of the fiber and a somewhat smaller quantity in the middle of the fiber. The whole preparation presents as nearly a fat-free section as has been observed of caudal dark muscle.

A number of fibers contain numerous spherical bodies measuring approximately 2 μ in diameter and having a dark-red color (1/12 oil immersion). These bodie are irregularly placed through the substance of the fiber, as are the brown pigment granules of degeneration. The stained bodies are spherical, and one might take them for fat bodies. However, if they are fat bodies then the color of the stain is distinctly different from the type. This color is a brilliant dark neutral red as against the usual lighter brick red characteristic of this stain. It is possible that we are dealing here with the reaction of some special fat which stains differentially, according to the observations of Bell.

Female salmon (no. 126), length 780 mm., weight not taken, Warrendale, Columbia River, August 24, 1911.

This salmon was fresh from the McGowan seining ground and was chosen as representative of the group of fish which show an advanced stage of migration change at this station.

Microscopic examination of the trunk pink muscle (transverse sections M1–3, L78).—The intermuscular fat is in relatively small amount. The myocommata contain a small amount of fat arranged as a narrow band of droplets on either side of the tendon. The larger fat drops measure 30 to 40 μ in diameter, seldom more. The intermuscular septa still contain a small amount of intermuscular fat. In the larger of these sheets of connective tissue a few fat droplets measure as much as 15 μ in diameter, most of them less. These are in the areas where in fish no. 118 the droplets were as much as 100 μ in diameter. Most of the fat drops are small and in relatively small group of 9 or 10 droplets in a group.

The intramuscular fat is very low in this specimen. It is present in the small and intermediate fibers, but difficult to distinguish in the larger fibers. The smallest fibers have their intracellular fat tolerably thickly sprinkled over the microscopic field. Most of these fibers show a somewhat greater amount of liposomes near the surface. The medium fibers show great variation. Certain ones are almost clear of liposomes, while others have a liberal sprinkling. In muscles of this size there is a condensation of fine liposomes under the sarcolemma. The same arrangement is true for the smallest fibers. These small intracellular liposomes are 0.4 to 0.6 μ in diameter.

In the largest fibers in the section one can scarcely find any liposomes in the body of the fiber. Around the border and immediately under the sarcolemma, especially in regions which have intercellular fat in the neighborhood, there are groups of liposomes.

These groups are shown in the figure 10, plate VII, and are characteristic. In one fiber 144 μ in diameter very delicate liposomes are rather thickly dispersed in the superficial layer and more scattering in the central portion of the fiber. This fiber is smaller than the average of the large size and has relatively more fat.

The teased pink fibers are filled with liposomes. These are in long chains, which are quite uniform in appearance. In the small fibers the liposomes are from 1 to 1.5 μ and occasionally 2 μ in diameter. In a number of instances observed adjacent liposomes have fused into oblong droplets. In certain isolated fibrillæ the liposomes are adherent but irregular in position. In a fiber about 150 μ in diameter I find that the central portion has only scattered chains of liposomes, while near the surface the chains are more numerous. In either case the liposomes are of irregular size in the chains and not of regular arrangement, as in the type from down river. The striations in this material are very narrow. I have not examined carefully enough to determine the exact relation between the liposomes and the striations. The number of liposomes in a given area varies in different portions of the fiber. There are irregular patches of fat droplets of liposomic size at the surface of the fiber. From this appearance and that noted in the cross section one comes to the conclusion that these patches of liposomes are the ones shown under the sarcolemma in the transverse section. The arrangement of these patches of fat under the sarcolemma is partly dependent on some pressure factor. At any rate, there is a fairly definite map shown by them. In some instances this may correspond with the capillary net. Also there are numerous areas, oftentimes two or three in the same field, in which the fat is arranged in a definite ring around a clear area. This suggests a relation to some relatively large intercellular fat drop. There is no other structure present with which such a definite arrangement of fat drops coincide, and the number of instances observed is too great to be a mere matter of chance. This pattern-like arrangement of fat under the sarcolemma was often noticed in preparing fresh material in the field. In one of three fibers of a group examined in this connection there were a number of fused liposomes in the chains. These fusions have taken place in the chains of liposomes near the surface, but are not of the subsarcolemmal group.

Microscopic examination of the caudal pink muscle (section M11).—The amount of intermuscular fat is insignificant in this section, almost exclusively limited to the myocommata. The intramuscular fat is also very small. In the very smallest fibers there is still present quite an appreciable amount of fat in small liposomes. These liposomes vary in size, about an average of 0.4 μ. They are not so numerous as in the same size of muscle fiber in the trunk region. There are small fibers which show groups of liposomes under the sarcolemma. In the medium-sized fibers such liposomes as are present are limited to the superficial layer of fibrillæ and to the space under the sarcolemma. In the large fibers the only trace of fat is under the sarcolemma, and that is present only in isolated regions where the liposomes are of scarcely visible size.

Microscopic examination of the trunk dark muscle (sections M7, 16, 25).—Section M7 shows a comparatively slight amount of intermuscular fat. That is chiefly along the thicker connective tissue strands. Among some of these strands which are more vascular the low magnification shows areas in which the bordering muscle fibers are almost free of fat. The appearance suggests that the fat is in process of removal. Under the 1/12 oil immersion it is noted that in the compact areas of the muscle there are scattered droplets of intermuscular fat. The drops are comparatively small in size, 3.5 to 4 μ, but occasionally as much as 12 μ in diameter.

The intracellular fat is present in medium amount. It is distributed less uniformly over the surface of the section of the fibers. It is noticeably less in amount in the center of the section of many of the larger fibers. The diminution in the amount of the fat in the middle of the fiber is primarily due to a great reduction in the size rather than in the number of the liposomes. In the center of a given fiber under observation the liposomes are from 0.4 to 1 μ in diameter, while at the surface of the same fiber they are 1.6 to 2 μ in diameter. In this fiber there are fat drops under the sarcolemma which measure 2.5 to 3 μ in diameter.

The examination of a fiber bordering on one of the lightly stained areas mentioned above shows no large fat droplets, and the liposomes are reduced to an average size of 0.3 μ in diameter. There is a group of liposomes about 1.2 μ in diameter under the sarcolemma of this fiber at the point farthest from the blood vessel. Bordering on the opposite side of the same area there are two fibers which show a very marked reduction in the number of liposomes next the vascular area, although the liposomes are not altogether absent. The parts of the fibers opposite the area contain larger liposomes, 1.6 to 2 μ in diameter.

There is a very great variation in the amount of fat in different parts of this section (M25, teased), if one is to judge by the microscopic size of the liposomes. Oil immersion examination of the dark fibers shows numerous chains of liposomes very similar in arrangement and appearance to slide M20 of trunk pink. The liposomes are more numerous than in the trunk pink, but the average size for the center of the fiber is about the same. Many of these fibers show fat droplets on the surface under the sarcolemma. Certain of the fibers show that the surface of the fiber has relatively large liposomes. In a certain case near the superficial focus are liposomes 2 to 2.4 μ in diameter and near the center of the fiber numerous smaller liposomes not over 0.6 μ in diameter with an occasional larger one 1.2 μ. The loading of liposomes varies along the length of the fiber. This might easily happen in a tissue where the fat was being eroded, since the arrangement of blood vessels can not be uniform with reference to the surface of the whole fiber (fig. 4, pl. IV).

Microscopic examination of the caudal dark muscle, slides M 16 and 17.—The intercellular fat has disappeared, or is limited to a tiny droplet here and there in the connective tissue (1/12 oil immersion examination). There are no larger drops or groups of droplets as in the trunk pink muscle. The myocommata still have some fat drops.

The intracellular fat is present in the dark caudal muscle, but the liposomes are extremely small in size. There are no fibers with the larger liposomes characteristic of the normal dark muscle. The smaller liposomes average only 0.4 to 0.8 μ. In a small area which contains more fat, the liposomes are larger, from 0.4 to 2 μ in diameter. These liposomes are in a group toward one side of the fiber in an area about 20 μ square. The center of the fiber has the smaller liposomes, and there is also a very marked irregularity in the number in different parts of the field.

In the above fiber and in four others in the immediate neighborhood there are small fat droplets under the sarcolemma, measuring 2 to 3 μ, but in each case these droplets are on the side opposite the adjacent blood vessels. The liposomes throughout the central portion of the fibers in fields in which the fat is evidently sharply removed are reduced to scarcely distinguishable size, but are comparatively numerous. On the surface of such fibers the liposomes are about 0.6 to 0.8 μ in diameter and also numerous. Certain portions of the section show the fibers turned horizontally. Liposome chains can be distinguished in these fibers. In one such fiber the liposomes of the chains are about 0.6 to 0.8 μ in diameter. There are no fused liposomes in this case. This caudal muscle does not have more than one-half to three-fifths the fat of the trunk muscle.

DISTRIBUTION OF THE FATS AT A LATE INTERMEDIATE STAGE OF THE SPAWNING MIGRATION.

A study was made of the amount of fat present in the tissues of salmon from the Columbia River at the Celilo Rapids. These fish have passed through a longer stretch of fresh water and through the relatively swift currents of the canyon of the Cascades. The famous fishery of Mr. Frank A. Seufert extends along the full extent of The Dalles of the Columbia.[a] The numerous fish wheels adapted for the different stages of the water make it an ideal collecting ground for scientific material. At the time of the visit to the fishery in August, 1911, active fishing was in progress on the lower Dalles, at Celilo Falls, and at the Tumwater seining grounds. The samples that were studied came from a point known as the Cement Wheel, also from the seining grounds at Tumwater immediately below the Celilo Falls. The Cement Wheel is about 300 yards above the mouth of the Government canal.

The Cement Wheel salmon will have battled only a short distance of the swifter portion of the rapids of The Dalles. Two salmon were taken from this point, a male and

[a] Mr. Seufert has always taken an active interest in the scientific questions concerning the propagation of the salmon and in work tending to develop and protect the industry. He aided the present work by putting at our disposal every facility for securing material in the best of condition.

female. The protocol for no. 127 is the male. By reference to the protocol it will be seen that the trunk pink muscle contains a medium amount of intermuscular fat. The fat droplets have a distribution that is normal, but the relative size of the droplets is small so that the amount of fat represented is evidently greatly decreased as compared with the standards from Ilwaco.

The intramuscular fat of the trunk pink muscle was present in much lower amount than in the fatter specimens from Warrendale but also in greater amount than in the poorer specimens. The characteristic of these Celilo fish in this regard is in the relatively low fat in the larger pink muscle fibers. Only scattered traces of intramuscular fat were found in any of these fibers, and those traces usually in the neighborhood of intermuscular fat.

Keeping in mind the condition of the dark muscle from the salmon from Warrendale it was to be expected that the corresponding tissue in the Celilo salmon would have a considerable loading of fat. This was found to be the case. The specimens from the Cement Wheel agreed in that the dark muscle fat was definitely diminished in quantity in comparison with average fish from Warrendale, and sharply diminished in comparison with Ilwaco specimens. The samples chosen were the average of the type running at that time, August 22 and 23. The grade of fish at this season is very much lower than at an earlier date, but this is a factor which characterizes the entire series studied and does not interfere with the comparison.

The caudal pink muscle was practically free of fat in each of the specimens studied. The caudal dark muscle had both intermuscular and intramuscular fat, but in each instance about 40 to 50 per cent as much as in the trunk muscle of the same specimen. The female of the Cement Wheel specimens showed less fat in both the trunk and caudal dark muscle than was present in the muscles of the male, notwithstanding the fact that the male gave other evidences of a greater retrogression in general than did the female.

Little or no variation could be shown between the salmon taken at the Tumwater seining grounds and those taken at the Cement Wheel. In each instance there was a fair showing of intermuscular fat in the pink muscle, and an amount of fat within the fibers of both the pink and dark muscle which characterize an approximately average grade running below the Cascades.

At the time of this visit no fish were running which were judged to be of as high a grade as no. 120 and no. 122, described in the protocols from Warrendale.

PROTOCOL.

Male salmon (no. 127), length 800 mm., taken at Seufert's Cement Wheel, The Dalles of the Columbia, August 22, 1911.

The fish was silvery in color, with dark dorsal surface; shape that of a half-exhausted specimen; flesh oily in appearance and to the touch; visceral mass small and degenerated.

Microscopic examination of the trunk pink muscle, I.—The intermuscular fat is medium in quantity. There are a few large droplets, the largest 40 μ in diameter, but many smaller droplets, especially of the size from 3 to 6 μ in diameter. The amount of fat is much less than in the specimens like no. 118 from Ilwaco, estimated at 40 per cent. The distribution of the intermuscular fat varies in different parts of the preparation. In certain parts the amount is not more than 25 per cent that described above.

The intracellular fat varies extremely in the different fibers. In the small fibers, size 40 to 70 μ in diameter, the fat droplets are fairly numerous but of larger size and greater number around the surface of the fiber. These fibers (1/12 oil immersion) show that the fat droplets aggregated around the surface are within the sarcolemma and superficial. In the center of the fibers the liposomes are much smaller, about 0.3 to 0.5 μ. There are droplets under the sarcolemma in practically all these pink fibers. They measure from 0.8 μ up to 2 μ in diameter. The average amount of intramuscular fat in the small fibers is not more than 40 to 60 per cent of that found at Warrendale. Many of the intermediate-sized fibers show only traces of fat in the center of the section. Around the circumference there is a somewhat greater quantity of fat, especially just at the surface. The largest fibers in the section have irregular outlines, look compressed and are very clear of fat, at least these fibers do not contain fat that stains in the usual way. I notice an occasional small group of liposomes at some point on the surface of the section, though these groups are few, often not present at all.

An appearance that is difficult to interpret is due to the presence of very small highly refractive granules in the protoplasm of the large fibers. These granules do not take stain, at least, if they are stained at all, it is very different in appearance from the normal, and they do not appear to be uniformly present in all the large fibers.

Teased muscle (M38 and 39) shows a comparatively small amount of fat. The smallest fibers and some of the intermediate fibers have chains of liposomes. The chains in the small fibers are not so numerous as one usually finds. The liposomes themselves are very small, and the picture is one of low content of fat. In the intermediate fibers the chains of liposomes are more numerous near the surface of the fiber. Just under the sarcolemma there are groups of small fat droplets rather irregular in arrangement. In the larger fibers of these slides it is difficult to distinguish the chains except at the very surface.

Microscopic examination of the caudal pink muscle.—The fibers of the caudal region are closely packed together and are very free of fat (section M46). The connective tissue septa have strands of fat droplets, most of them small but some medium in size. These measure from 2 to 15 μ, chiefly the former size.

The caudal pink muscle fibers are practically free of intracellular fat. Certain ones show traces of fat at points on the surface, but these are only traces and are limited to areas bordering on the fat-bearing septa. Extensive areas with no septa between the fibers are free of fat.

The fibers are so compact that their outlines in cross section are irregular polygons.

The caudal pink teased muscle shows practically no fat in the fibers, traces only appear. Certain of the fibers have a slight bluish tinge and through their substance are opaque granules which are difficult to identify. These granules are in the interfibrillar spaces.

Microscopic examination of the trunk dark muscle.—The trunk dark muscle still retains a large amount of fat (slide M41 under the oil immersion). Drops between the fibers measure on an average from 6 to 9 μ, occasionally as much as 15 μ.

Fat is distributed throughout the substance of the fiber. The droplets vary greatly in size. The largest ones run from 2 to 2.6 μ in diameter. These are more numerous around the superficial portion of the fiber in most of the material, though groups of fibers are found in which these large liposomes are quite evenly distributed through the substance. In numerous fibers the central portion is relatively free of liposomes and the fibers look lighter in color under the microscope. In the light areas, however, there are liposomes present, though they are very small for dark fibers, 0.6 to 0.8 μ, and they are not as numerous as in the superficial border. Different portions of the section vary greatly in the amount of fat. The muscles freest of fat are those which lie along the septa which carry blood vessels. Under a low magnification these areas are sharply limited.

Teased fibers (section M44) give a good view of the amount of fat along the course of individual fibers. There is much variation in different lengths of one and the same fiber. In the fatter areas numerous groups of fat droplets lie over the surface of the teased fibers. These groups have a configuration such as was noted in similar muscle from specimens from Warrendale. Undoubtedly the arrangement of fat bears a definite relation to the arrangement of blood vessels. The chains of liposomes are continuous in some areas for long distances. The individual liposomes will measure 2 μ in diameter in the larger chains, but vary through a range of much smaller sizes according to the relative amount of fat present. The larger chains are obviously near the surface of the fiber. Occasionally a chain is noted in which the majority of the liposomes have fused, forming a fat rod such as has previously been described. These rods, as observed, lie near the surface of the fiber.

Microscopic examination of the caudal dark muscle.—The dark tail muscle has enough fat to give it a relatively deep stain (M56, oil immersion), but this fat is much less in quantity than in the trunk muscle of this salmon. The fat is condensed around the superficial areas of the fiber. Apparently there is some intermuscular fat in droplets 3 to 6 μ in diameter.

