THE FAT-ABSORBING FUNCTION OF THE ALIMENTARY TRACT OF THE KING SALMON

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REVIEW OF THE LITERATURE.

The absorption of fats by the alimentary tract of man and animals has been a subject for discussion and investigation for many decades. Our present views concerning the topic have been arrived at almost exclusively by the study of the higher mammals. Few observations along this line, especially of an experimental nature, have been made on fishes. The setting of the problem which has led to the investigations here presented is found in our current views of fat absorption. These views have been concisely and admirably summarized by Wells.[a]

In the intestines fat is split into a mixture of fat, fatty acid, and gylcerin; but as the fatty acid and glycerin are diffusible, while the fat is not, they are separated from the fat by absorption into the wall of the intestine. Hence an equilibrium is not reached in the intestine, so the splitting continues until practically all the fat has been decomposed and the products absorbed. When this mixture of fatty acid and glycerin first enters the epithelial cells lining the intestines there is no equilibrium, for there is no fat absorbed with them as such. Therefore the lipase, which Kastle and Lœvenhart showed was present in these cells, sets about to establish equilibrium by combining them. As a result we have in the cell a mixture of fat, fatty acid, and glycerin, which will attain equilibrium only when new additions of the two last substances cease to enter the cell. Now another factor also appears, for on the other side of the cell is the tissue fluid, containing relatively little fatty acid and glycerin. Into this the diffusible contents of the cell will tend to pass to establish an osmotic equilibrium, which is quite independent of the chemical equilibrium. This abstraction of part of the cell contents tends to again overthrow chemical equilibrium, there now being an excess of fat in the cell. Of course, the lipase will, under this condition, reverse its action and split the fat it has just built into fatty acid and glycerin. It is evident that these processes are all going on together, and that, as the composition of the contents of the intestines and of the blood vessels varies, the direction of the enzyme action will also vary. In the blood serum, and also in the lymphatic fluid, there is more lipase, which will unite part of the fatty acid and glycerin, and by removing them from the fluid about the cells favor osmotic diffusion from the intestinal epithelium, thus facilitating absorption.

Quite similar must be the process that takes place in the tissue cells throughout the body. In the blood-serum bathing the cells is a mixture of fat and its constituents, probably nearly in equilibrium, since lipase accompanies them. If the diffusible substances enter a cell containing lipase, e. g., a liver cell, the process of building and splitting will be quite the same as in the intestinal epithelium. The only difference is that here the fatty acid may be removed from the cell by being utilized by oxidation or some other chemical transformation.

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[a] Wells, H. G.: Chemical Pathology, p. 67, Philadelphia, 1907.

This point of view has been arrived at through certain classic researches which will be reviewed briefly and which for the present purpose may begin with the work of E. H. Weber.

In 1847 Weber[a] demonstrated that during fat absorption the superficial epithelium of the duodenal villi was filled with fat granules. He says that the cylindrical epithelial cells "swell up and contain chyle granules" and that "the cylindrical cells are no longer cylindrical or prismatic, but are round, and many become whitish-opaque while others are filled with transparent oily fluid." This he explains as due to the power of the cells to absorb the food materials. Weber showed that some of the parenchymal cells also became opaque, containing oil-like fluids. This seems to have been the first microscopic observation of the absorption of fats by the intestinal epithelium.

Kölliker[b] in 1857 made similar observations, showing that fat was absorbed in the stomach. He also used the histological method. Kölliker found highly refractive fat granules in the fresh tissue. On page 175 is the statement, "I have never failed to find fat in the stomach epithelium in the dog, cat, or mouse from the second day after birth on. The mass of fat was indeed very variable. The cells may contain only slight masses of fine granules or they may be gorged with fat in which not only the finer but also larger fat drops are present." It is significant that he found the fat only in the cylindrical cells, i. e., not in the pavement epithelium of the mouse's stomach. This definite work of Kölliker on the ability of the gastric mucosa to absorb fat has apparently been overlooked by physiologists until the last few years.

The question of the method by which fat is absorbed has been inseparably associated with the observations of the actual physical processes of absorption. Our current view, that fat must be dissociated before absorption, received its first experimental support, so far as the stomach is concerned, by the work of Marcet[c] in 1858. This author delivered a course of lectures in London, discussing in the last of the series the evidence of the digestion of fat in the stomach. The keynote to his work is explained in the following quotation: "The experiments were undertaken upon dogs, and repeated four times with the same result. The animals were made to take a meal, consisting of cooked meat and sheep's fat, and were killed from one to five hours later; the contents of the stomach being at once submitted to examination yielded in every case fatty acids."

This chemical work of Marcet gave an admirable support to the histological observations of Kölliker, showing that the gastric mucosa is capable of producing fat-splitting ferments, or as we now call them, lipolytic enzymes. In the light of our present knowledge, these two pieces of work, that of Kölliker and of Marcet, should have established the fact of the fat digestion and absorption in the stomach. But no further reference along this line seems to be available from the literature until the work of Cash[d] in 1880, who, without reference to the previous work of Marcet, again investigated the fat-splitting properties of the gastric juice and of the extracts of the gastric mucosa.