The intramuscular fat is arranged practically the same as in the trunk muscle, except that the size of the droplets is smaller throughout. Droplets 2 μ in diameter are relatively rare and the liposomes in the chains in the middle portion of the fibers run about 0.5 to 0.8 μ in diameter, about the size of the liposomes in the relatively fat-free fibers of the trunk.

The teased fibers give an explanation of the diffuse appearance of the stain in this section (M58–59). It is due to the extremely small but numerous liposomes. Liposomes are also present in groups just under the sarcolemma in many places. In the largest chains observed in fibers that seem relatively better supplied with fat the liposomes measure from 1 to 1.2 μ in diameter.

FAT IN THE TISSUES OF SALMON FROM THE SPAWNING GROUNDS.

Salmon at the spawning grounds in the Clackamas River, at Cazadero, Oreg., have been fasting from three to four months. During the entire time there is no food source for the energy which the salmon have expended other than in the tissues and in the stores of fat. One would certainly expect the great fat storehouse to be sharply drawn on if not exhausted by the time this stage of the life cycle is reached.

We have taken fish from the spawning grounds so emaciated and so weakened that they were scarcely able to maintain equilibrium in the river currents. One knows that some of these fish would die in a day or so even if they were kept in the water in the most carefully protected condition.[a] Fat is still present in the muscular tissues of such salmon. Its percentage is low, yet the microscope reveals an unexpected quantity.

There is considerable variation among the different individuals present at Cazadero. All of the early September spawners are undoubtedly of the so-called spring run of salmon. They have been in the river at least since May or June (estimated). Yet there are specimens that have very much more fat than the dying salmon mentioned above. These variations are shown best in a comparative study of the various types of muscle.

Trunk pink muscle.—The preparations of pink muscle show a sharp contrast with the loading of fat observed in the normal fish and in fish from the mouth of the Columbia River. Instead of the large intermuscular fat globules there are only small droplets present and these are few in number. They are located in the angles in the larger connective tissue masses which mark the points where several fibers are grouped. If two fibers are compactly pressed to each other there are no fat droplets between them. Where several fibers are separated by masses of connective tissue there may still be small groups of fat droplets. In such cases the fat droplets are small in size and few in number. In one specimen, no. 131, the number of intermuscular fat droplets averages about one droplet to ten fibers, and the largest droplet present measures only 6 μ in diameter. This quantity of intermuscular fat is insignificant.

The presence of a significant amount of intramuscular fat in these most exhausted specimens from the spawning grounds is in striking contrast to the disappearance of the intermuscular fat. The intramuscular fat is in minute liposomes less than 0.5 μ in diameter. The largest liposomes are in the smallest fibers. They are scattered throughout the substance of the fibers except in the surface band of fibrillæ, where the absence of interfibrillar fat gives the appearance of a clear band of fibrils around the superficial border of the muscle fiber as seen in transverse section. Around the surface of the pink fibers and under the sarcolemma are groups of fat droplets. Where

[a] Males were selected from those retained in the spawning pens. In one instance salmon for study were selected from those most advanced in retrogression yet with no obvious fatal lesions. Of those not used three had died by the next morning. This is positive evidence that the salmon selected were at the dying stage.

two fibers touch each other a double row of droplets slightly separated can be easily distinguished. The separating line, of course, is the section through the sarcolemma. The largest fat droplets in this series under the sarcolemma measure as much as 2 μ in diameter. Often the droplets are slightly compressed, evidently by the pressure of the sarcolemma, since the radial diameter is a little less than the diameter tangent to the fiber.

Taking the pink fibers as a whole it seems that in the better specimens the liposomic fat is present in greater number of droplets, also in larger droplets, than in the poorest specimens from Warrendale (compare fig. 10 and 11). Certainly this fat is greater than in no. 125 and no. 126. In fact, the comparison is close to those fish which have the highest quantity of intracellular fat at stations lower down the river. In the poorest fish taken from the spawning grounds the fat is almost completely eliminated both from the intermuscular and intramuscular regions. This is true for fish no. 140, which has the lowest amount of fat observed in the lateral pink muscle.

The pink muscle fibers themselves are not plump and round in the fish from the spawning ground. On the other hand, they form irregular polygons in cross section. Even the smaller fibers have lost their cylindrical shape. The fibers are more compact and the whole appearance suggests a diminution in volume (fig. 19, pl. XI).

In teased preparations there is one rather striking deviation from the typical arrangement of liposomes, namely, the deep-lying liposomes are no longer in such regular spindle-shaped rows as are found in the normal. The chains have the appearance of broken rows, in which the smaller liposomes are absent, thus giving the chains an irregularity that is rather constantly observed in the fish of this station. Those chains that are most definite and least interrupted are clearly located near the surface of the fiber. In the teased material from no. 139 there is a marked difference in the appearance of the liposomes present in the small fibers as compared with the larger. In the larger fibers the chains are less numerous and the droplets in the chains smaller. In this fish the small fibers, 40 to 50 μ in diameter, have very evenly distributed liposomes, the diameters varying from 0.3 to 0.7 μ. In certain fibers of this section there are irregularly placed highly refractive bodies which (1/12 oil immersion) are only lightly stained. These granules are probably associated with an early stage of degeneration.

These teased fibers also show irregular patches of liposomes over the surface of the fiber and under the sarcolemma. These fat droplets are a trifle larger than those within the sarcoplasm. In salmon no. 140 we still have a small amount of fat under the sarcolemma. In the larger fibers the intracellular fat is present only in traces, the liposomes being not over 0.2 μ in diameter and in very short, irregular, and scattered chains. In the smaller fibers of the material the number of liposomes is still relatively slight, but the size of the individual liposomes is somewhat larger. Where a liposomic chain is present it is noted that the arrangement of individual liposomes is very irregular, giving the chain the appearance of being broken.

Caudal pink muscle.—The pink fibers of the caudal region have as nearly no fat as in any specimen examined. The intermuscular fat is completely eliminated, while only a trace of intracellular fat is to be found. The teased preparations show that this trace is made up of definite but tiny liposomes which are only sufficient in quantity to give a faint stain to the section. Now and then in a fiber near the surface one can note a few small liposomes in irregular groups. At best this quantity and distribution can be called only a trace.

Trunk dark muscle.—In the dark muscle there is no intermuscular fat, or if any is present at all it is in tiny droplets in those localities which contain the greatest amount of connective tissue. In a cross section and in a low-power field one might see two or three such areas.

The intramuscular fat in the dark muscle has markedly changed in its appearance and arrangement. The relatively large droplets characteristic of the normal tissue, often measuring as much as 6 μ in diameter, have practically disappeared in this stage. In place of the large droplets the dark muscle now contains a much greater number of relatively very small sized droplets of the type described as liposomes. Also the fat droplets under the sarcolemma are now reduced to liposomic size, not larger than 2 μ in diameter and averaging about 1 μ. In a cross section these tiny fat drops around the surface of the fiber and within the sarcolemma form definite rings which mark the outlines of the fibers. Examination under the 1/12 oil immersion shows that many of the droplets entering into the composition of this superficial layer of fat are wedged in between fibrillæ. Within the central substance of the muscle the liposomes are now interfibrillar in arrangement. They are small in size but numerous and comparatively evenly distributed through the sarcoplasm. This description applies to the trunk dark muscle of the fatter spawning salmon. The poor salmon do not have so many liposomes in the deep sarcoplasm and different individual fibers vary greatly in their fat content.

In the teased dark muscle fibers from the trunk it is noted that the most fat lies just under the sarcolemma, but that it is very irregularly placed. The droplets are small and seldom exceed 2 μ in diameter. There are a few chains of liposomes in the body of the fiber, but these chains are widely separated and consist of extremely small liposomes. The largest liposomes are about 0.4 μ in diameter in fish no. 139. In no. 140 the surface of the fibers has irregular fat droplets often running as much as 3 μ in diameter. But distributed through the substance of the fiber there are only traces of fat except at the very superficial sheet of sarcoplasm where the liposomes are measurable.

Caudal dark muscle.—In the caudal dark muscle there is still some considerable quantity of intercellular fat, especially in salmon no. 138, although this fat is less than in the lateral dark muscle of the same specimen.

Within the sarcoplasm of the fibers there are numerous areas in which there are only traces of fat. Even at the surface of the fiber there is often only a trace of fat. Under the sarcolemma the fat is in isolated groups of liposomes measuring only a fraction of a micron.

In fish no. 139 there is more fat in the caudal dark muscle, especially under the sarcolemma, where the droplets measure from 1 to 3 μ. Different portions of the section show great variation in the amount of intracellular fat. These areas are similar to that noted in Warrendale fishes no. 125 and no. 126. In fibers bordering on these vascular areas the fat droplets are removed from under the sarcolemma, and are absent except for traces in the body of the fiber.

In comparing the amount and distribution of fat in the Cazadero specimens it is obvious that the total percentage amount of fat is profoundly reduced. On the other hand, it is apparent that this reduction has taken place chiefly in the intercellular fat. The extreme case of exhaustion shows practically no fat either between or within the fibers. Yet the majority of the salmon taken at Cazadero show on the average as much intracellular fat as is shown in those salmon taken from the Columbia River at a much earlier stage in the migration. Certainly they show as much fat in the fibers as all but the very fattest of the earlier specimens. It is this showing which presents such a striking factor in the comparison between the pink muscle of different salmon at the various stages. The pink muscle maintains a surprisingly large amount of intracellular fat throughout the whole series of stations, even when the fat is practically eliminated from the great storage depots.

In the case of the dark muscle, which at the early stages is surcharged with fat, there is an obvious gradual diminution from the mouth of the river to the spawning ground. On the other hand, there is no complete elimination of fat below that stage of smallest liposomes which characterizes the pink muscle as a type. The fat may be eroded from the dark muscle; that is to say, the large drops will gradually decrease in size but will never be completely eliminated. There is some factor operating which maintains a supply of liposomes in the active muscle of the major portion of the body. It is true this supply is not kept up in the caudal muscle, but this undoubtedly is due to the great and continuous activity of that musculature.

Cheek muscle.—The amount of fat in the cheek muscle has been described for fish from Ilwaco, but this particular type of muscle has not been studied in all the intermediate stations. However, in one specimen, no. 140 from Cazadero, this muscle has been carefully reexamined. The fibers of the muscle of this fish are even more compact than noted at Ilwaco. There is only a small amount of interstitial connective tissue and this carries a few scattered but small fat droplets.

The intracellular fat is present only in traces. In a large proportion of the fibers no fat can be distinguished; yet in a few of the smallest fibers merest traces are discernible.

The striking characteristic of the cheek muscle of no. 140 is found in the evidences of degeneration. Certain of the fibers take a definite stain not due to the fat, but due to characteristic degenerative changes in the fibers. These fibers stain a light rose pink. Under the oil immersion the fibers that take this special stain show signs of disintegration or atrophy. The bodies of the fibers have greatly shrunken. Their outlines show that they are compressed as if between adjacent fibers. The fibrillar structure has likewise disappeared. Slight vacuoles are present. The most diagnostic feature of the change consists in the pigment granules of muscular atrophy. These pigment granules are irregularly placed and vary greatly in size. Measured with the 1/12 oil immersion they vary between 0.1 and 0.2 μ in diameter. The degenerative changes noted are typical of simple atrophy. The changes in this particular muscle are the most advanced that have been noted. There is some slight indication of atrophy in the trunk musculature even at an earlier stage of the journey, but nowhere else have I found definite degenerative pigments, unless the highly refractive bodies noted in no. 125 and no. 139 be such.

The further details of these degenerative changes are being studied and will be presented later in a special report.

ANALYTICAL DETERMINATIONS OF THE PERCENTAGE OF FATS IN THE SPAWNING SALMON.

The three Cazadero salmon of this series from which samples were taken for fat percentage determinations reveal a larger per cent of fat than one would, a priori, expect.

On the whole, they confirm the microscopic findings. It is unfortunate that no fat percentage determinations were made on no. 140, the only salmon that presented in the masseter muscle definite and unmistakable evidence of muscle degenerations. This series should be compared with the Ilwaco series in table 1, page 92.

TABLE II.—ANALYTICAL DETERMINATIONS OF FATS IN THE TISSUES OF CERTAIN SALMON FROM CLACKAMAS RIVER, CAZADERO, OREG., TAKEN SEPTEMBER, 1911.

No. and sex of fish.	Muscle fats in percentage.		Remarks.
	Pink muscle.	Dark muscle.	
134♀	2.870	6.719	Spawned.
137♂	6.139	9.662	Spawning.
138♂	3.332	7.962	Spawning, one-half to three-fifths spawned.

PROTOCOLS.

Spawning female salmon (no. 132), length 960 mm., weight 6,840 grams (after artificial spawning).

External appearance first class, body slender in form. An appreciable oedema of the inner surface of the body cavity walls. Visceral mass exceptionally small.

Microscopic examination of the trunk pink muscle.—In a small portion of section N47 there is a very small quantity of intermuscular fat, but in general neither the connective tissue septa nor the thicker strands separating the fibers have more than tiny liposomes. There is a sharp contrast between this fish and no. 127 from Celilo in this regard. These fat droplets in the connective tissue measure a maximum of 5 to 6 μ. The average size is very much smaller, between 1 and 2 μ. The trunk pink fibers of this salmon are somewhat more plump and round in outline than in no. 131. All the larger fibers, however, are compressed and irregular in outline, suggesting the same type of change noticed in other relatively fat free tissues.

There is intramuscular fat in the smaller fibers and in the medium in the form of liposomes. These liposomes are distributed rather uniformly through the substance of the smaller fibers. In the medium-sized fibers there are not so many liposomes and they are much fewer in the center of the fibers. Around the circumference of the fibers and under the sarcolemma there is more fat, especially under the sarcolemma. These small and intermediate fibers have sometimes almost complete rings of fat droplets under the sarcolemma. The largest fibers have a greatly reduced quantity of fat. The fat is in very much smaller liposomes, often scarcely visible. Around the surface and under the sarcolemma there are groups of fat droplets, but not so plentiful as in the medium fibers.

This material on the whole is characterized by the small amount of intermuscular fat and the relatively great amount of intramuscular fat. In some portions of the sections the intramuscular fat is as great as in fish no. 118 from Ilwaco, far greater than in no. 126 from Warrendale or no. 127 from Seuferts Cement Wheel. The uniformity of distribution of the fat is not so great as in the Ilwaco specimens.

Microscopic examination of the caudal pink muscle.—There is no intermuscular fat in this section (N59). In the myocommata shown there is a trifle of fat just at the surface.

The muscle fibers have some intramuscular fat, but the relative amount in the different types of fibers is difficult to determine, on account of the excess of precipitate present. Some of this so-called precipitate is in characteristic round granules like unstained fat, but some of it is in the characteristic scarlet red color, interpreted as a less successful staining manipulation.

Microscopic examination of the trunk dark muscle (slides N53–57).—These sections all show a relatively small amount of fat in the myocommata.

The muscle tissue as a whole shows much fat in the critical region at the surface of and between the fibers. Under the oil immersion it is apparent that a large amount of this fat is between the fibers in droplets from 2.5 to 3 μ in diameter. The muscle fibers are so compact in arrangement that it is difficult to identify the exact limits of the fiber. Certain unquestioned regions show this fat between the fibers. In other regions one can as definitely say that there is fat beneath the sarcolemma but outside the substance of the fiber. At the border of the section a number of fibers have been slightly split apart and some turned in a horizontal position. These fibers confirm the above.

As a rule, through the section there is only a small amount of intramuscular fat in chains of liposomes through the bodies of the fibers. In the horizontal fibers the size of the liposomes is shown to be from 0.4 to 1.5 μ in diameter. The majority of the liposomes are of the larger sizes.

The striking characteristic of this tissue is the great differentiation as between the amount of fat in the body of the fiber and at the surface. There is apparently more fat in the dark muscle of this fish than in fish no. 128 and no. 129 from Celilo, undoubtedly more than in fish no. 131 of Cazadero, but the bodies of the fibers contain relatively less.

Microscopic examination of the caudal dark muscle.—The amount of intermuscular fat is greatly reduced over that of the trunk muscle, the droplets are smaller, and they are not so numerous as in that region. They are, however, sufficient to give a mosaic-like appearance to the section.

The intramuscular fat is extremely small except such as lies just under the sarcolemma. Certain of the sections show the ends of the fibrillæ clear and sharp. The pattern is the same as shown in figure 7, plate v, except that there are no fat spaces present. This certainly indicates that the muscle has not degenerated, yet nearly all the fat characteristic of the normal muscle is absent and there are only a very few liposomes in the fibers near the surface.

Spawning male salmon (no. 138), length 840 mm., weight 7,730 grams, from the spawning pens of the United States Fisheries Station, Cazadero, Oreg., on the Clackamas River, September 4, 1911.

This male salmon was one-half to three-fifths spawned. Color brassy, with black spots. Soft dorsal decayed and one fungus spot on dorsal fin. A fish in good condition but in late stage of exhaustion. It would probably have died in the course of 24 to 36 hours.

Microscopic examination of the trunk pink muscle.—The pink fibers of salmon no. 138 are not plump and round as in fish no. 118. On the other hand, their outlines form irregular polygons. Even the smaller fibers have this shape. The larger fibers bear histological evidence of great decrease in size (samples one day in formalin). A set of cross-sectional measurements show the following: 40 by 80, 60 by 70, 80 by 140, and 100 by 220 μ, outlines all very irregular. The cross sections show both the striations and the fibrillæ very nicely.

The sections are free of intermuscular fat except for a few of the finest droplets and liposomes. In a large section there is one such group of fat droplets in a large mass of connective tissue. There are a few thin strands of connective tissue between the fibers, and these carry occasional fat droplets not over 3 to 4 μ in diameter. The larger size is rare, though fat drops in the same locality in no. 118 from Ilwaco measure 100 μ and more. There are a good many tiny liposomes in this connective tissue, though the mass is extremely small.

In contrast to the dearth of fat between the fibers there is an unexpectedly large quantity of intramuscular fat within. As usual this fat is in liposomes. The size and number are both greater in the smallest fibers, yet the largest fibers have a pretty even sprinkling of liposomes of extremely minute size. I can not find a single fiber but that has some fat within its protoplasm. In the smaller fibers there is a great amount of fat around the surface in the region just under the sarcolemma. Taking the small fibers as a whole, it seems that the liposomic fat is present in a greater number of droplets and in slightly larger droplets than in the poorer specimens from Warrendale. There is decidedly more intramuscular fat than in fish no. 126 from Warrendale.

The teased fibers give a beautiful confirmation of the notes made on the cross sections. They show one variation from the typical arrangement of liposomes, namely, that the deep-lying liposomes are not in such regular rows as in the normal. The liposomes have a very irregular appearance, as if the smaller liposomes had disappeared. Those liposomes that are left are unusually large and uniform in size for pink fibers. The rows that are most definite and uninterrupted are located near the surface of the fiber. The larger liposomes are from 2 to 2.5 μ in diameter.

Microscopic examination of the caudal pink muscle.—The caudal pink muscle sections are free of all but traces of fat. The intermuscular fat is limited to a few droplets in the myocommata. Only a few irregular chains of very small liposomes are present in the fibers. These are more distinct in the smaller fibers. The striations are distinct and clear, but the fat is faint and scarcely distinguishable.

Microscopic examination of the trunk dark muscle.—The dark trunk fibers are very compactly arranged, so that it is difficult to distinguish between the intermuscular fat and subsarcolemmal fat. But it is apparent that there is a small amount of intermuscular fat. This is confirmed by the connective tissue of teased muscle. The drops are of liposomic size, rarely more than 2 to 2.5 μ in diameter.

The intramuscular fat is present in the usual localities. Under the sarcolemma the droplets are medium in size, 1.7, 2.8, 3, and 5.4 μ in typical droplets. The sarcoplasm of the fibers is rather evenly studded with unusually small liposomes for this type of muscle. The liposomes are even smaller than in the trunk pink muscle, are condensed about the surface of the fibers and are larger there, 0.4 to 1.2 μ. In the body of the fiber there are fewer liposomes and the size is from 0.2 to 0.8 μ.

The teased dark fibers confirm the above notes as to the fat disposal. The fibers have exceptionally clear striations which average 2.6 μ in length. The fat is irregular and the liposomes of the chains can not be followed in relation to the striations. The fat under the sarcolemma is in patches in these fibers. The whole appearance of the dark fibers closely approaches that of the pink fibers of this salmon.

Microscopic examination of the caudal dark muscle.—In the caudal dark muscle there is still enough fat between the fibers and under the sarcolemma to give a distinct mosaic-like marking of the outlines of the fibers. In some portions of the sections this surface fat is gone.

The intramuscular fat is still much less than in the trunk dark muscle. There are fibers that have the merest trace of liposomes in the muscle substance. Even in the fattest fibers only minute liposomes are present, the smallest amounts observed in this type of muscle, except in no. 140. There is the sharpest contrast between this tissue and the Ilwaco type.

Spawning male salmon (no. 140), length 980 mm., weight 8,070 grams.

This fish was selected as a type of spawning fish in good physiological condition but at the last stages before death. It was taken at the United States Fisheries station at Cazadero, Oreg., on the Clackamas River, September 6, 1911.

Microscopic examination of the trunk pink muscle.—There are traces only of intermuscular fat, which is in fine liposome-like droplets in the larger connective tissue strands and in the myocommata.

The small fibers still contain a sprinkling of liposomes, enough to give them a decidedly pink appearance under the low power. The 1/12 oil immersion shows that these liposomes are gathered chiefly around the superficial border of the fiber and that they are in groups in the neighborhood of intermuscular fat. The largest liposome observed in a small fiber measured 1.4 μ in diameter, but such are few in number. The average size of liposomes for these small fibers is only a fraction of a micron.

In a large fiber under the oil immersion there are a few areas of fat in irregular-sized droplets under the sarcolemma, traces in contrast with Ilwaco salmon. Through the body of the fiber there are chains of finest liposomes 0.1 to 0.2 μ in diameter, but the chains are short and irregular. In two other typical fibers in the field, one medium and the other rather small, the liposomes are present in about the same number of chains as in the large fiber, but are slightly larger in size. In all these fibers the particular characteristic feature is the irregularity in the chains of liposomes. The individual chains have not the usual arrangement of larger liposomes in the middle of the chain and the size tapering down to small ones at the end of the chain. They are irregular in size throughout the chain.

Microscopic examination of the caudal pink muscle.—There is practically no fat in this caudal muscle. The trace that is present (1/12 oil) is only enough to give a faint stain to the superficial border of occasional fibers. There are scattered and irregular groups of a few small liposomes just at the surface of the fibers. Several fibers that have been turned horizontally show no chains of liposomes.

Teased fibers show no liposomes in the body, but occasional traces of liposomes just at the surface of the fibers. These traces are definitely between the sarcolemma and the sarcoplasm, an arrangement most often noted in pink fibers poor in fat.

Microscopic examination of the trunk dark muscle.—The outlines of the trunk dark fibers are marked by rather heavy rings of fat droplets. These markings are least prominent in the neighborhood of vascular areas. It is not always possible to distinguish between intermuscular and subsarcolemmal fat. Both are present. Most of the fat observed is judged to be under the sarcolemma. The fat drops between the fibers range in size from 3 μ to 8 μ.

The fat under the sarcolemma seems to be in rather small but numerous droplets. This fat runs from 2 to 4 μ in diameter. Out in the body of the muscle fibers there is a variable arrangement of liposomes. In some fibers they are uniformly distributed through the substance of the fibers; in others there is apparently no fat in the middle of the fiber. In general, liposomes are present in the superficial areas even in those fibers which have the least fat. The whole appearance suggests a nutritive balance in which it is just a question whether all the fat will be used or whether there will be an excess sufficient for deposit.

The teased muscle shows great variation in the quantity of fat in the individual fibers (1/12 oil). At the surface of the fiber is a good deal of fat in small droplets, the subsarcolemmal fat. The liposomes in the chains are small like those in normal pink muscle. The number of chains is also low. In the fibers carrying the least fat these chains are all but absent.

Microscopic examination of the cheek muscle.—The muscle fibers of the cheek muscle are very compactly arranged. In comparison with the Ilwaco type the fibers are less rotund in outline. The histological structure is indistinct. In many fibers the fibrillæ can not be seen because of a disintegration which marks the first stage of degeneration. Certain fibers scattered irregularly through the section show a definite protoplasmic degeneration with vacuoles and pigmentation. The pigment granules are small in size, 0.1 to 0.2 μ in diameter. They are unevenly distributed through the fibers and apparently somewhat greater near the surface. There is great variation in the quantity of pigment in different individual fibers.

The fat in the cheek muscle is limited to a very few small groups of intermuscular fat. No traces of fat could be distinguished within the fibers themselves.

SIGNIFICANCE OF THE OBSERVED CHANGES OF THE AMOUNT OF FAT.

It is obvious that the salmon fat furnishes the food during the migration fast. The revelations of the microscope are convincing on this point, even if there were no collateral supporting evidence.

My unpublished chemical analyses of the tissues have revealed a dearth of carbohydrates in the salmon tissues at all stages of the migration. This fact is of vital significance in connection with the fat problem. The lack of carbohydrates and the abundance of fats support Miescher's assumption that fats furnish the source of the muscular energy expended by the salmon during the migration. In connection with a series of salmon-feeding experiments [a] it was shown that the salmon liver exercises a distinct lipogenic [b] function during the feeding and growing stage. Noël Paton has found that the amount of fat in the liver of the frog is increased after fat feeding.[c] It seems to me that in animals like the salmon the lipogenic function of the liver becomes a primary function, taking a rôle quite comparable to that of the glycogenic function of the organ for many mammals. Fishes of this class are carnivorous. Their food is of a highly oily character, as is also that of certain birds, and is continuously so. The food is rich in proteins and fats and in inorganic constituents, but it is poor in carbohydrates. In the adaptations to such a diet, if for no other reason, the salmon has reached the point in its phylogenetic development where fats furnish a direct and primary source of foods for the energy

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[a] Now in manuscript.

[b] It was Loevenhart (American Journal of Physiology, vol. 6, p. 331, 1901) who first advocated the idea that we might have a "lipogenesis" in the body comparable in character to the "glycogenesis" of Claude Bernard. He suggests that wherever there is fat storage there will be lipase, and proves it by investigations on a number of tissues that contain fat, for example, the liver, mammary gland, pancreas, brain, spleen, heart muscle, blood, adipose tissue, etc. He says: "In the case of fats the areolar tissue is the great primary store, secondary deposits being found in all the tissues. In some animals even this difference in the storing of fats and carbohydrates is not to be noted. In many fish, notably the cod, the liver, at certain seasons of the year, becomes the great depository for fat. The liver we have found to possess powerful lipolytic activity, and hence, under proper conditions, it should be capable of storing fat. Moreover, this is in accordance with the experiments of Noel Paton, who showed that the fat contained in the liver of frogs is increased after a fatty meal. It is believed that both phases of lipogenesis are induced by lipase, a fat-splitting and fat-forming enzyme." From my observations I am convinced that lipogenesis is a definite and specific function of the liver in certain carnivorous animals whose normal food consists of a high percentage of fat, as is the case in the king salmon.

[c] Paton, D. Noël: On the relationship of the liver to fats. Journal of Physiology, vol. XIX, 1896, p. 167.

liberating tissues. The tissues, in short, can utilize the energy of fats by direct oxidations. It remains to examine the facts submitted and to discover, if possible, the mechanism whereby this great store of salmon fat is rendered so labile and so wonderfully efficient in the execution of the activities of this last lap of the salmon life cycle.

Of all the facts presented it seems to me the most significant are:

I. The appearance of intramuscular fat in the pink muscle at the beginning of the spawning migration, and

II. The maintaining of a relatively uniform distribution of this fat in the fibers until the death of the animal.

Just as soon as the salmon ceases to feed, and the products of digestion no longer reach the active musculature, then, and not until then, there is thrown into the pink muscle fibers a supply of fat adequate to the energy needs of this most critical period in the salmon life cycle. The excess of fat is deposited in an extremely finely divided state and is brought into intimate contact with the fibrillæ, one can almost say with the sarcous elements. Certainly in many rows of fat droplets between the fibrillæ the individual liposomes are in close approximation with corresponding sarcous elements in the fibrils. One can not escape the inference that this microscopic emulsion of the fat, its general distribution throughout the muscle fiber, and the intimate relation with the elemental fibrillar structure, all point to an immediate utilization of the fats in the production of muscular energy.

This hypothesis is further supported by the observations on the cheek muscle and on the fin muscles, all muscles in much more uniform, though less intense, activity than the lateral muscle. These muscles carry a light but strikingly persistent load of minutely divided intramuscular fat during the entire migration. They never have a great excess of storage fat, either intermuscular, as in the pink muscle, or intramuscular, as in the dark muscle.

The great fat storehouses are the intermuscular fat of the great lateral pink muscle, the inter- and intra-muscular fat of the dark muscle, and the fat of the adipose connective tissues. With the cessation of feeding no further fats, proteins, etc., are brought in as foods. With the external food supply now shut off the physiological mechanism of the salmon must turn to the food materials on hand, to the internal food supplies of the salmon's own body. The internal supply is limited to body tissues as such, and to the fats. It is the fats that are immediately drawn upon. From the fat deposits the fat is gradually but regularly transported to the active muscles, where it is maintained in a uniform and favorable distribution, and in amount adequate to supply the energy expended by the salmon in the migration fight against the currents and rapids of the rivers on its way to the spawning beds.

TRANSPORTATION OF FATS IN THE FASTING SALMON.

HISTORICAL.

The histological observations on the king salmon have given every confirmation of Miescher's original assumption, based on his study of the Rhine salmon, that the fat of the salmon can be transported from one part of the body to another; i. e., from tissue to tissue. He laid special emphasis on the utilization of the muscle fats in the building up of the fats of the ovaries, but he also suggested that fat was the source of the nourishment of the animal.[a] It is irrelevant for the present purpose that Miescher considered the source of the fat to be a fatty degeneration of the muscle tissue. The fact remains that he demonstrated the presence of intramuscular fat microscopically and for this he should receive full credit. He must also be given full credit for the conception that the salmon fat can be transported for purposes of tissue growth, ovaries, and for use in energy production, muscles. I can not find that he has offered any explanation of the detail of the processes involved in the fat transference or that he has discussed the matter, but this work is so important that the three statements he makes are quoted in full in their setting, and in his own words. On page 186 he says:

Dass wirklich der Seitenrumpfmuskel die wesentlichste Stoffquelle ist, sowohl für die Ernährung des Thieres, als für die Gesclechtsreifung, wird evident bestätigt durch das Mikroskop. Schon die Winter- und Frühjahrssalmen zeigen nämlich zwischen den feinen quergestreiften Elementarfäden (Fibrillen) der ungleich dicken Muskelfasern, besonders in den dünneren, bald mehr bald weniger ausgesprochene Fetttröpfchenreihen, wie man sie als Anzeichen sogenannter Entartung des Muskelgewebes kennt. Die Menge dieser Fetttröpfchen nimmt gerade im Hochsommer, wenn der Eierstock rascher zu wachsen beginnt, beträchtlich zu und kann bis zur Undurchsichtigkeit mancher Fasern führen. Am stärksten degenerirt eine gesonderte dünne Muskelplatte, die an der Seite des Körpers direct unter der Haut liegt (Hautmuskel). Dagegen bleiben sozusagen völlig intact und fettfrei alle übrigen Muskeln, Brustflosse, Bauchflosse, Rücken- und Afterflosse, Kiefer- und Zungenbeinmuskeln, der obere und untere Längsmuskel und die Schwanzmuskeln im engern Sinne. Nur die Bauchflosse zeigte an einigen Stellen schwache Anzeichen von Degeneration.

The one comparison as to the intramuscular loading of the fibers with liposomes as the migration time continues is given on page 213.

So findet man denn bei den Frühsalmen jene schwache, hauptsächlich die dünneren Muskelfasern in mässigem Grade betreffende Durchsetzung mit Reihen feiner Fettkörnchen, bis dann im Frühsommer das Wachsthum des Eierstocks in seiner geometrischen Progression zu einem absoluten monatlichen Stoffverbrauch führt, dessen Anforderungen neben der eigentlichen Selbstzehrung sich gebieterisch in den Vordergrund drängen und wirksamere Hilfsmittel verlangen.

When the Rhine salmon spawn they begin the return migration to the sea with the associated recuperative processes. Concerning this stage, Miescher makes the following final statement on page 215 of his monograph (page 171 of the reprint):

Wie ganz anders das Bild, wenn wir Gelegenheit haben, Thiere zu sehen, die auch nur um 10 Tage, besser um ein paar Wochen das Laichen hinter sich haben (leere Weibchen, zu Ende December oder im Januar gefangen, aber auch eines aus Herrn Glaser's Fishkästen, gewiss nicht mehr als 10 Tage von seinen Eiern befreit). Die Haut ist wieder bläulich glänzend und klar, die Geschwüre übernarbt oder in Heilung, das Fleisch durchscheinend, von Fettkörnchen völlig oder fast völlig befreit; auch die Herzfasern in Reinigung begriffen; im Darm keine Spur von Nahrung. Dagegen enthält der Eierstock bald mehr bald weniger Eier, die, in einen serösen oder auch etwas eitrigen Erguss der Follikelhaut eingebettet, sichtlich zusammenschrumpfen und aufgesogen werden.

Mahalanobis[b] in Noël Paton's report on the life history of the salmon also calls attention to the storage of fat in the muscular tissues, and to the use of this fat in the reproductive organs and in the production of energy. This author calls attention to two important things, viz, (1) he observes that the fat is present in largest amount in the muscles of fish entering the estuaries, and in least amount at spawning stage, and (2) he refutes Miescher's degeneration theory as a means of accounting for the presence of

[a] Miescher: Statistische und biologische Beiträge zur Kenntniss vom Leben des Rheinlachses im Süsswasser, s. 186, 1886; also reprinted in Die histochemischen und physiologischen Arbeiten, s. 145, 1897.