[a] Weber, E. H.: Ueber den Mechanismus der Einsaugung des Speisesaftes beim Menschen and bei einigen Thieren. Müller. Archiv für Anatomie und Physiologie, bd. 6, 1847, p. 400–402.

[b] Kölliker, A. von: Einige Bemerkungen ueber die Resorption des Fettes im Darme, ueber das Vorkommen einer physiologischen Fettleber bei jungen Saugethieren und ueber die Function der Milz. Verhandlungen der physikalisch-Medicinischen Gesellsch. Bd. 7, 1857, p. 174–193.

[c] Marcet: A course of lectures on the chemistry, physiology, and pathology of human excrements. Lecture v. The Medical Times and Gazette, vol. 17, 1858, p. 209.

[d] Cash, Th.: Ueber den Antheil des Magens und Pankreas an der Verdauung des Fettes. Archiv für Anatomie und Physiologie (Phys. Abth.) 1880, p. 323–333.

In 1890 Krehl [a] made a restudy of the question of fat absorption from the intestinal tract. His drawings showing different stages in the microscopic loading of the epithelial cells with fat granules have become classic in the literature. The most significant fact on which Krehl lays emphasis is "the fat is not taken up from the intestine in globular form, but is absorbed in solution, and is resynthesized" giving rise to the droplets observed in the pyloric epithelial cells which the author presents in his figures. The conclusion that fat is absorbed in the dissolved state was later advocated by Pflüger (1900), after which it received general acceptance.

In 1901 Schilling [b] again observed fat in the gastric epithelium of the calf, in this case after a meal of milk. Schilling noted that the epithelial cells were thickly studded with microscopic fat droplets and that fat deposits appeared in the connective tissue of the tunica propria and parenchyma. He also noted fat in the lymphatic glands during absorption. He apparently did not investigate the presence of fat in the lymphatic radicles from the stomach.

In 1908 Van Herwerden [c] published the results of extensive and valuable studies on the gastric digestion in fishes. This subject he investigated under two heads, the second of which, namely, "Enzymes in the gastric mucosa," concerns us here. Van Herwerden made his observation chiefly on sharks, but also on bony fishes. These fishes he fed with olive oil or egg-yolk emulsion, the food being introduced into the stomach by way of the mouth. Having previously determined that fasting animals were relatively free of fat granules, he states that upon killing animals after a certain number of hours following feeding, "one finds fat drops in great numbers in the superficial epithelium." He states further that "the fishes contained fat granules everywhere in the submucosa between the musculature and especially in the lymph vessels which accompany the blood vessels. In hungering fishes I have never found this to be the case."

Van Herwerden also tested the activity of glycerin extracts of the gastric mucosa. He found in *Scyllium* a decided increase in the formation of fatty acids; also, in teleosts, his tables show the presence of an active lipolytic enzyme. Extracts previously boiled gave always negative results, as did also extracts from the muscle walls of the alimentary canal.

These interesting observations of Van Herwerden seem to be the first that have been made along this line upon the fishes. This splendid article had escaped my search in the literature until after the publication of the preliminary report of the present work.

Three previous communications have been made with reference to the present work; the first relating briefly the observations on fat absorption from the pyloric cœca of the king salmon,[d] and the last two, one a preliminary [e] and the other a brief statement of the facts of fat absorption from the stomach of the king salmon.[f]

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[a] Krehl, Ludolf: Ein Beitrag zur Fettresorption. Archiv für Anatomie und Physiologie (Anat. Abth.) 1890, p. 97.

[b] Schilling, F.: Die Fettresorption im Magen. Fortschritte der Medicin, bd. 19, 1901, p. 613.

[c] Van Herwerden, M.: Zur Magenverdauung der Fische. Zeitschrift für Physiologische Chemie, bd. 56, 1908, p. 453-494.

[d] Greene, Charles W.: The absorption of fats by the alimentary tract with special reference to the function of the pyloric cœca in the king salmon, *Oncorhynchus tschawytscha*. Read before the St. Louis meeting of the American Fisheries Society, 1912. Transactions American Fisheries Society, 1912, p. 261.

[e] Greene, Charles W.: The absorption of fat by the salmon stomach. Preliminary notice. Proceedings American Physiological Society, American Journal of Physiology, vol. 29, 1912, no. 4, p. XXXVI.

[f] Greene, Charles W.: Absorption of fat by the salmon stomach. American Journal of Physiology, vol. 30, p. 278, 1912.

During the progress of this work a preliminary notice and final paper have been published by Greene and Skaer[a] reinvestigating the fat absorption from the stomach in mammals; also a paper by Weiss[b] briefly presenting the fact of fat absorption by the gastric mucosa in the snake and in mammals.

EXPERIMENTS DEMONSTRATING THE ABSORPTION OF FATS.

METHOD.