[b] Paton, D. Noël: The life history of the salmon. Article 9, by Mahalanobis, S. C., Microscopical observations or muscle fat in the salmon. Fishery Board for Scotland Report, 1898, p. 106.

the fat in the fibers. Mahalanobis considers the fat deposition as a "fatty infiltration due to increased accumulation of fat from diminished utilization in the tissues."[a] In the same paragraph he says:[b] "Figure 3 is from a fish fresh from the sea—one that had been actively feeding, and consequently its blood and lymph were rich in fat, whence, in all probability, the muscle cells absorbed fat and stored it between the fibrils." And "as already pointed out, the fat granules in fish leaving the sea are more crowded immediately under the sarcolemma (fig. 3, pl. III)." These quotations include all the remarks tending to show the author's views as to fat transferences in the salmon tissue. It is evident that he has given attention only to the mechanism whereby the fat is originally laid down in the muscle, and his conception is that the process is one of "infiltration" or absorption from the blood and lymph.

Mahalanobis is hopelessly confused in his studies by the fact that he has failed to recognize the dissimilarity of two strikingly different tissues. This is shown in the following quotation and by the figures referred to therein: "In the fish leaving the sea this accumulation of fat in the fibers sometimes reaches an enormous amount, and a thick layer occurs under the sarcolemma. This will be evident from a comparison between figure 2 and figure 3, the former being from a late fish, no. 69, and the latter from no. 79." [c] The figures given by him exhibit differences both in structure and in fat disposal which bear no relation either to the seasonal type or to the stage of fasting which Mahalanobis observed, as the studies presented here on the king salmon conclusively show. The basis of this matter is more fully discussed in this paper in connection with the description of the tissues in question. Mahalanobis compared the dark muscle fiber of his fish no. 79 with a pink fiber of no. 69. The former muscle is normally loaded with enormous fat droplets in the fiber, whereas the latter muscle never has fat in larger size than liposomes. His figure 3 from fish no. 69 is from the intermediate zone of pink fibers. Had he chosen a deeper group of fibers the dearth of fat would have been undoubtedly greater. The figures are illustrative of the two normal extremes, are from wholly different types of muscle, and are, therefore, not directly comparable. This fact he apparently fails to recognize, though his first quotation from Miescher should have helped in the identification of the muscle types he used.

On the comparative side of the question involved here the recent brilliant work of Bell should be presented.[d] Bell has studied the liposomes in the muscle fibers of the ox, dog, cat, rabbit, rat, and the frog. He has also examined the moth (*Phlegethontius*), and the fly (*Musca*). He presents a good historical statement of the work that has been done along this line, from the discovery of interstitial granules of muscle fibers by Henle in 1841, down to the publication of his own work in 1911. Bell calls the muscle interstitial granules that are of a fatty nature "liposomes," a term introduced by Albrecht.

Bell, in discussing the granules in the muscle fibers, says (p. 310), "All agree with Kölliker that the granules lie in the sarcoplasm between the fibrils," and later:

In the skeletal muscles of vertebrates, when the cross markings are wide and distinct, it can usually be seen that the granules occupy the J-band. But when the striations are narrow the granules seem to extend the entire distance between adjacent Krause's membranes. Large granules nearly always lie

[a] Paton, op. cit., p. 110.

[b] Paton, op. cit., pl. I.

[c] Mahalanobis, op. cit., p. 108. His fish no. 79 was "a fish fresh from the sea."

[d] Bell, E. T.: The interstitial granules of striated muscle and their relation to nutrition. Internationalen Monatschrift für Anatomie und Physiologie, bd. XXVIII, s. 297, 1911.

partly at least in the Q-band. In many fibers the granules are arranged in distinct transverse rows, being apparently limited by Krause's membrane.

The nature of the muscle granules and particularly of the liposomes has been extensively studied by Bell. But as the question of the kind of fat has not been especially investigated in the salmon muscle the review of the discussion will be omitted at the present time. The contributions by Bell that are of special and far-reaching value in relation to the questions involved in this paper are two: First, the influence of starvation on the fat content of the muscle tissue; second, the influence of fat feeding on the number and size of the liposomes of the muscles. Under the subject of lack of food, Bell says:

In every animal there is a gradual disappearance of the liposomes during inanition. As the animal loses weight the liposomes gradually become smaller and less refractive; and they also stain with decreasing intensity. The muscle fibers of a well-nourished cat are usually full of coarse deeply stained droplets such as is shown in figure 1, from the frog.

Also:

In the rat there is a very rapid decrease in the number, size, refractive power, and staining intensity of the liposomes. A well-fed rat may contain a large number of strongly refractive liposomes in its muscle fibers, many of which may be stained with osmic acid. After a reduction in the body weight of 15 to 20 per cent only a few faintly refractive liposomes are usually left. After a reduction of 25 to 30 per cent, it is often found that no liposomes at all can be demonstrated. Every liposome has disappeared.

The remarkable sensitiveness of the liposomes in rat muscle to the food supply undoubtedly accounts to a considerable extent for the large variations one finds in animals gathered at random. It will be shown, however, later that the quality of the food is a factor of almost as much importance as the quantity. A rat whose body weight has been reduced 25 to 30 per cent may develop a large number of deeply staining liposomes in its muscle fibers (if fed on a diet largely composed of fat meat) though the body weight remains far below normal.

There is, as has been shown, a marked difference in the number and character of the liposomes of a well-nourished normal animal and those of an emaciated animal; but the liposomes of an animal in ordinary condition may not differ essentially from those of a very fat individual. No particular differences were noted between the muscle liposomes of steers, in which the subcutaneous fatty layer was 6 cm. thick, and those of steers in which this layer was only 5 mm. thick. It was also noted in rats and dogs that excessive amounts of connective tissue fat are not coordinated with excessive development of the liposomes.

It is however clear from the above-described disappearance of the liposomes during inanition that they consist of some form of reserve food substance. This conclusion is in accord with the view that they consist of true fats or fat-like substances. The gradual decrease in the refractive power and staining-intensity of the liposomes indicates that the fats are mixed in the liposome with some substance other than fat.

Under the topic of "The effect of special feeding on the liposomes" Bell says:

Some interesting results were obtained by feeding summer frogs on special rations. It has been pointed out above that in the summer months (June, July, and August) the muscle fibers contain very little fat. In a great many animals, in July and early August at least, no liposomes at all can be demonstrated in the light fibers, and those in the dark fibers are very small and faint and can only be stained with Herxheimer's solution. Some young frogs were found in which no liposomes at all could be shown. It was found that when frogs in this condition were fed heavily on olive oil or fat meat for a few days the fibers become loaded with liposomes, giving a picture similar to that found in winter animals.

Bell also tested the fat content of the muscles of frogs caught in the field showing that the muscles of leopard frogs before feeding had a "few faint liposomes in the dark fibers, none in the light fibers," but after feeding with fat meat and olive oil all the fibers are loaded with liposomes. Those in the dark fibers are large and stain with considerable intensity.

After a series of experiments on leopard and bullfrogs he says: "It is apparent that if the frog be fed an excessive amount of fat the fat will be rapidly stored up in the muscle fibers." Similar experiments were performed on rats, which were kept on a low ration until the liposomes were removed, then were fed on a ration of fat meat. Under this diet the rats gained in weight and the muscle "fibers filled with liposomes."

By these brilliant experiments Bell has conclusively proved that the liposomes in the muscles of vertebrates, frogs, and mammals bear a distinct relation to the state of nutrition. The liposomes decrease in number and quantity under a low state of nutrition and they can be increased in size and number when the animals receive a favorable food. These experiments are of peculiar importance to the problem of the present paper, since they prove that the presence of the liposomes in muscle tissue is to a certain extent an index of the nutritive condition of the animal in question. It does not of necessity follow that the liposome content of the tissues of an animal in the fasting condition, as in the case of the salmon, will have the same significance. From my previous work, however, and from numerous field observations, I had arrived at the working hypothesis that this was the case in the salmon, a position strengthened by the conclusions of Prof. Bell, which he kindly communicated to me before his results were published.

The salmon muscle fat is a filtration fat, not a fatty degeneration. It may be stated here that the studies on the king salmon tend to disprove Miescher's theory that the intracellular fat of the salmon muscle, of whatever type the muscle, is a fatty degeneration, a "Fettentartung";[a] and support the observations of Mahalanobis that the process is an "infiltration." In short, the observations made on the king salmon have tended to confirm the view expressed above that the intracellular fat of the king salmon is an expression of the nutritive state of the muscle. It is a loading of fat by a process of infiltration, as will be explained more fully, and is not a degeneration of the muscle substance.

It seems surprising that the test of degeneration versus infiltration should not have been applied to the material under discussion by Miescher and by Mahalanobis. Any examination of histological sections ought to have shown that there was no appreciable and adequate conversion of cell proteins into fat, and this observation would have settled the matter. Transverse sections of dark muscle taken at a late stage in the migration journey show great regularity of structure, and this structure is of the normal type. If the muscle protoplasm had undergone fatty degeneration commensurate with the amount of fat found in this tissue at the time of its greatest load of fat, it is evident that there would be little normal protein left, and that this little would show pathological structure. This pathological condition I have never seen except in the extreme emaciated condition found at the time of death. Even then it was found to be extensive in only one tissue, the great masseter muscle, and this muscle contained no fat.

If argument were still lacking to establish an alibi for the "fatty degeneration" process of laying down fat in the salmon muscle, it ought to be supplied by the fact that the young and actively growing dark muscle fibers of the superficialis lateralis muscle bear a heavy load of intracellular fat. These fibers take on a rich deposit of intracel-

[a] Miescher, op. cit., p. 207.

lular fat, both when the muscles are small and immature and when they are larger, also at a time when they are undergoing longitudinal cleavage.

I have several stages of relatively young fish, from 7 to 16 cm. long, all of which show a rich deposit of fat in the fibers of the superficialis lateralis. In the older fish,[a] as measured by the standard of size, there is a heavy loading of fat in the dark muscle with corresponding separation of the bundles of fibrillæ. There is, however, no disappearance of fibrillæ or other unusual characteristic than the distortion that comes from the presence of such enormous quantities of fat. These remarks all apply, of course, to the dark type of muscle. In the pink muscle there is little or no intracellular fat in the muscle fibers during any phase of the growth cycle. This fat appears only after the feeding ceases.

It seems obvious that intracellular fat of the muscle can not, in the salmon, be attributed to "fatty degeneration" in any true sense as signifying a protein degeneration, or, for that matter, a protein cleavage. The amount of protein present does not justify such a conclusion.

MECHANISM OF FAT TRANSFERENCE IN THE SALMON BODY.

Fat metabolism in most animals, in the Mammalia for example, always involves the two intertwined problems of most nutrition experiments, namely, fat intake and fat mobilization. The former carries with it the detail of fat digestion, absorption, and the laying down of the fats in the fat-storing tissues. The latter involves the taking up of the fats from the storage tissues and their utilization in the production of new tissue or in the liberation of energy, as the case may be, and such transferences in the body as either method of utilization may entail. In most animals complete separation of these two groups of processes involves more or less abnormal conditions for the animal. But for the salmon the long fasting period is a perfectly normal process. We, therefore, can make observations under the grim assurance that the salmon will not, in fact, can not, eat. There is no added fat being absorbed during this fasting period, hence we have present at this period only the uncomplicated mobilization and utilization processes.

The discovery of a fat-splitting enzyme, or lipase, was made by Claude Bernard in 1846, and it was early suggested that the fats of absorption might be resynthesized in the intestinal epithelium. It was not, however, until 1900, when Kastle and Loevenhart [b] announced their brilliant discovery of the reversible action of lipase, that we have had an adequate and thorough comprehension of the mechanism whereby the animal body can transport fats from tissue to tissue. In light of the reversibility of lipase action it is easy to see how a fatty infiltration can make its appearance in a tissue so stable in structure as striated muscle, without assuming a disintegration of its protoplasm, as in the fatty degeneration theories.

In the problem before us I have already discussed at length the comparison with regard to the actual loading of fat in the two chief types of tissue, the lateral dark muscle and the lateral pink muscle. These are very different types of muscle, and, while the problem and the controlling factors are essentially similar, it will greatly simplify the

[a] I have during a number of years found salmon of various sizes entering the fresh waters, all of them exhibiting a great variation in maturity of sex organs. These two facts, i. e., size and maturity, are independent of each other.

[b] Kastle and Loevenhart: American Chemical Journal, vol. XXIV, p. 491, 1900.

presentation of the matter to consider the phenomena of fat mobilization in these tissues separately. I will take the simpler case first, i. e., the pink muscle tissue of the lateralis profundis muscle.

TRANSFERENCE OF FAT IN THE PINK MUSCLE.

Let the reader recall the characteristics of this muscle up to the time of the entrance of the salmon into fresh waters, viz: (1) The intermuscular fat of the pink muscle is slight in the early growing stage as represented by fish 10 to 15 cm. long which are migrating toward the sea. The intermuscular fat increases in quantity after they reach the sea, and reaches its maximum when the fish cease feeding—that is, when they begin the adaptation process preparatory to entering the estuaries. (2) The intramuscular fat is absent in the young salmon of fresh water, also in the voraciously feeding sea forms, up to the time the salmon cease to feed, except for traces of fat in the smaller fibers just at the end of this period. (3) The intramuscular fat after the beginning of the spawning migration makes its appearance throughout the substance of the pink muscle fibers of all sizes. It appears in short chains of very small liposomes that are quite evenly interspersed among the groups of fibrillæ of the muscle cells. This intracellular fat is present within the pink muscle fibers throughout the migration and at the time of death after the spawning.

The special contrast is in the distribution of muscular fat just before and just after the salmon cease to feed. The important change is in the relatively sudden appearance of the liposomes among the fibrillæ of the pink fibers. For this phenomenon the following explanation is offered:

Active feeding salmon are also rapidly growing salmon. While growth is taking place all excess of fat is laid down in the connective tissue or in the dark muscle and never in the muscle fibers of the pink muscle. The concentration of the fatty products never exceeds the oxidations in the fibers of the pink muscle, hence no intracellular deposit occurs.[a] The transition from a feeding to a fasting state is associated with numerous tissue changes in other parts of the body, changes which are accompanied by equally important functional readjustments. Among the functional changes the one that most concerns the present argument is the increased production of the fat-splitting enzyme, lipase. Assume for the moment that the products of the last digestion have been absorbed into the blood and have already been utilized by the tissues. Assume also that this state has reached a point where the expenditure of energy must be done by drawing on the body reserves. Then what can happen?

The salmon tissue glycogen is a negligible quantity. There is no adequate supply in either muscle or liver, as in the mammalia. Glycogenesis can not, therefore, come to the support of the body in this crisis.

There is an abundant store of fat in the intermuscular depot, great quantities of it, and a lipogenesis[b] comes to the support of the salmon in a way quite comparable to the glycogenesis of the mammal as conceived by Claude Bernard. Under these conditions the activity of the muscular tissue is directly dependent on the fat as a source of energy. The muscle oxidizes fatty bodies in the salmon, just as it oxidizes carbohydrate bodies in certain other well-known animals.

[a] An exception may be found in the border zone of fibers between the pink and the dark muscle.

[b] Loevenhart: On the relation of lipase to fat metabolism, lipogenesis. American Journal of Physiology, vol. VI, 1901, p. 331.

It is to be assumed that the muscle fibers absorb the fatty bodies from the lymph and blood, presumably as soluble fatty acids and glycerin. The fatty bodies of the blood and lymph are derived from the stored fat by a process of lipolysis. To that extent to which the store of intermuscular fat of the pink muscle is eroded by this process of lipolysis will the percentage concentration of the cleavage products of the pink muscle lymph be high. From the lymph the fat cleavage products dialyse directly into the pink fibers and become available for oxidation. In the early stages of the fast there are numerous tissues besides the muscle containing an excess of stored fat; the digestive tube, the pancreas, the liver, the skin, etc., as well as the connective tissue and the muscles. Loevenhart [a] has stated that the limits of lipogenesis are "nearly proportional to the amount of enzyme acting" and "nearly independent of an excess of ethyl butyrate" in the experiments of his series. Hence, with increasing production of lipase in the blood there is an ever-increasing percentage of fatty bodies thrown into solution in the blood and lymph.

Hand in hand with the increase of fatty bodies in the blood and lymph will go an increase in fatty products in the substance of the muscle fibers. Muscular oxidations are not rapid enough to keep down the increasing quantity of fatty bodies, hence they will diffuse through the muscle protoplasm in considerable excess. The lipase of the blood and lymph will also diffuse into and be present in the muscle fiber, a fact demonstrated for other muscle tissues. Under the law of reversible lipase action this excess of fatty cleavage bodies is bound to be reconverted into and deposited as neutral fat. Thus arise the chains of microscopic liposomes of the pink muscle at the beginning of the salmon fast.

The number and size of the chains and of the individual liposomes in the pink muscle, therefore, is a result of the interaction of a number of factors, chief of which are the following:

a. The relative abundance of the stored fat in the tissues of whatever source, i. e., the gross amount of fat available for lipolytic erosion in all parts of the body.

b. The relative abundance of lipase throughout the body, chiefly of the blood and lymph, but having origin in lipase producing tissues.

c. The structural and physical factors controlling the rapidity of the absorption from the blood and lymph into the muscle fibers; i. e., the sarcolemma, sarcoplasm, etc.

d. Especially the rapidity with which the fatty bodies are utilized, oxidized, by the muscle sarcoplasm.

The constants of lipase action have not yet been determined sufficiently to enable one to apply to this specific instance definite governing laws. It is hoped that something may be accomplished along this line as this work progresses. At present, however, one may say that in a general way e, the size and number of the liposomes in any given fish's pink muscle, will vary directly as a, the abundance of stored fat, b, the relative abundance of lipase, c, the structural and physical factors governing the diffusion of the lipolysed products, and inversely as d, the rate of oxidation of fats in the muscle fibers. The relation may be expressed as follows:

$$\frac{a \times b \times c}{d} \times k = e$$

where k is a complex constant representing the unknown facts and relations referred to above.

[a] Lovenhart, op. cit., p. 350.

TRANSFERENCE OF FAT IN THE DARK MUSCLE.

It is stated earlier in the paper that in the active feeding and growing salmon large quantities of fat are laid down in the dark muscle fibers. This deposit of fat begins in the earliest stage observed in the young salmon. It reaches its maximum somewhere near the time when the salmon begin their migration journey. At the Ilwaco station the amount of fat deposited in this type of muscle is astoundingly large. (See fig. 1, pl. III.) The amount is especially significant when it is remembered that the deposit has taken place as a storage process in a tissue that is supposed to be most active in the giving off of mechanical energy.

The variations noted in the dark muscle at the different stages in the migration journey are variations in the amount and character of the distribution of fat. Extensive discussion has been presented showing the facts as regards this picture at the different migration stages. Attention is here called especially to two points, first, the striking variation in the amount of fat of the dark muscle of different parts of one and the same animal as given in such fish as no. 125 and no. 126. The second factor is the relatively large amount of fat present as liposomes in the dark fibers at the death of the salmon.

As regards the first point, it is obvious that the diminished quantity of fat along the courses of certain of the smaller blood vessels, as shown in fish no. 120, also no. 126, represents a process of fat erosion. It would seem that the fat in the process of being removed is taken up first along the course of the blood vessels. Apparently we have to do here with the simple process of lipolysis. If this be the correct view then it is evident that the fat products of the dark muscle are handled in a way analogous to the fats in the pink muscle in so far as the process of solution and utilization goes. Therefore there is nothing peculiar about this tissue in this particular regard.

In an animal in which the fats have reached a certain stage of consumption and in which the processes of fat solution are going on rapidly we will have the greatest contrasts as between the highly vascular and the less vascular areas. The former favor in every way the rapid solution and removal of the fats. In a comparatively large section of dark muscle through, say, the trunk region of such an animal, one will notice a decided mottled or marbled appearance of the section viewed under comparatively low magnification. The less fat areas will be lighter, with less of the scarlet red stain, while the fatter regions will be relatively deeper red in appearance. Often the light areas form distinct patterns which conform to the smaller veins and arteries.

In the salmon at this stage the contrasts as between the trunk muscle and the caudal muscle are always sharp. The dark muscle, like the pink muscle, will contain relatively less fat in the caudal region than in the trunk region. The microscope will show that this caudal muscle fat is in smaller droplets which are fewer in number than in the trunk region. In the less vascular areas of the caudal region there will often appear fibers that have only traces, sometimes no fat. These factors are attributed to the more rapid using of fat for the production of energy in the caudal muscles as the more active tissues.

The utilization of the fats of the dark muscle does not present as acute a problem as regards the numerous smaller liposomes which we found in the case of the pink muscle. The salmon begins the fast with the dark muscle fully loaded with intracellular fat. Therefore, the first change that will occur in this muscle will be a process of using up the fat on hand in the cell. When at any time or for any reason this intracellular dark muscle fat is wholly consumed, then the dark muscle will be in the same category as the pink muscle in so far as its source of material for the production of energy is concerned. Regions of dark muscle which have reached this stage are found with the arrangement of liposomes that is described as typical for the pink muscle. On the other hand, the dark muscle will have the chains of relatively small liposomes rather uniformly distributed throughout the muscle mass. These liposomes at this stage are relatively small, as for example in the fishes described from Cazadero. Rarely will fibers be found with no liposomes. It seems to me that should a certain area of dark muscle fibers through excessive activity consume all of its liposomes, then fat would be thrown into those fibers by the process of lipolysis and fat transference in exactly the same way that it is thrown into the pink fibers. This detail is fully described in connection with the discussion of the pink muscle.

As regards the second factor mentioned above, namely, the high percentage of fat still present in the dark muscle at the time of the death of the salmon, it seems to me the matter is more complicated. The operation of no ordinary factor would maintain a higher percentage of fat in the dark muscle at a time when the fats were almost consumed. One is led to suspect that there is some special factor operative in the dark muscle. In all probability this factor is the same in the late stage in the life cycle as that operating in the earlier stage in the salmon development which results in the loading of fats into this type of muscle. I have observed no special facts which of themselves explain this situation. There are, however, certain accessory facts which permit of an explanation which will be offered as a tentative hypothesis. Of these facts the most important is the fact of the loading of the dark muscle during the embryonic stage of its development.

Undoubtedly such deposits of fat as occur in no. 97, fig. 7, represent a perfectly normal process which is to be interpreted as a function of this muscle. Histologically the dark muscle differs slightly in its structure from the pink muscle. The dark fibers contain more sarcoplasm and somewhat larger and fewer fibrillæ. At an early embryonic stage this difference between the dark and pink muscle is rather more striking than it is later. This suggests that the dark muscle is a less highly differentiated type of muscle than the pink. One may assume, therefore, that it retains more primitive characteristics. In the sections which cut the borderland between dark and pink muscle a few of the pink fibers of the intermediate zone are found to be filled with liposomes. This loading of liposomes is greatest in the fibers nearest the surface of the great muscle mass and is totally absent in the deeper portions of the muscle. The fibers in question are of the pink fiber type. Their loading of fat must therefore be due to some special factor. These three facts, namely, (1) the excessive loading of fat in the growing dark muscle, (2) the more generalized type of dark muscle, and (3) the tendency of the neighboring zone of pink muscle to load intramuscular fat, all suggest that the dark muscle has still strongly developed one of its general functions. This function is the production of lipase. It may be anticipating a bit in the following discussion, but it is evident that the presence of a relatively high concentration of lipase results in the seizing of the fats during the growing stage and their concentration in the lipase-producing tissues. This view is borne out by the deposit of large amounts of fat in the pancreas as well as in the dark muscle. A relatively high concentration of lipase in the dark muscle would lead to a greater concentration of lipase in the tissue lymphs in those tissues which surround the dark muscle, namely, the connective tissue of the skin and the superficial layer of the trunk pink muscle. The deposit of liposomes in the intermediate zone of pink muscle fibers is occasioned by this greater concentration of lipase.

If it can be granted that the dark muscle retains this assumed power of lipase production throughout its whole life, then it will follow that at the spawning grounds we will still have in this particular tissue more than the average amount of lipase in the muscle fluids. If so, there will be a tendency to hold fats and fatty acid in solution in this tissue, and, other things being equal, the tendency to maintain a somewhat higher content of fat in the form of liposomes.

The liposomes will form in those tissue spaces in which there is greater stagnation of the tissue fluids. In so far as the dark muscle is concerned, this point is immediately under the sarcolemma. Should the cleavage products of fatty acid and glycerin diffuse from the sarcoplasm through the sarcolemma, then it would be picked up ultimately by the blood stream and washed away. If, on the other hand, fats are coming into the pink muscle, then the diffusion will pass first through the sarcolemma and then the sarcoplasm, which is using fat in its oxidations. Therefore, the central portion of the muscle fiber will contain fewer liposomes and smaller ones while the superficial portion and the region immediately under the sarcolemma will contain relatively larger liposomes. This picture corresponds to the facts whether or not the theory offered in explanation be true. The factors discussed in previous pages which determine the number and size of the liposomes are therefore the same for dark muscle as for the pink, except for one, namely, the factor *b*, given on page 126, the relative abundance of lipase in the dark muscle. This factor *b* is greater than in the pink muscle, since the dark muscle itself is presumed to be producing lipase.

RELATIVE ABUNDANCE OF STORED FAT AND ITS DISPOSAL IN THE ORGANS.

My chemical analyses have shown that there is always a great variation in the percentage of fat taken from any standard region of different individual salmon from any particular station. No school of salmon contains individuals of uniform characteristics as regards either size or condition, for example, the specific loading of fat. Considering only the region under discussion here, namely, the mid-lateral portion of the body, I find that there is great variation in the total amount of fat present. In the histological examination of the variation of fats this will show itself in the number and especially in the relative size of the fat droplets. The loading of fat, as indicated by these facts, is most constant in the salmon obtained at the mouth of the Columbia River. It is to be assumed that if one could obtain salmon just in the region where and at the time when they cease feeding, then the loading of fat would be most nearly constant. Even then a great variation might be expected, since in salmon of the same size it can not be assumed that there has been uniformity of opportunity in obtaining food during the long period of development. Therefore, there are great variations present in the total loading of fat in the lateral pink muscle, even at the Ilwaco station, variations that are the expression of a multiplicity of factors.

As salmon pass up the rivers in their migration and since the stored fat forms the total source of the energy-giving material, it follows that there will be a diminution in the total fat directly proportional to the length of time and the relative expenditure of energy since the beginning of the fast.

Instances of variations in fat between the fibers of the lateral pink muscle at the first station, Ilwaco, are to be obtained by reference to the protocols of fishes nos. 111, 113, 115, and 118, representing very fat fish taken during early August, 1911. The commercial fishermen report that the very fattest specimens are obtained earlier in the season. Fish no. 114 and especially no. 117 represent the opposite extreme, i. e., the poorest fish for the August season. Undoubtedly no. 117 was at a stage in which there was considerable diminution of fat below its maximum. This diminution may have been due to any one of a number of factors, but one is led to suspect that the fish has been long without food, even at this low station on the river. The marking experiments of 1908 [a] give evidence that some at least of the August fish remain long in the waters of the lower river.

It is self-evident that where a fish has a low percentage of stored fat to begin with there will be a less abundant erosion by the action of the lipases; therefore, other things being constant, one can not expect in such an animal as high a percentage of concentration of the fatty products in the blood and tissue fluids as in fish that contain a greater fat content. This factor *a*, the variation of the storage fat, has its influence on the factor *e*, the number and size of the liposomes, as is indicated by the variations noted in fishes nos. 120 and 125.

SALMON LIPASES.

The rapidity with which the stored fat of the salmon tissues is eroded when the fasting begins will, of course, depend chiefly upon the second factor mentioned in the section on the transference of fat in the pink muscle, namely, the amount or concentration of the lipases.

The phenomena of fat mobilizations in the body belong strictly within the group of enzyme actions, for which the general law is so admirably stated by Wells: [b]

> All metabolism, then, may be considered as a continuous attempt at establishment of equilibrium by enzymes, perpetuated by prevention of attainment of actual equilibrium through destruction of some of the participating substances by oxidation or other chemical processes, or by removal from the cell or entrance into it of materials which overbalance one side of the equation.

The presence of lipase in various animal tissues has been demonstrated often enough. Hanriot [c] demonstrates the presence of lipase in the following fluids and organs: Blood, lymph, urine; liver, pancreas, testes, spleen, thyroid. The lipase was in greatest amount in the blood, liver, and pancreas. Kastle and Loevenhart [d] showed the presence of lipase in intestinal epithelium, which on account of its position as an absorbing tissue is of more than passing interest. The evidence as regards the presence of lipase in the blood plasma and in the lymph is somewhat contradictory, yet such lipase is not only demonstrated, as given above, but its quantity has been shown to vary under certain pathological conditions. Pathologists have followed the variations in the quantity of lipase in necrosis [e] with fat formation. The pancreatic cells are well-known lipase producers. It is to be expected, therefore, that there will be a variation in the quantity of lipase that will reach the body fluids from this tissue. In confirmation of this point lipase has been found in the urine of a clinical case of fatty necrosis associated with inflammation of the pancreas. [f]

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[a] Greene, C. W.: The migration of salmon in the Columbia River. Bulletin U. S. Bureau of Fisheries, vol. XXIX, 1909, p. 131.

[b] Wells, H. G.: Chemical pathology, p. 68. Philadelphia, 1907.

[c] Hanriot: Comptes rendus de la Société de biologie, 1896, p. 925.

[d] Kastle and Loevenhart: American Chemical Journal, vol. XXIV, p. 491, 1900.

[e] Flexner: Journal Experimental Medicine, vol. II, p. 194, 1904.

[f] Opie, Eugene L.: A case of hemorrhagic pancreatitis. The occurrence of a fat-splitting ferment in the urine. Johns Hopkins Hospital Bulletin, vol. 13, p. 117, 1902.

I have examined the pancreas of the king salmon for lipase, testing extracts of the fresh normal glands of salmon caught in active stages of digestion. The experiments were preliminary only, yet the tests were positive and the reactions vigorous. The salmon pancreas secretes an active lipase. In my fat-absorption experiments there was always a vigorous loading of the epithelial cells of the intestinal mucosa, especially in younger fish. There was a greater mass loading than I have ever seen in mammalian intestinal epithelium. This, by our current theories of fat absorption, is as strong circumstancial evidence of the presence of lipase in the salmon mucous epithelium as one could well expect to discover. Aside from these tests no studies have been made on the lipases of any of the Salmonidæ. It is, of course, highly desirable that such studies should be made. The amount and the variations of the lipase content of the salmon blood, and especially of the muscles, should be determined by a series of quantitative tests. These are the tissues of peculiar interest to the problem in hand. There are other tissues directly concerned in the fat metabolisms of the salmon—the liver, the divisions of the alimentary tract, the pancreas, etc.

However, on a priori ground, there is every reason for assuming that the salmon is well supplied with lipase in its fat metabolizing tissues, particularly in the alimentary mucous epithelium (the gastric epithelium is also fat absorbing), the muscles, the liver, etc. The blood and the lymph can not escape a lipase content in an animal in which so many of the tissues are concerned in the lipolytic processes.

In previous discussions the conclusion has been reached that there is a marked increase in lipolysis at the particular time the salmon cease feeding and begin the migration journey. This carries the assumption that there is at this time an increase in the amount, i. e., percentage, of lipase in the blood and the tissues, including the muscular tissues. Whence come these lipases?

SOURCE OF THE LIPASES.

The presence of a greater percentage of lipase when the cessation of feeding occurs may be explained on two physiological grounds, both of which are probably active; first, there may be an absolute increase in the lipase produced in a given time; and, second, it is possible that with the cessation of feeding the lipase that is usually consumed in the intestine and pyloric cæca in the processes of digestion and absorption is now thrown more fully into the blood stream. To this extent it raises the percentage content of lipase in the blood.

The pancreas.—The salmon pancreas is one proven source of lipase. The pancreas is morphologically of the type described by Legouis[a] as the diffuse pancreas. The gland filaments are quite separate from each other. They form an open meshwork running over the pyloric cæca, the blood vessels and mesentery of the stomach and intestine, the inner loop of the stomach, and the mesentery of the spleen.

These pancreatic filaments are richly supplied with blood vessels, and these vessels anastomose with the blood vessels of the cæca, intestine, etc., of the region. Pancreatic ducts have been described by Legouis as converging to a common duct that enters the intestine either with, or in the neighborhood of, the bile duct.

[a] Legouis, P.: Recherches sur les tubes de Weber et sur le pancreas des poissons osseau. Annales des Sciences Naturelles Zool., 5th ser., t. 17, 1873. See also t. 18, 2d article.

My histological observations, which will be given in detail in a later paper, show that the pancreatic cells are not all compactly arranged in acini, as is usually the case, but that they are more or less scattered. Every cross section of the pancreas reveals the fact that a large amount of adipose tissue is present in association with the pancreatic tissue. The cross section of a pancreatic lobe is somewhat triangular in shape. The central portion of the triangle in an adult organ always contains one or two blood vessels of relatively large size, together with a considerable amount of fatty tissue. The pancreatic cells are arranged around the surface of the gland and in the interstices among the fat cells and blood vessels. There are many acini where definite arrangement of pancreatic cells around a central lumen can be shown. This lumen rarely forms a space as large as one sees in the corresponding region of a mammalian pancreas. In these groups, in favorable preparations, that portion of the pancreatic cell bordering on the lumen is highly granular and the granules stain in a way characteristic of zymogen granules. The pancreatic cells around the surface of the gland and in many portions of the deeper region have a rather scattered arrangement in which it is extremely difficult to show definite relation to ductules. Such relation is questionable in a large proportion of the gland. The diminutive size of the pancreatic ducts, the presence of the large amount of fat in the gland, the rich vascular arrangements of the gland, and the diffuse arrangement of the gland cells of the salmon pancreas have led me to the conclusion that the secretion of this gland is largely internal, i. e., that the pancreatic secretion is largely thrown into the lymph and blood stream of the organ. In this statement it is not meant to minimize the importance of the gland in the production of the pancreatic juice. Rather, the intention is, to emphasize the importance of the internal lipase secreting function.