The method of determining the character and degree of fat absorption from different portions of the alimentary tract of the king salmon has been that of microscopic examination. Tissues were examined fresh and after formalin fixation followed by the newer fat stains, Sudan III, scarlet red, etc. The chief reliance for staining the fat in the cells has been on the alkaline scarlet red. These methods of observation have been confirmed by more careful tissue fixation in Flemming's osmic acid mixture and by the corrosive bichromate method of Bensley. Flemming's solution not only fixes the tissues but gives the characteristic osmic acid staining of the fats. The Bensley fixation, when followed up by paraffin sections and differential staining, gives a negative picture, since the fats are dissolved out by the clearing fluids, leaving only fat vacuoles.

The detail of procedure for staining with scarlet red is as follows: The perfectly fresh material, living tissue if possible, was dropped into a 10 per cent formalin for two hours or more. Precautions were taken to insure penetration and proper fixation. The material fixed in formalin was then frozen in a freezing microtome and cut as thin as possible. The frozen sections were cut directly into 70 per cent alcohol, and stained in alcoholic solutions of scarlet red. The stain was made by heating an excess of scarlet red in 70 per cent alcohol containing 2 per cent sodium hydroxide to a temperature of about 80° C. This procedure, which is recommended by Bell,[c] gives a stain which on cooling leaves a saturated solution of greater staining powers than the ordinary alcoholic scarlet red. The stain was always filtered into shallow dishes just before using. Shallow oval bottom salt cellars were used, and these immediately covered to prevent evaporation. The sections were lifted from the 70 per cent alcohol, the excess of fluid quickly removed, and then they were dropped into the stain. Staining is comparatively rapid and requires only from 5 to 15 minutes for a successful impregnation.

Sections were taken from the stain, the excess of adherent stain being removed by a momentary immersion in 70 per cent alcohol, and then were immediately plunged into a large dish of water. When the particular tissues were delicate, an intermediate grade of 35 per cent alcohol was used. In this case the sections must be in contact with the alcohol only long enough to remove the adherent stain, otherwise the stain in the tissue itself will be drawn. As a matter of routine practice it was found desirable to add to the wash water bath a couple of drops of hydrochloric acid. The faint acidity was found favorable to the more rapid removal of the traces of alkali. This step contributes decidedly to the keeping powers and clearness of the sections after they are mounted

[a] Greene, Charles W., and Skaer, William F.: Absorption of fat by the mammalian stomach, Proceedings American Physiological Society, American Journal of Physiology, vol. 29, no. 4, 1912, p. xxxvii. Evidences of fat absorption by the mucosa of the mammalian stomach, American Journal of Physiology, vol. 32, 1913, p. 358.

[b] Weiss, Otto: Die Resorption des Fettes im Magen. Pflüger's Archiv für die gesamte Physiologie, bd. 144, 1912, p. 540–543.

[c] Bell, E. T.: The staining of fats in epithelium and muscle fibers. Anatomical Record, vol. 4, 1910, p. 199–212.

in glycerin. Pure glycerin was used to make the mounts. Sealing with a mixture of paraffin and beeswax around the cover glass was the final step in the mounting and preservation of the sections.

The more permanent sections of the tissues fixed as described were made by the paraffin method, in which no special features in technique were introduced.

The previous fixation in formalin was found to be decidedly advantageous in the preparation of frozen sections. The brief time of immersion in the formalin does not introduce a change in the character and distribution of the fats. On the other hand the tissues are coagulated, hence firmer, and can be carried through the technique with a much more satisfactory result. When the frozen sections were made directly from fresh living tissues, then at the moment the frozen section was immersed in the alcohol preliminary to the scarlet red staining, considerable contraction and sometimes tearing took place. It was found that the distortion of the sections by this step was detrimental to the securing of normal pictures of the structure and relations of the contained fat.

SELECTION OF SPECIMENS.

Two types of fish were used for the determination of the points detailed in this report. First, salmon of various sizes and presumably of different ages collected from the markets in the city of Monterey. The fish selected were those delivered directly from the fishing boats, which had made their catch by trawling on the ocean fishing banks in the vicinity. These fish came into the market with living tissues, a fact that could easily be determined. The alimentary tracts were taken from the salmon at the slaughtering tables of the fish-packing establishments of the Booth Packing Co.[a] If the tissues were proved to be alive in material chosen then histological samples were selected and placed in fixative immediately, so that the question of prefixation changes does not enter into consideration.

The second class of material is that derived from young salmon collected from two stations. The first collecting ground was that of the Brookdale hatchery maintained in the town of that name on the San Lorenzo River in the Santa Cruz Mountains. Young salmon were also obtained from the McCloud River in the Shasta Mountains in northern California. Both these groups of young salmon had never been in salt water. The ages of the young salmon varied from one to two years, the latter being those obtained at the ponds from Brookdale.

NORMAL-FEEDING SALMON.

The class of adult salmon mentioned above, which were secured at Monterey, were in an active aggressive stage of ocean feeding. These salmon come into the markets often with the stomach and intestinal tract gorged with food. The natural food is of a varied class, but at Monterey consists mainly of three kinds: First, the squid; second, the local species of herring; and, third, marine Crustacea, chiefly a rather large amphipod.[b] The king salmon is a voracious feeder and his ability to capture a great variety of food

[a] For the courtesy extended by this company I am indebted to Mr. Frank E. Booth, the president.