When digestion stops and the animal begins its fast, the pancreatic gland undoubtedly continues to function. This is shown especially by the histological appearance at different stations of the migration journey. There is obviously no digestive function to be accomplished by the secretion during the fast. Therefore, during this phase of the life cycle the internal secretive function becomes the main, in fact, the only one. The vascularity of the pancreas and of the pyloric cæca does not change in proportion to the amount of retrogressive change shown in the cæca and in the rest of the alimentary tract. Putting these facts and deductions together they may be summarized thus:

1. The pancreatic gland is abundantly active during the migration fast.

2. The activity consists in the production of an internal secretion which is not essentially different in character from the normal secretion produced at an earlier stage in the life cycle. It is therefore rich in lipases.

3. The pancreatic lipase produced during the fasting period is chiefly, if not wholly, discharged into the tissue spaces, from whence it reaches the blood stream.

4. The circulation carries the lipase to the tissues of the body, which includes both the fat-storing tissues and the active fat-using muscles now under especial consideration.

Lipase from the granule cell layer.—There is a second tissue filled with zymogen granules which I believe to be an active source of lipase, namely, the granule cell layer of the alimentary tract. I have already called attention to the presence of a layer of granule cells in the mucous coat of the alimentary tract. This layer is especially richly developed in the pyloric cæca. In every section of the retrogressing cæcum, one is strongly impressed with the continued large size of this granule cell layer. Even in fish from the Ilwaco station, specimens which represent the first stage in the changes following the beginning of the fast, this layer is notably large. The granule cell layer is much thicker and more prominent than in the normal Monterey salmon tissue. I have made computations that indicate an actual increase of the mass of this granular layer.[a] The greater apparent increase in mass may be explained on the ground that the normal volume of the granule cell layer is retained while the volume of the various other structures of the alimentary tract is sharply diminishing, a question that is now under investigation. The structure of the cells in this layer does not materially change while the other tissues around it are sharply degenerating. The cells remain loaded with granules which have a strong affinity for the basic dyes. The loading of granules remains characteristic of the cells even in the most degenerated salmon examined. Here, again, one must interpret the granules as zymogen granules. Gulland [b] has given the name eosinophile leucocytes to cells of this region, an identification to which I can not subscribe. By implication he would ascribe to the granule cells a very different function from that which appears to me to be the true one. The granules, which do stain sharply with eosin as Gulland observes, are too large to be compared with the granules of eosinophiles. The granule cells themselves are larger, differently arranged, and have a very different type of nucleus from the typical eosinophile. Also one never finds granule cells of the type characteristic of the cells of this layer in the salmon blood vessels of either the cæca or of the intestine. The matter is more fully discussed in another paper, but the points are mentioned here in order to meet objections to the view which is offered for the function of these cells; namely, the granule cell layer of the pyloric cæca and of the alimentary tract of the salmon is an internal secreting organ which has for its probable function the production of lipase.

In the normal organ, when active digestion is going on, the lipase produced within the granule cells is thrown out into the tissue spaces. Some of this lipase quite probably reaches the epithelial layer and supplements the lipase produced by the epithelial cells. Such lipase will, of course, facilitate the absorption and the resynthesis of the fats in the epithelial cells, according to the laws of fat absorption. A considerable portion of this layer lies outside the stratum compactum. It seems to me improbable that the internal secretion of those cells will diffuse through the stratum compactum with the same facility that it will pass out into the tissue spaces of the adjacent muscle coats. The granule cell layer itself is almost free of blood vessels. The muscle coats, on the other hand, are richly supplied with blood vessels. It would follow that any secretion diffusing into the muscle coat would quickly be taken up by the blood, and would be carried throughout the body. During the fasting period this lipase-producing function of the granule cell layer is no longer of use in the absorption of fats but is of great supplementary aid to the pancreas in maintaining an adequate supply of lipase in the blood. This offers an explanation that would account for the persistence of the organ.

Increase in lipase from change in isotonicity of the tissues.—In the recent brilliant work of the Rockefeller Institute in isolating and growing tissues of pure culture, it has been shown that physiological activity, as measured by growth, is influenced by the degree of concentration of the nutritive fluids. Tissues growing in physiological solutions of

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[a] These determinations were made by computing the cross-sectional area of the granule cell layer of cæca from Monterey and from Ilwaco salmon. The Ilwaco specimens exceed the Monterey by an average of 11 per cent. But the probable error is high.

[b] Paton, Noel: Life history of the salmon. Article 3. The minute structure of the digestive tract of the salmon, and the changes which occur in it in fresh water, p. 13, 1898.

slightly lower tonicity than that to which the tissues are adapted, undergo a more active growth than those in fluids of relatively high tonicity. These factors have been worked out on several tissues, but very strikingly on the rate of healing of wounds in the skin of frogs which had their body fluids decreased in tonicity by injections of water.[a]

I have no doubt that these principles are operative in the salmon during the migration. The salmon tissues in this stage of the life cycle are most of them long past the period where growth is the prominent physiological activity. There is, however, one marked exception, namely, the reproductive organs. These organs are quite immature at the time the migration begins. They suddenly take on an active stage of growth which proceeds readily till complete development at the time of spawning. Other tissues show their variations in physiological activity in ways other than growth, the muscular tissues in increased production of energy, the glandular tissues by variation in secretory activity, etc.

In work on the Sacramento River[b] I showed that there is a marked decrease in the depression of the freezing point of salmon serum as the fish migrate from the sea to fresh water. The depression of the freezing point is a direct measure of the tonicity of the blood. As this diminution begins with the process of migration from salt water to fresh water it is evident that it will have the same type of influence on the tissue activities of the salmon as that shown in the experiments on tissue growth. This is undoubtedly a factor in the stimulation of the reproductive organs to the sudden increased growth which takes place in the salmon at this time. It is a safe inference that this change in isotonicity of the blood and tissue fluids if exerted on other tissues of the bodies will cause variation in their physiological activity. Applying this principle to the pancreas and to other tissues where lipase is produced we have a factor accounting for an increase in metabolites, of which lipase is one, at the critical time of the beginning of the migration.

Lipase from tissue degenerations.—Another source of lipase may arise through tissue degenerations. Extensive degenerations are taking place in the alimentary tract. At Ilwaco the mucous membrane of the intestine and pyloric cæca has already reached a considerable degree of disintegration. On the current theories explaining degenerative change one may assume that these cells have already passed through a stage of physiological hypertrophy. Kastle and Loevenhart have shown that these cells are active lipase producers in the normal condition. Therefore, one may assume that the inflammatory condition preliminary to the actual necrosis is associated with an increase in lipase production. The disintegration of the cells and especially the chromatolysis is the final step in the process. These pathological changes accelerate the physiological production of lipase in the mucous epithelium of especially the pyloric cæca just at the time when such an increase in lipase is of most value to the salmon, namely, at the beginning of the fast. Lipase from this source would almost cease at a quite early stage. However, the epithelium is rarely wholly disintegrated.

Lipase from the liver and other tissues.—There is evidence that other tissues of the salmon are more important in producing lipase. Of these one may mention the liver, whose function in this regard will be presented in another paper.

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[a] Ruth, E. S.: The influence of distilled water on the healing of skin wounds in the frog. Journal of Experimental Medicine, vol. 13, p. 422, 1911.

[b] Greene, C. W.: Physiological studies of the Chinook salmon. Bulletin U. S. Bureau of Fisheries, vol. XXIV, 1904, pp. 446, 449.

Also the group of dark muscle fibers represented in the musculus superficialis lateralis has already been mentioned as lipase producing tissue. But when all the less important sources which have been discussed are left out of account there still remains an adequate lipase producing mechanism in the pancreas and in the granule cell layer of the alimentary tract to account for the presence of sufficient lipase in the blood and tissues to meet the need of the fat transference that we have under discussion. Histological evidence has been given to show that there is no diminution of the activity of the pancreas at the inauguration of the fasting period. If the pancreatic lipase production even remain constant then the amount of lipase which this gland will produce as an internal secretion will tend to raise the total lipase of the blood and tissues. The lipase that is consumed in the process of digestion during the feeding period will now be left to be thrown into the circulation. It follows that there will be an increase in the percentage of lipase in the blood, therefore, according to Loevenhart, an increased solution of the fats with which this lipase comes in contact. These fats are the stored fats. An increased solution of the stored fats will raise the fatty acid and glycerin content of the tissue fluid and the blood. The inevitable result will be an increased supply of these fat cleavage products to the active muscular tissues. This supply will diffuse through the muscle spaces, the sarcolemma, and throughout the sarcoplasm of the muscle fiber in an ever increasing quantity. Since the relative amount of activity of the muscles can not be assumed to change, i. e., is comparatively constant, it follows that the percentage amount of fat will increase within the active muscle fibers.

It is shown on page 81 that the pink muscle fibers contain no intramuscular fat during the feeding stage, or at most, only a trace of such fat at maturity. This is only another way of saying that the consumption of fatty substances in the muscle fibers of the feeding salmon is in balance with the fatty bodies penetrating the fibers. There is never a sufficient excess of fatty acid and glycerin within the fibers to produce resynthesis and deposition of the fat in visible form. But with the increasing percentage of these substances penetrating the fiber after the fast begins there will be a synthesis of neutral fats and these will be deposited and can be identified. The liposomes present in the lateral pink muscle of the salmon taken at Ilwaco represent such deposits that have taken place since the beginning of the migration. The amount of neutral fat present in the pink muscle fibers is a measure of the excess of fatty acids and glycerin brought into the fibers over those oxidized in the muscular activity. If oxidation diminishes, then fats will be deposited and the excess is expressed in the number and size of the liposomes (fig. 8, pl. VI).

The character of the liposomes, that is, their number, size, and arrangement in the pink muscle depends also on one other very different group of factors. This is the structure of the muscle (fig. 13, pl. VIII). That the fat is laid down in chains of liposomes of the minute sizes that have been described must depend largely upon the structural arrangement of the fibrillæ and of the interfibrillar sarcoplasm. It is not desired, however, to discuss this factor beyond merely calling attention to it.

RÉSUMÉ.

The points made in this investigation that call for special mention may be categorically stated as follows:

1. Fat is the prominent and immediate source of the energy of the salmon expended during the spawning migration.

2. The salmon fat is stored in the body during the stage of feeding and growth, and reaches a maximum at the time the feeding stage ends, i. e., at the beginning of the migration fast. This fat can not in any proper sense be looked upon as a fatty degeneration.

3. The fat storage tissues are primarily the muscles and intermuscular connective tissues. Storage tissues of minor importance are the cutaneous and other adipose tissues, the liver, the alimentary tract, and the skeleton.

4. There are two distinct and characteristically different types of muscle—the superficial lateral or dark and the deep lateral or pink muscle. The latter represents the major portion of the great lateral muscle mass.

5. The pink muscle is characterized (*a*) by the enormous load of fat between the fibers, intermuscular fat, and in the myocommata at the time of maturity; (*b*) by the great variation in the size of its fibers.

6. The pink muscle fibers have no intramuscular fat, or at most only traces of fat, during the feeding stage.

7. Immediately at the beginning of the spawning migration the pink fibers are loaded with numerous chains of very small liposomes. This loading of liposomes increases during the early stage in the journey, and then decreases somewhat up to the spawning time. The fat never wholly disappears even in dying salmon.

8. In the active caudal pink muscle the liposomes are much less constant and are often completely absent as advanced stages of exhaustion appear.

9. The pink muscle fibers are plump and cylindrical at the time the migration begins. But at the spawning time the larger fibers have the appearance of being shrunken by decrease in mass. They become polygonal in cross-sectional outline. The sides of the polygon are often concave to the exterior, as if compressed by the adjacent smaller fibers.

10. The dark muscle is characterized (*a*) by the enormous loading of intramuscular fat at all stages of the life cycle, but especially at the time the spawning migration begins; (*b*) by the relatively small and uniform size of the fibers.

11. The stored fat of the dark muscle is gradually eroded during the migration until the fat reaches a quantity and distribution comparable to but still greater than that in the pink fibers. The fat is never completely eroded and is present in considerable quantity at the death of the salmon after spawning.

12. The smaller muscles of the fins and of the head of the salmon take little part in the fat storing. The food supply of these muscles, however, is the same, namely, the fats.

13. Distinct degenerative changes were found in the adductor mandibulæ muscle of a spawned male at the dying stage. This degeneration is a simple atrophy with pigmentation.

EXPLANATION OF PLATES.

The drawings presented were all made from camera lucida outlines. Fat is represented in the characteristic red color obtained by the scarlet red method of staining fat: All the drawings and outlines were made for me by Mr. George T. Kline, biological artist of the University of Missouri.

PLATE III.

FIG. 1. The transparency of a segment of dark muscle fiber of salmon no. 115 from the mouth of the Columbia River, Ilwaco, Wash. The most superficial liposomes and fat droplets are represented somewhat darker, while the paler colored droplets are deeper in the fiber. Magnification, Leitz ocular 2, objective 7.

FIG. 2. A small segment of dark muscle fiber from a young salmon, from the Columbia River, Warrendale, Oregon. Magnification, Leitz ocular 3, objective 7.

FIG. 3. Section of trunk dark muscle of salmon no. 120, adult in prime condition from the Columbia River at Warrendale, Oreg. The amount of fat present is almost as great as in the Ilwaco fish no. 111 and no. 115. Magnification, Leitz ocular 2, objective 7.

PLATE IV.

FIG. 4. Trunk dark muscle of salmon no. 126 from the Columbia River at Warrendale, Oreg. This salmon is representative of a late stage in the fat removal from the tissues. Certain fibers near the large blood vessel to the right are free of all but the smallest liposomes. Other fibers are still well supplied with fat. Magnification, Leitz ocular 2, objective 7.

FIG. 5. Dark muscle from salmon no. 138, a spawning salmon from the Clackamas River, Cazadero, Oreg. This figure represents the latest stages in fat removal from the trunk dark muscle. Magnification. Leitz ocular 2, objective 7.

PLATE V.

FIG. 6. Transverse section of dark muscle from an exhausted, naturally spawned salmon no. 108, McCloud River, Baird, Cal. This figure represents the extreme exhaustion of fat from the dark muscle. The salmon was an enormous male which was taken just at the time of natural death. The fat is in finest liposomes condensed at the surface of the fiber but absent between the fibers. The representation of the size of the liposomes is somewhat strong. Magnification, Leitz ocular 2, objective 7.

FIG. 7. Dark muscle from young fish no. 97. The preparation is a paraffin section stained with Mallory's analine blue connective tissue stain. The figure presents well the excessive number of clear spaces which represent vacuoles produced by extracting the fat in the imbedding process. One fiber has recently divided longitudinally into two. This fiber shows no fat along the new portion of sarcolemma. Magnification, Leitz ocular 3, objective 1/12. (From American Journal of Anatomy, vol. 13, 1912, p. 175.)

PLATE VI.

FIG. 8. Trunk pink muscle of salmon no. 118 from the mouth of the Columbia River, Ilwaco, Oreg. Attention is called to the great variation in the size of the fibers, to their characteristic outlines, the great amount of fat between the fibers, and to the general distribution and extreme fineness of the liposomes in the fibers, which have come out rather too strong in the reproduction, many of them being actually just perceptible. This figure without the liposomes in the muscle fibers would represent the normal condition of the salmon pink muscle at the beginning of the migration fast. Magnification, Leitz ocular 2, objective 4.

FIG. 9. Segments of two trunk pink fibers with adherent intermuscular fat drops from salmon no. 118. Magnification, Leitz ocular 2, objective 4.

PLATE VII.

FIG. 10. Trunk pink muscle of salmon no. 126 from the Columbia River at Warrendale, Oreg. This salmon is the one presented as a typical poor condition fish. The intermuscular fat is reduced to groups of droplets in the stronger connective tissue septa. The intramuscular fat is extremely low, limited to the smallest and medium sized fibers. These fibers retain their normal histological structure as shown in figure 13. Magnification, Leitz ocular 2, objective 4.

FIG. 11. Trunk pink muscle from salmon no. 132, a spawning female from the Clackamas River, Cazadero, Oreg. The intermuscular fat is practically eliminated, yet all the fibers except the largest show a considerable sprinkling of liposomes. In the small fibers these droplets are quite uniformly distributed, in the medium fibers concentrated around the surface, and in the largest fibers present only in traces at the surface. The outline of the largest fiber to the upper right-hand side of the figure indicates that it is approaching a degeneration stage, though the microscopic fibrillar structure is still normal in appearance in this particular fiber. Magnification, Leitz ocular 2, objective 4.

PLATE VIII.

FIG. 12.—Cheek muscle of salmon no. 140, a spawned male from the Clackamas River, Cazadero, Oreg. Fat is present in a few groups of small droplets in the connective tissue septa. There is no intramuscular fat. One fiber in the center of this group is in an advanced stage of atrophy with pigmentation, shown in the granules of this fiber (not to be confused with similar appearance of liposomes in other figures). The three fibers to the right of this pigmented one show the first stages of degeneration represented by a swelling and blending of the fibrillæ. This detail of structure is not shown in the figure. Magnification, Leitz ocular 3, objective 4.

FIG. 13. A highly magnified portion of a trunk pink fiber from salmon no. 126, Columbia River, Warrendale, Oreg. This small segment of a medium-sized pink fiber shows the normal fibrillar arrangement. The amount of fat present is indicated in figure 10, plate VII. Traces of fat were present in this particular segment just under the sarcolemma and between the outer series of fibrillæ. This figure is offered in evidence as showing that the elimination of fat from the pink muscle is not accompanied by any immediate breaking down or degeneration of the finer structure of the tissue. Magnification, Leitz, ocular 3, objective 1/12 oil immersion. Camera lucida outlines.

PLATE IX.

FIG. 14. The trunk dark muscle of young salmon no. 97 from the McCloud River, Baird, Cal. The muscle fibers are drawn in outline to show the compact arrangement and relative size of the fibers as compared with the adult. One particular fiber in this figure showed an exceptionally large fat drop in the middle of the fiber. Magnification, Leitz ocular 4, objective 3.

FIG. 15. Dark trunk muscle of salmon no. 126 from the Columbia River at Warrendale, Oreg. Drawing to show the outlines of the fibers of the adult fish after the fat is largely removed. This figure should be compared with the preceding. Magnification, Leitz ocular 4, objective 3.

PLATE X.

FIG. 16. Outline of the trunk pink fibers of the young fish no. 97 from the McCloud River, Baird, Cal. The figure shows outlines of the fibers at a stage in which active growth is taking place. The large number of relatively small fibers have recently split off the larger in the process of fiber multiplication. Magnification, Leitz ocular 4, objective 3.

FIG. 17. Trunk pink muscle fibers from adult salmon no. 118 from the mouth of the Columbia River at Ilwaco, Wash. The outlines of the fibers show the relative symmetry of the adult prime condition muscle. The separation of the fibers is due to the loading of fat in the interstitial connective tissue. Should be compared with figure 8, plate VI. Magnification, Leitz ocular 4, objective 3.

PLATE XI.

FIG. 18. Trunk pink muscle from salmon no. 122 from the Columbia River, Warrendale, Oreg. This outline figure shows the more compact arrangement of the fibers of pink muscle that has lost most of its intermuscular fat. The fibers themselves are normal in outline. In this fish the pink fibers in general seem somewhat smaller in size than the average for adult fish of mature size. This point should be kept in mind in comparing the absolute size of the fibers shown in this figure and the preceding. Magnification, Leitz ocular 4, objective 3.

FIG. 19. Trunk pink muscle from fish no. 140, a spawning male from the Clackamas River, Cazadero, Oreg. The outlines of the fibers shown in this fish are typical of the stage just before natural death. The larger fibers do not show any unquestioned structural signs of degeneration, though they have the decrease in plumpness. Magnification, Leitz ocular 4, objective 3.

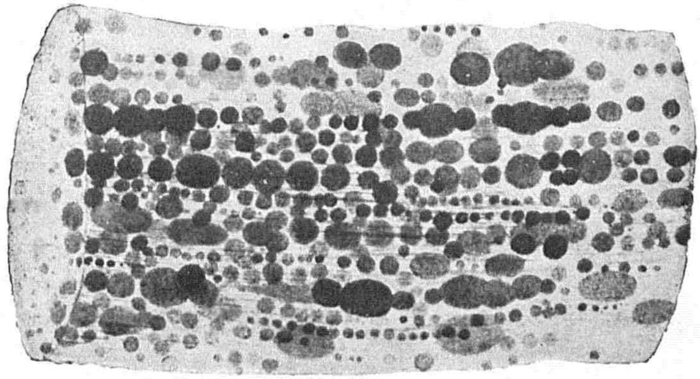
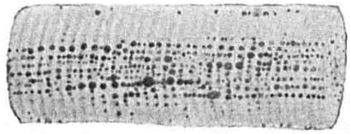


FIG. 1.



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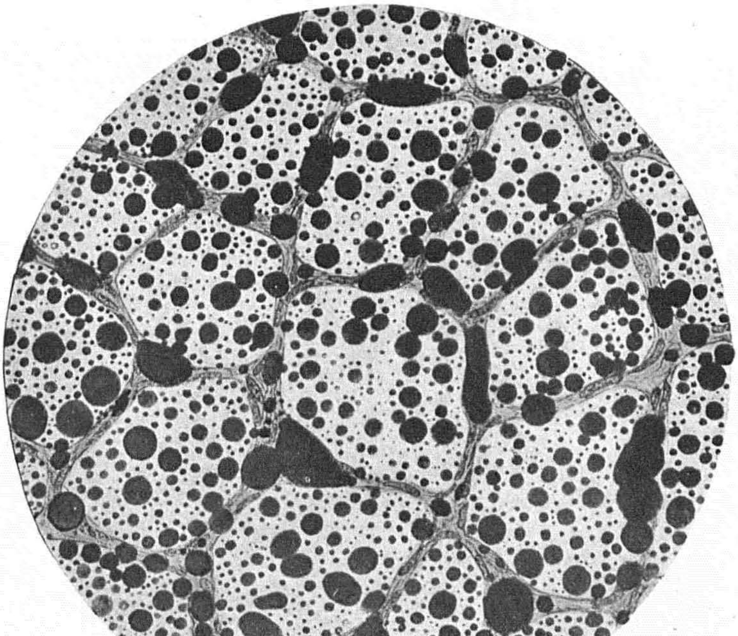
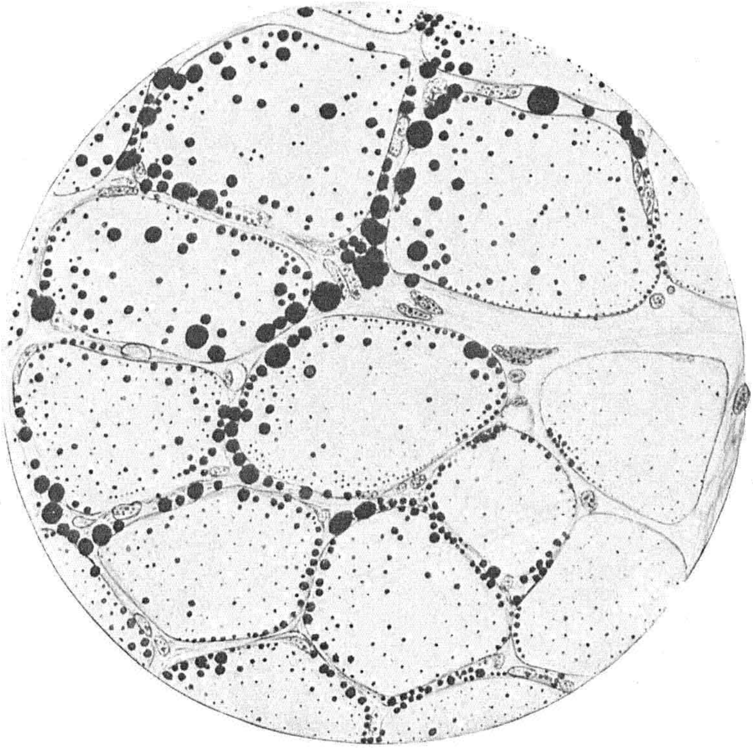


FIG. 3.



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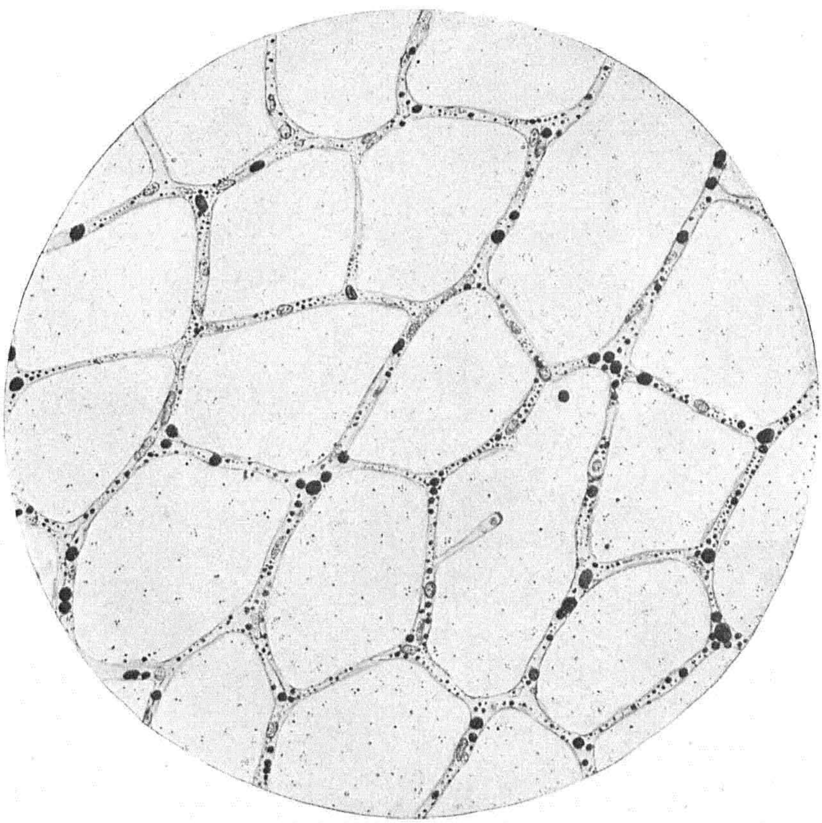

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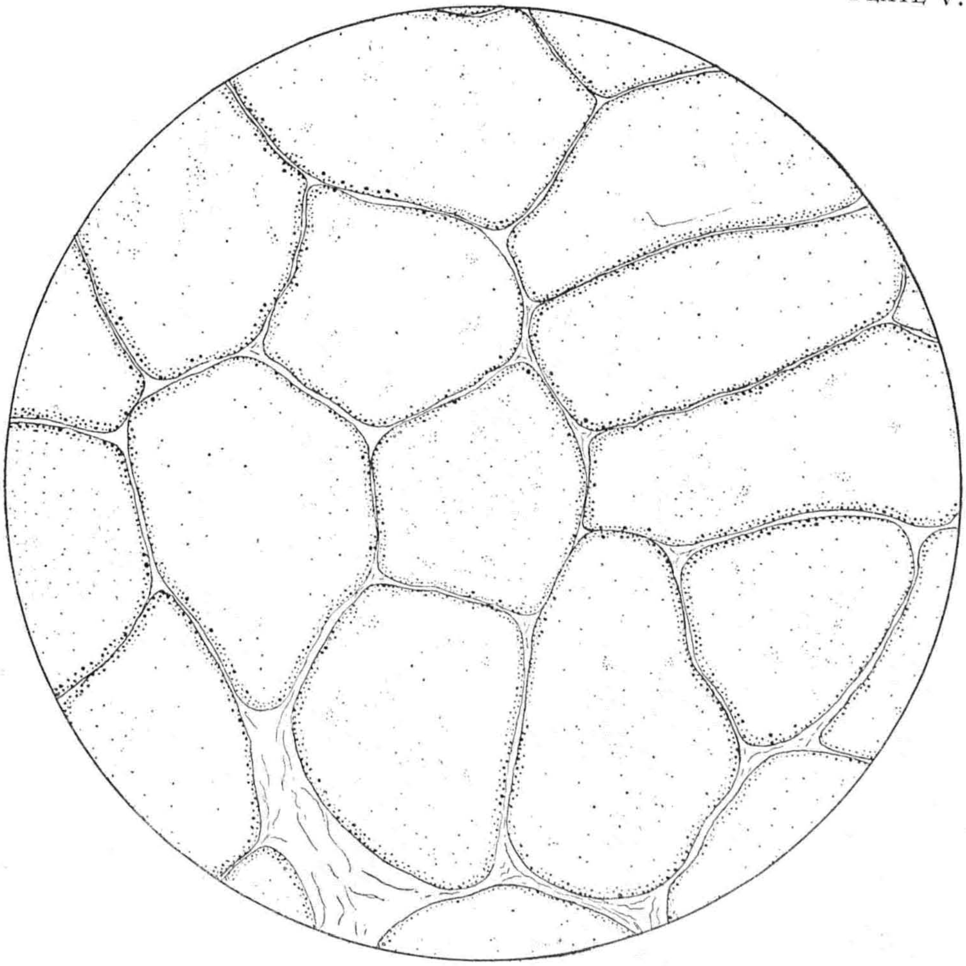

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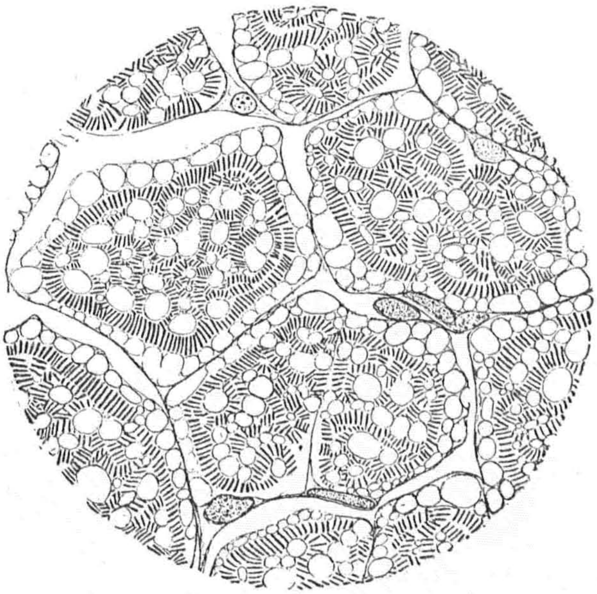

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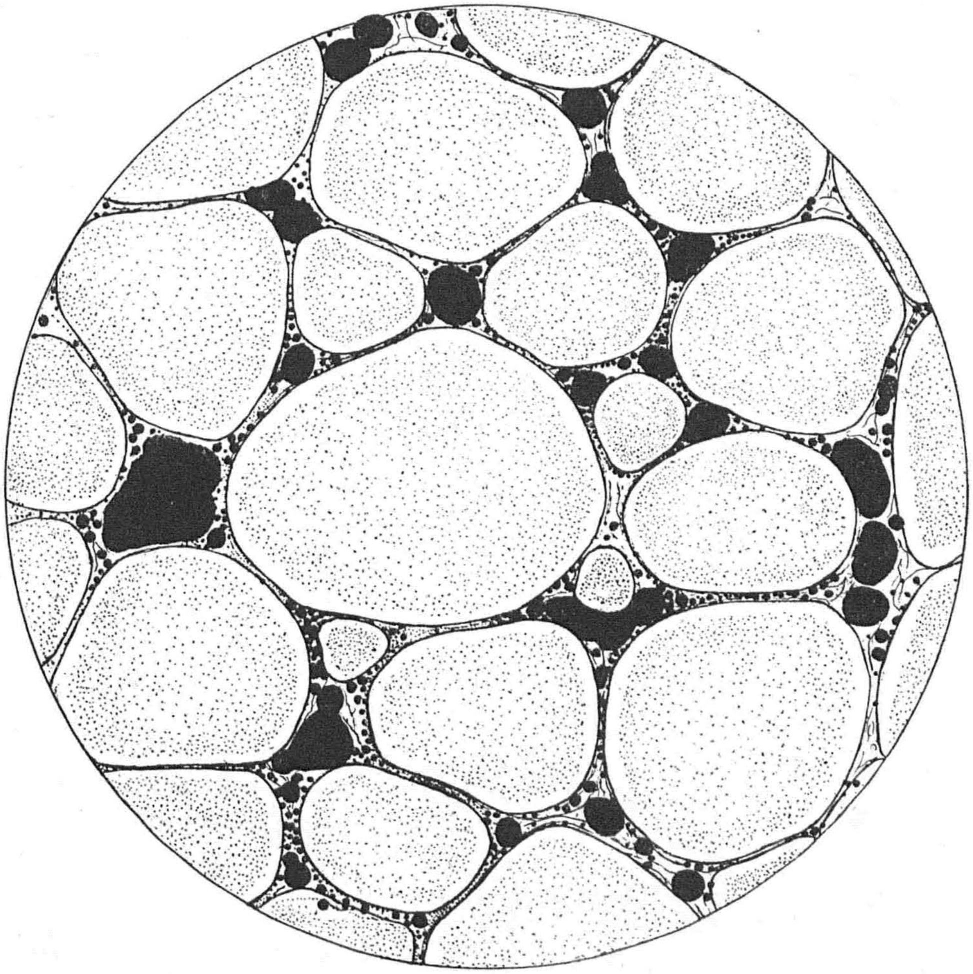