[b] In July, 1911, quite a number of salmon were noted with large numbers of these Crustacea in their stomachs. One salmon stomach in particular contained 4 or 5 (estimated) ounces of such food. It would have been interesting to have counted the actual number of crustaceans present, but the content of the stomach was partly lost before the thought occurred to make such an enumeration.

material besides the forms mentioned above is shown by the various species of fishes occasionally noted in the food at Monterey. These natural foods are all relatively oily, the point which particularly concerns us here. As digestion proceeds and the protein framework is dissolved away these oils are liberated in the alimentary canal and form no inconsiderable portion of the food of the king salmon. When one remembers the characteristics of the salmon flesh, charged with oil as it is, and evidently storing great quantities of oil, the interest which attaches to the question of the source of the oil in the food and the method of digesting and absorbing oils is obvious.

As a matter of fact it was in the course of a study of the character and microscopic distribution of the fats in the salmon tissue that I instituted observations on the alimentary tract of the king salmon which made it obvious that large quantities of oils were absorbed from the foods in these normal feeding salmon.

FAT-FED SALMON.

The inability to control the relation between the time of taking food and the chance of securing the fish and making observations of the stage of absorption in the normal feeding salmon renders it extremely difficult to settle the question of the characteristics of fat absorption in such. As a matter of fact, my observations made it very clear that much absorption of fat was taking place in salmon feeding under natural conditions, yet it was found next to impossible to determine the nature and details of the process from the specimens available. For this reason the idea of feeding salmon in the aquaria was conceived and its immediate execution was made possible through the courtesy of the directors and superintendent of the Brookdale hatchery. Young salmon were transported in live cans from Brookdale to the Hopkins' Seaside Laboratory at Pacific Grove, Cal. Two sizes of salmon were available, one group of yearlings from 6 to 7 centimeters long, and a group of small 2-year-olds from 14 to 16 centimeters long.

These young salmon were fed olive oil by rectal injection. This was found to be an extremely reliable and easy way of introducing the oil into the alimentary tract in such a way as to give one confidence in the accuracy of the results. A medicine dropper was drawn out in the flame to a slight cone of proper size. A desirable quantity of oil was then taken into the dropper, the tip inserted into the anal aperture and gentle pressure maintained until the oil was emptied into the alimentary tract. It is comparatively easy to hold the young salmon by a firm grip of a lobe of the caudal fin rays, the fish resting in the palm of the hand in such a way that the head and gills remain under water to prevent asphyxiation. Under these conditions the fish does not struggle as much as might be expected. The slight contractions of the muscles of the anal sphincter occurring when the pipette is first introduced soon relax, but one has always to maintain a gentle pressure on the pipette for a moment before oil begins to flow into the tract. The alimentary canal of the salmon is a simple S-shaped tube, as has been described and figured in a previous paper.[a] When the oil is injected into the posterior end of the canal in sufficient quantity it flows into the different limbs of the intestine and into the stomach, and from the stomach will be discharged from the esophagus into the mouth if an excess of oil is used. In my later experiments this fact was adopted as an index of when the proper quantity of oil was administered.

[a] Greene, Charles W.: The anatomy and histology of the alimentary tract of the king salmon, *Oncorhynchus tschawytscha*. Bulletin, Bureau of Fisheries, vol. XXXII, 1912, p. 73–100, pl. XXV–XXVIII.

A series of artificial feeding experiments was executed at the Hopkins' Seaside Laboratory, followed by a more extensive series at the Federal salmon hatchery at Baird, on the McCloud River in northern California. In this later series the question of absorption in relation to the time following the administration of oil was especially investigated. Furthermore, in the Baird series it was possible to maintain the young fish without food an adequate time to insure the complete elimination of the fat from the alimentary canal which might previously have been derived from natural foods.

The Monterey series consisted of two salmon of the 2-year-old group with confirmations on two salmon of the small 1-year-old group, with different time allotments for absorption, ranging from 20 to 70 hours. Careful examinations were made extending over the stomach, intestine, and various pyloric cœca of each of these series.

GENERAL RELATIONS OF THE ORGANS OF ABSORPTION.

The critical regions for the study of the absorption of fat in the salmon are three, namely, the stomach with its two divisions, the cardiac and the pyloric ends; the intestine with its two great divisions, the pyloric and post pyloric; and the numerous pyloric cœca which have their origin from the pyloric intestine.

These great divisions are of necessity to be described separately. Logically, one might take them in the order, stomach, intestine, cœca; but because of the way in which the evidence was accumulated and other questions attached to the subject it is more convenient to discuss the details in the reverse order, i. e., absorption in the pyloric cœca, in the intestine, and in the stomach.

ABSORPTION OF FATS BY THE PYLORIC CŒCA.

The gross anatomy and the normal histological structure of the alimentary tract of the king salmon have both been presented in a previous paper.[a] Figure 1 of that paper is an illustration showing the general relations of the cœca to the pyloric end of the intestine from which they arise in such profuse numbers. Those cœca which originate from the beginning of the intestine, that is, in the neighborhood of the pyloric valve, are much longer than those that arise from the posterior end of the series. These cœca often reach a length of from 10 to 15 centimeters and even more in the adult feeding salmon. They have a normal diameter of 5 to 8 millimeters. In the sea salmon taken at a time when food is abundant and digestion has been going on actively for some time the cœca are always gorged with material and distended to their full length and diameter.

The content of the pyloric cœca under these conditions is peculiar in appearance. One never finds solid particles of food. Instead, there is only, as Gulland and others have mentioned, a creamy, yellowish, puslike mass which has a viscid adhesive consistency. This content is never very fluid, i. e., of limpid character. The exact color of the contents varies with the class of food material which the salmon is digesting at the time. If the food is made up of Crustacea then the content of the cœca has a darker color, often of a deep orange red. It is apparent that the viscidity of the mass is due to the secretion of mucous by the epithelial lining of the cœca themselves.

In the younger salmon the pyloric cœca have the same relative size, but of course are smaller in proportion to the gross size of the fish. In no instance have I observed

[a] Greene, Charles W., op. cit.

any extensive mucous content of these young cœca. In the specimens that were fed fat there was an occasional increase in the transparency, which was interpreted as due to the presence of oil. In the intestine of such fish the excess of oil was easily and often shown.

EVIDENCES FROM SALMON FEEDING NORMALLY.

Fat droplets were always observed in the epithelial lining cells of the pyloric cœca of the Monterey salmon. However, the fat was not present in all cells. Certain portions of the epithelium were filled with fat droplets, while other portions were relatively free. In almost every animal observed, and in different regions of the same animal, certain extended portions of the epithelium were observed to contain no fat droplets, while in the neighboring regions, often in the same section or perhaps in the next mucous fold, fat would be present. These facts could not readily be explained by the assumption that fat was loaded into these cells by way of storage, being brought in from other portions of the body. On the other hand, such observations strongly suggest a process of fat absorption. Previous observations on fat absorption in fishes are apparently very limited; at any rate the search in the literature has thus far revealed to the writer only the observations of Van Herwerden [a] "On Gastric Digestion in Fishes." This splendid paper deals largely with digestion and the digestive enzymes. But it definitely demonstrates fat absorption in *Scyllium*. It follows that the chief guide in the interpretation of the present results is that to be found in the comparative literature on fat absorption in other animals, a portion of which has been referred to and reviewed in a previous chapter.

The mucous epithelium of the salmon cœca is very extensive, considered in proportion to the size of the tubes. The measurements of the superficial extent of the mucous coat show that it is from 6 to 8 times the extent of the external surface of the cœcum itself. These folds are very complex in arrangement, though the epithelial coat itself is of uniform and simple type, a matter that is discussed in the paper presenting the normal structure of these organs. It is this complex folding, and therefore the relative variation in the contact of the epithelium to portions of the contained food mass, that explains the fact of unequal loading of fat in the epithelial cells. Hence there is no doubt that the fat observed was absorption fat.

HISTOLOGICAL APPEARANCE OF FAT IN THE EPITHELIAL CELLS.

A cœcum containing fatty food material in an advanced stage of digestion and absorption will almost always present epithelial cells in all the stages of fat loading. The appearance of the cells loaded with fat is characteristic and changes progressively as absorption proceeds. In a general way, though some allowance must be made for the comparison, the histological character of the cells would suggest three stages.

Fat absorption, stage 1.—The earliest stage of absorption is that of the passage of fat into and through the superficial border of the epithelial cells. The methods of staining, whether they be direct staining of the tissues with scarlet red or fixation and staining of the fat by the osmic acid mixtures, show a large number of very fine granules in the most superficial layer of the protoplasm of the cell. These fat granules are extremely

[a] Van Herwerden, M., op. cit.

small, the largest being less than 1 μ in diameter. In some instances they appear in such minute size that they are only just distinguishable under the oil immersion. As absorption proceeds the fat granules make their appearance deeper and deeper in the cell, loading up the zone between the free surface and the nucleus. Here the fat droplets are relatively large, ofttimes being 4.5 to 6 μ in diameter. In the intermediate area and between the superficial zone and the extra-nuclear zone are all sizes of fat droplets from the extremely minute ones just described to the large ones in the extra-nuclear zone. This picture is shown very clearly in figures 6 and 12.

Fat absorption, stage 2.—The second characteristic cellular appearance, which is designated as stage 2, consists in the filling of the inner or basal end of the cell with fat droplets. Not only that portion external to the nucleus will be loaded with fat, but the portion between the nucleus and base of the cell will also contain an excess of fat droplets.

The sizes of the droplets in the end of the cell are similar to those just external to the nucleus, but the number of droplets is rarely so great. When the cell is fully loaded it generally happens that fat will be found in the connective tissue of the tunica propria beneath. If fat absorption is continuous at this stage, as one might legitimately assume from the histological appearance, it is obvious that as the fat is entering the outer zone it will at the same time be discharging from the inner zone and passing into the channels which distribute fat through the body. Knoll[a] has recently reported experiments on fat absorption in the mammalia in which this condition is shown to hold.