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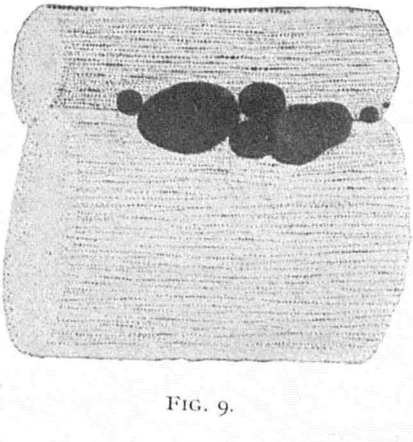

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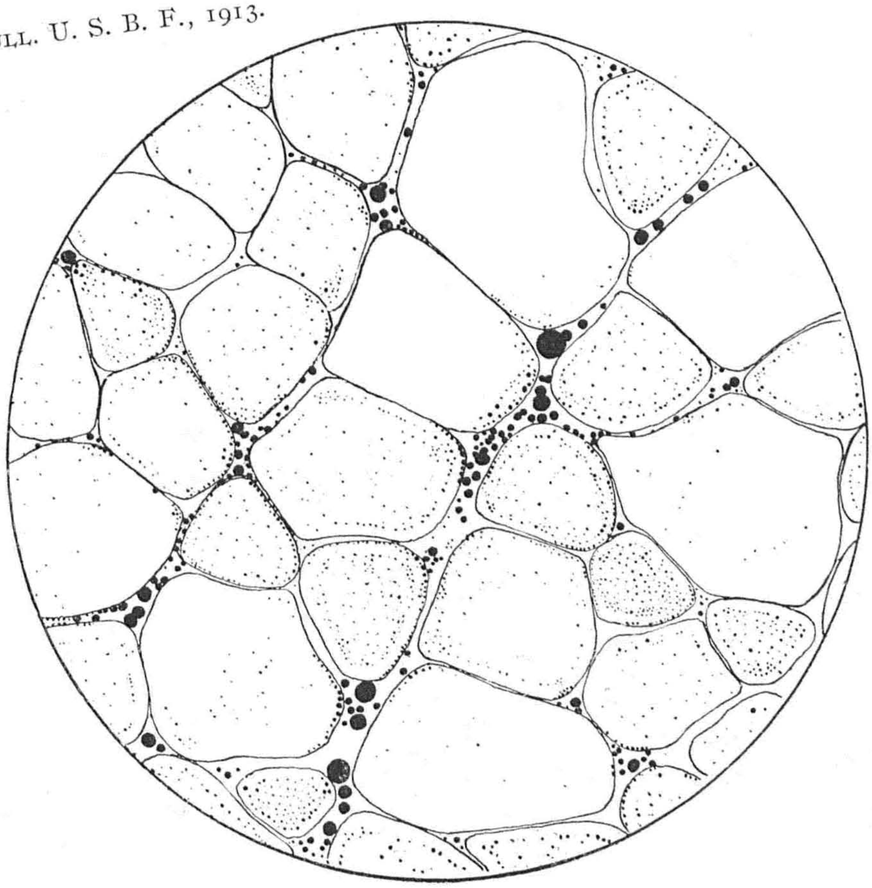
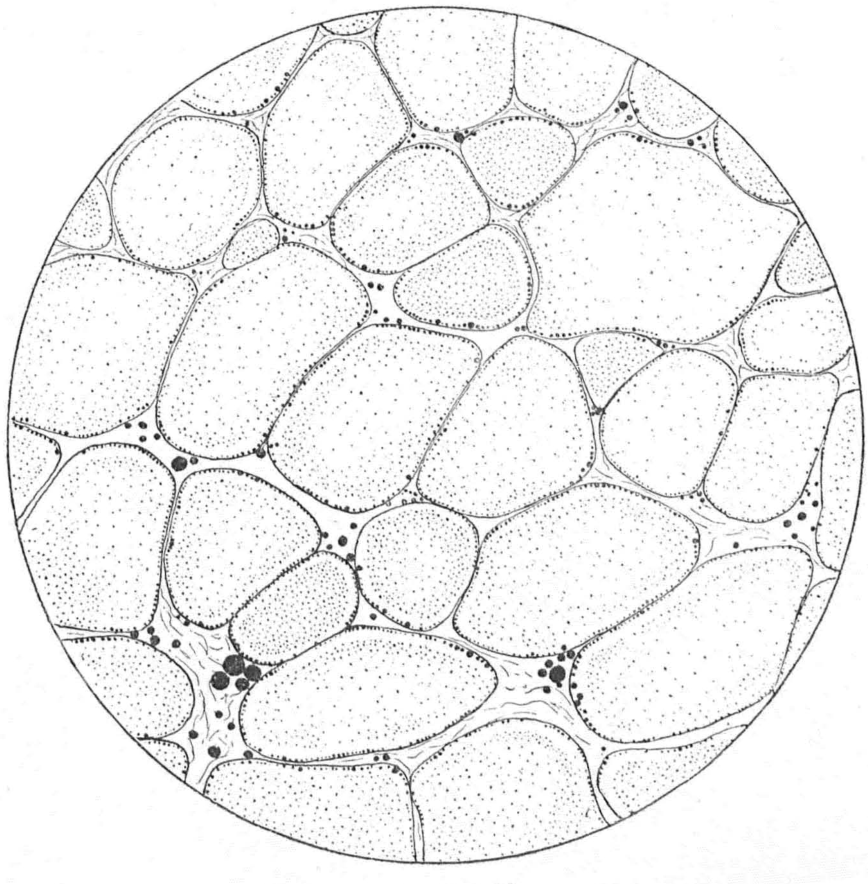


FIG. 10.



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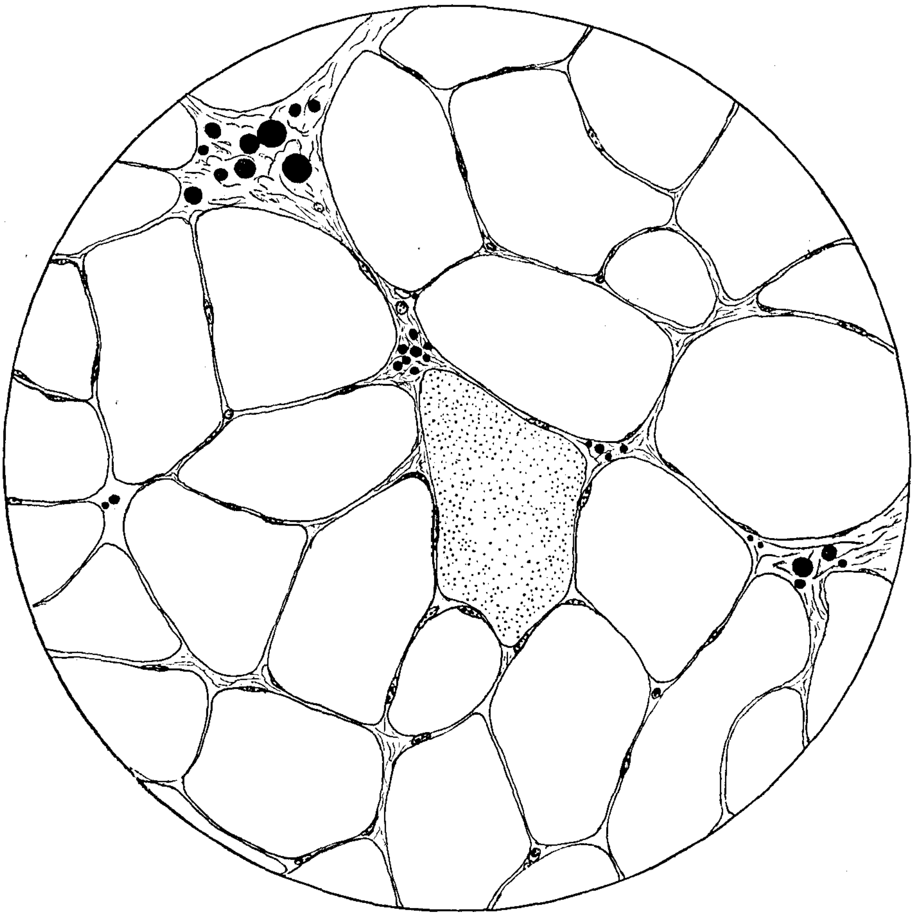

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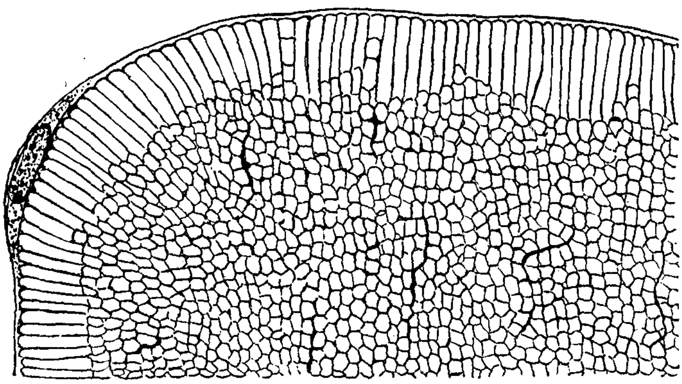
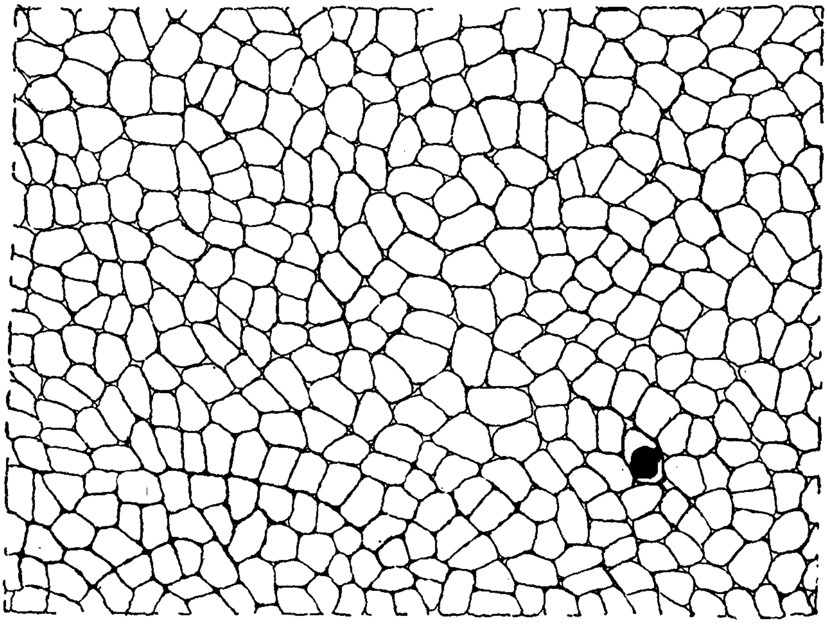


FIG. 13.



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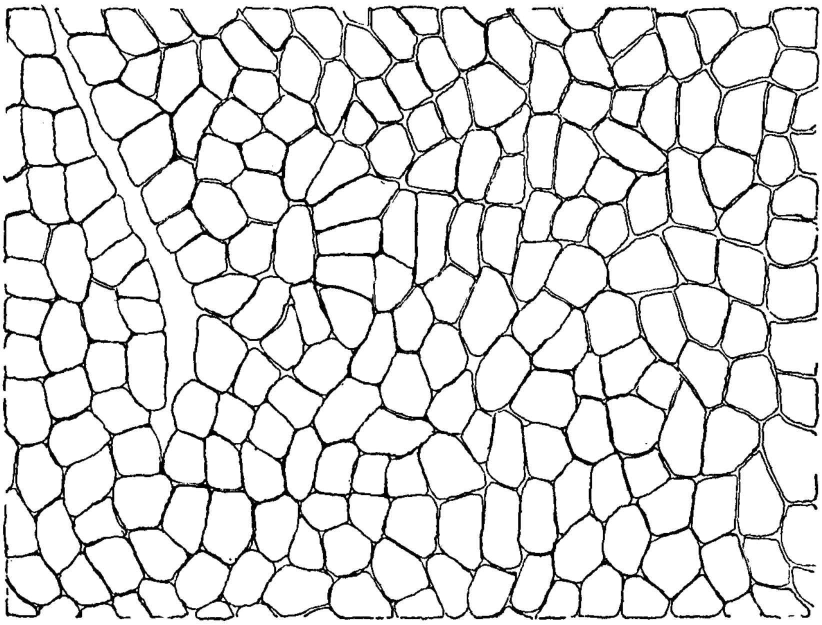
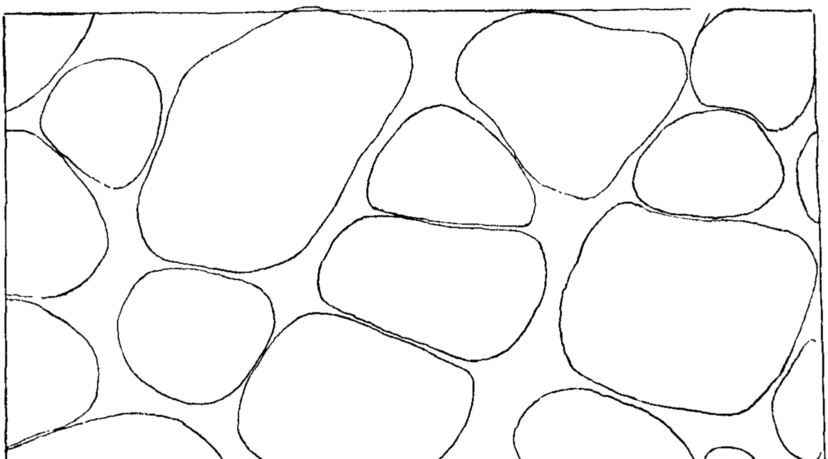


FIG. 15.

FIG. 16.



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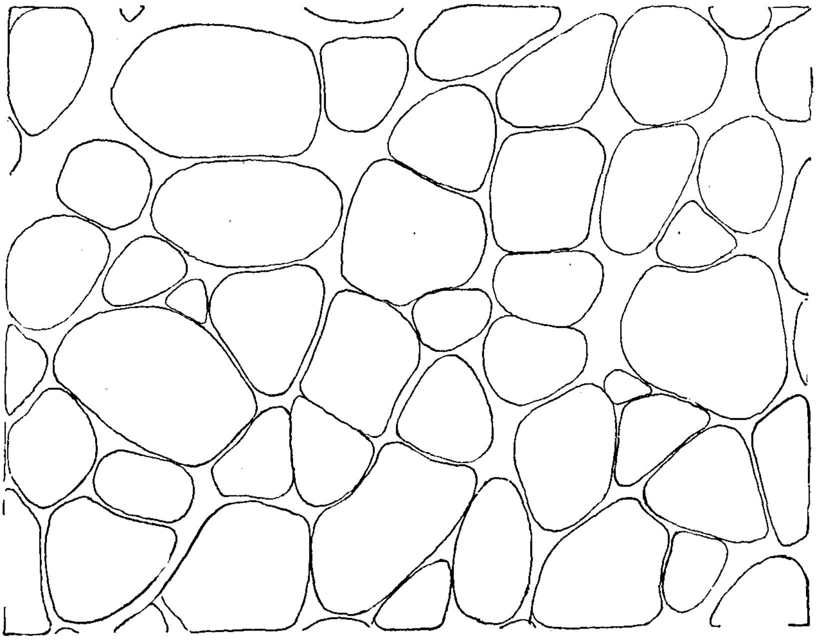

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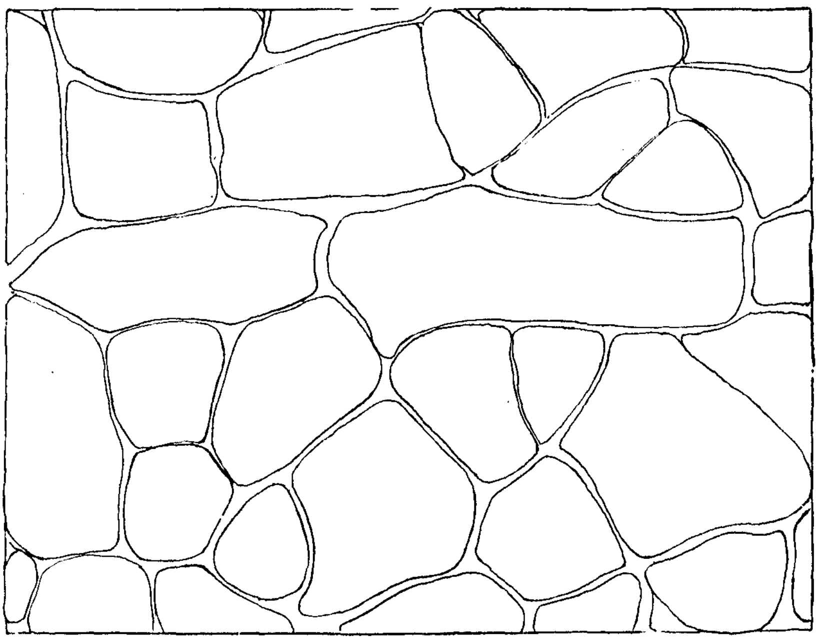

FIG. 19.