Fat absorption, stage 3.—When absorption from the lumen of the cœcum ceases, the outer margin of the cell begins to clear of fat. This disappearance of fat apparently slowly and gradually extends over the whole area of the cell external to the nucleus. In favorable material in this stage epithelial areas will be found in which the outer or extra-nuclear zone of the mucous epithelium is almost, sometimes entirely, free of fat droplets. Still, fat droplets will be present in considerable quantity in the inner or basal zone. As a rule the basal portion of the cell will contain relatively large droplets in this stage and the connective tissue supporting the cell will be similarly loaded with fat droplets. However, some groups of cells are found in which the fat droplets in the basal portion of the cell are extremely minute, as shown in figure 13. In this particular figure the basal areas are heavily loaded with fat in extremely fine subdivision. The adjacent connective tissue of the tunica propria contains a similar distribution and size of fat droplets.

These three stages of course are only phases of an orderly and progressive process in which the fat enters the outer zone of the cell, is disposed within the substance of the cell in droplets, and is ultimately distributed from the cell to the basal pole, the opposite from which it entered. The variations in the size of the droplets in different zones of the epithelial cells, especially the extremely small droplets in the outer portion of the cells and the fine droplets in the bases of the cells at the time the discharging is almost complete, are very interesting when considered in relation to the theories of fat absorption. But the discussion of these theoretical points will be taken up again in a later section of the paper.

[a] Knoll, A.: Chemische und mikrosopische Untersuchungen über den Fetttransport durch die Darmwand bei der Resorption. Pflüger's Archiv, bd. 136, 1910, p. 208–247.

EVIDENCES IN ARTIFICIALLY FED SALMON.

A brief report on these experiments has been presented.[a] The first series of experiments carried on by the method of fat feeding described on the preceding page contains two young salmon, one 14 and the other 16 centimeters long.

Figures 6 and 7 present the histological picture of the amount of fat in the superficial epithelium of frozen sections of the cœca from salmon 45. Absorption proved to be extremely rapid and vigorous in this young salmon, not only in the cœca, but in the intestine, as will appear later. The epithelial cells, especially of those mucous folds which extended out into the lumen, were simply gorged with fat. The fat droplets were extremely large and filled not only the superficial portion of the cells but the basal portion as well. If the adjacent membranes of a deep fold were in contact with each other, thus preventing a free contact with the fat of the cœcal content, such places would show a relatively small amount of absorption fat in the cells. On the free loops of the mucous folds this situation did not exist, hence these portions were gorged with fat in all the sections examined. This fact is shown especially well in the high magnification of figure 11. In some portions of the tissue in the neighborhood of the areas drawn in this figure the fat was present in so great a quantity as to burst the cell membrane. It was believed at the time of the preparation that the fat absorption continued until the quantity within the cells produced a pressure greater than the cell surface could stand, hence the break, though one can not exclude the possibility of mechanical pressure during manipulation. Drops often reach a diameter of from 8 to 10 μ, or even more in the young, which is greater than the normal diameter of the epithelial cell, even at its largest end.

In figure 6, showing the fat in salmon No. 45 stained with osmic acid, a number of cells are shown in which the fat droplets of the outer portion of the cell are large enough to take up the entire diameter of the cell. In different regions of this particular histological preparation other than shown in the figure there are numerous confirmations of the above statement.

Both the positive staining and fixation of fat by osmic acid and the arrangement of fat vacuoles in corrosive fixed and paraffin sectioned material give confirmation of the direct observations of the fresh material stained with scarlet red. *The series of studies show that fat absorption takes place abundantly in the pyloric cœca.* Whatever else these organs accomplish, it is perfectly clear that the absorption of fat is one of their chief functions.

FAT IN THE TUNICA PROPRIA OF FAT-FED SALMON.

One of the most interesting confirmatory lines of observation which is largely cleared up by the fat-feeding experiments is the fact of the presence of fat in the tunica propria. In fasting salmon, especially in those used for control in the Baird series, practically no fat is present in the tunica propria. One must be guarded in such statements because this tissue holds on to its fat with great persistence. Fat will persist in the tunica propria when one can demonstrate absolutely no fat in the epithelial cells. But when absorption begins, as judged by the amount of fat in the epithelial cells, then

[a] Greene, Charles W.: The absorption of fats by the alimentary tract, with special reference to the function of the pyloric cœca in the king salmon, *Oncorhynchus tschawytscha.* Transactions of the American Fisheries Society, 1911, p. 261.

fat begins to appear in the tunica propria in an increased quantity. After 18 hours or more (see figs. 6, 7, and 8), the connective tissue layer supporting the epithelial cells becomes extremely full of fat droplets. The fat appears first in the vicinity of the bases of the epithelial cells, then is distributed through the substance of the tunica propria. The stratum compactum always forms a definite and striking boundary to the fat containing tissue of the tunica propria. This is shown especially well in the figures, particularly figure 6.

The tunica propria acts as a sort of reservoir for the fat immediately following periods of active absorption. The matter has not been sufficiently studied yet, but it seems obvious that the tissue building up this stratum of the cœcal wall seizes and holds fat with unexpected persistence. Fat will be found here in a relatively considerable number of droplets at a time when the epithelial cells are completely discharged.

The stratum compactum, as described in the discussion of the normal structure, forms a continuous sheath around the tunica propria. It is a continuous membrane with no discernible openings other than at the points where blood vessels enter. Any fat passing through the stratum compactum would have to pass through in solution or else be carried within in the lumen of the blood vessel. In either case no definite fat globules as such get by this membrane from the tunica propria.

PROTOCOLS.

Brookdale Salmon, Field Series No. 45, Length 14 Centimeters, Taken July 6, 1911.

This young salmon was a 2-year-old reared by the Brookdale hatchery, California. It was taken from an aquarium and transported to the Hopkins Seaside Laboratory, Pacific Grove, Cal. This salmon was fed fat. In this instance it received first a fat emulsion consisting of 20 per cent olive oil in coagulated milk injected into the stomach through the mouth. This feeding did not seem to be very successful and was followed later by an injection of olive oil into the rectum. This method of feeding proved to be very successful, convenient, and satisfactory. The salmon was killed after allowing 18 hours for absorption of the olive oil (22 hours, counting time from the first attempt to feed by way of the mouth). Frozen sections were made of the fresh tissues, and certain portions of the tissues were fixed by different histological methods for later examination.

Fat in the pyloric cœca.—The epithelial cells are simply gorged with fat droplets. Especially are the outer ends of the cells so filled that the cell boundaries are obscured. The diameter of the droplets varies widely. The larger drops distort the cells. The basal ends of the cells contain a much smaller amount of fat.

The tunica propria is also well filled with fat, but not so great an amount as in fish 46. The drops are more uniform in size. This fat extends deep into the folds of the stratum compactum, but is never present in its substance.

Fat in the intestine.—The epithelial cells are as much crowded with fat as in the cœca, as shown in figures 2 and 4. The deeper folds of the intestinal mucosa are not always filled with fat, at any rate the amount of fat is not nearly so great as in the outer folds.

There is less fat in the intestinal tunica propria than in the cœca of the same animal.

Brookdale Salmon, Field Series No. 46, Length 16 Centimeters, Taken July 6, 1911.

This young salmon was a mate to no. 45 and was transported at the same time. It was fed fat by rectal injection only, and was killed after 42 hours of absorption. Frozen sections were prepared and tissues were also fixed for permanent histological mounts.

Fat in the pyloric cœca.—The amount of fat in the epithelium of the pyloric cœca varied in different preparations. Those cells on the tips of the folds were crowded with fat, while those in the grooves between folds were relatively free. Figure 8 and figure 11 are from this specimen.

The tunica propria, as shown in figure 8, was more crowded with fat than in no. 45.

Fat in the stomach.—Sections of the gastric division of the stomach showed the outer ends of the epithelial cells medium full of fine fat droplets, shown in figure 1. These droplets are in the extreme outer ends of the cells just within the striated border. They are strikingly smaller than the droplets present in the intestinal and cœcal epithelium of the same specimen. A sprinkling of fat droplets is present in the inner limbs of the gastric epithelial cells.

McCloud River Salmon, Field Series No. 88, Female, Length 84 Millimeters, Taken July 23, 1911.

This young salmon was seined from the McCloud River and was 1 year old as verified by scales. It was fed fat by the method of rectal injection and killed after 20 hours.

Fat in the pyloric cœca.—Frozen sections were made of the pyloric cœca and these stained with scarlet red and counterstained with hæmatoxylin. Absorption fat was present in moderate quantity, see figure 9. The greater portion of the fat is limited to the outer ends of the cells, but a few droplets were present in the inner ends of the cells and a small amount in the tunica propria.

Fat in the stomach.—This specimen showed an unusual amount of absorption in the gastric epithelium. Particularly was the pyloric epithelium loaded with fat. (See fig. 1.) Many of the deep folds of the pyloric epithelium were practically free of fat, but those cells dipping deepest into the cavity of the stomach were unexpectedly filled.

Two sections of cardiac stomach were fat-stained only. The slender cylindrical epithelial cells of the mucous ridges bordering on the lumen of the stomach and those cells extending down into the crypts of this somewhat contracted stomach all show numbers of droplets. The fat is greatest in amount in the cells of the free folds. The fat is finely divided in appearance; that is, in minute droplets. It is greatest in quantity in the outer thirds of the cells. There is a transparent superficial border of the epithelial coat in which the fat is in the form of finest liposomes, requiring the oil immersion lens for resolution. (See fig. 1 of osmic acid staining of no. 46, Greene, American Journal of Physiology, vol. 30, p. 280.) There is also fat in the inner limbs of the cells down to their bases, and this is more or less continuous with small amounts of fat in the tunica propria.

The gland cells of the secreting portion of the stomach in this section are granular in appearance and slightly pink with scarlet red. In several regions very small fat droplets, the size of which varies around 0.5 μ, are found in the basal portion of many of the gland cells. In connection with the large majority of the gland tubes in this slide there are cell areas over the surface of the tubes which seem quite thickly studded with finest fat droplets. This section is not counterstained, so it is difficult to determine to exactly what tissue these cells belong. In some instances they undoubtedly belong to the connective tissue of the tunica propria surrounding the gland tubes.

Several of the submucous areas just within the circular muscle and through which blood vessels run are finely punctate (oil immersion lens) with liposomes. Vascular areas in the longitudinal muscle coat are also stippled with liposomes, the droplets being located in the endothelial cells and in the walls of the blood vessels.

McCloud River Salmon, Field Series No. 91, Male, Length 83 Millimeters, Taken July 25, 1911.

A young salmon seined from the McCloud River, fed olive oil by the method of rectal injection and killed after 70 hours.

Fat in the pyloric cœca.—Transverse sections of the pyloric cœca were made by the freezing method and stained for fat. Figure 10 shows a characteristic section from this fish. The amount of fat in the outer portion of the epithelial tissue is unusually great, though a very small amount had penetrated the inner limbs of the epithelial cells, and practically none to the tunica propria. The length of time that had been allowed for absorption would justify the expectation that the tunica propria would be loaded as shown in figure 8, from no. 46. Such was not the case. Possibly fat was late in entering the particular group of cœca examined.

The amount of fat in this material; as in the cœca of all of the fat-fed salmon, is unquestionably from fat absorption. The control materials, salmon no. 82 to 86, presented no fat in the epithelium of the cœca and only traces in the tunica propria.

FAT-ABSORBING POWER OF THE SALMON INTESTINE.

In the paper on the normal structure of the alimentary canal it has been shown that the salmon intestine has a histological structure relatively simple. It possesses the same epithelial lining coat, the tunica propria, the stratum compactum, and muscular membranes which are found in its diverticula the cœca. The mucous membrane itself is shown to be somewhat more complexly folded than in the cœca, a complexity that increases with the size of the fish. No differentiations are found in the different portions of the epithelial coat of the mucosa. Even in the deepest grooves or pits of epithelium the cells have the same general form and structural characteristics as in the most superficial folds.

The intestinal epithelium of the salmon is also a fat-absorbing tissue. Fat is taken from the lumen of the intestine with the greatest avidity by these cells. The judgment in this case is based on the histological showing made by the epithelial folds after fat-absorbing experiments. Unfortunately, no observations were made on the normal-feeding salmon and no opportunity has arisen to repair the deficiency. The facts presented here are wholly those derived from the studies of the young salmon which had been fed fat by the methods described above.

Figure 2, plate XII, from young salmon no. 45 presents a general view of the relations of the fat under a low magnification. Figure 3, plate XIII, is a highly magnified drawing showing the fat of one of the loops of one of the mucous folds of the section shown in figure 2. These figures show the epithelial cells gorged with fat for their whole extent external to the mucosa. In some instances the fat drops are large and have a diameter equal to or even greater than that of the normal cells. Often this fat appears in chains of drops extending from the surface of the cell to the nucleus. In other instances the droplets are somewhat smaller, but nevertheless crowd the cell body to the margin These statements are made on the basis of observations in both the pyloric and the post-pyloric loops of the intestine and on sections of the pyloric portion of the intestine. The section from which figure 6 is drawn, representing the fat stained with osmic acid in a fold of the cœcal epithelium, also contains a section through the pyloric intestine. The intestinal epithelium, too, is crowded with fat. In these epithelial cells the beaded arrangement of fat droplets is especially prominent. Where the section is accurately longitudinal through the epithelial cell, the rows of droplets of the larger size are shown filling up the whole body of the cell and to extend from the free surface to the region of the nucleus. It is comparatively seldom that fat is present in the basal portion of the cells, i. e., within the nuclear zone, in any such massive quantity as is so often found in the external limb of the cell. Here the fat is scattered along in fewer droplets, generally of fairly large size. In figure 5, plate XIII, the amount of fat in the inner nuclear zone is comparatively small.

It is to be emphasized that no fat droplets are present in the nuclei themselves. We have not observed any nuclear fat either in the epithelial or in the connective tissue nuclei supporting the epithelium of any portion of the alimentary canal. We are inclined to think that the nucleus does not for some reason ever receive a deposit of fat.

The intestinal epithelium discharges its fat into the connective tissue of the tunica propria just as observed in the pyloric cœca. In the material from which figure 3 is drawn this fact is very patent. Here the tunica propria contains a comparatively heavy loading of fat. Considering the whole of figure 2, the showing of fat in the loop chosen for figure 3, plate XIII, is if anything too low for the tunica propria. In the intestine also the fat that makes its appearance in the tunica propria is not distributed over the whole of that structure down to the stratum compactum. The stratum compactum forms a very definite and limiting boundary to the fat-containing tissue. However, it is believed that this fat present in the tunica propria is not a true storage fat. No characteristic areolar fat cells are present such as are found in such numbers in the pancreas and in certain other definitely adipose tissues of the salmon. The tunica propria fat of the intestine is in comparatively small drops, rather evenly distributed over the structure, and bears all the histological evidences characteristic of the fat in the epithelial cells which is so obviously transient in its character. The fat in the tunica propria of the intestine is also retained with greater persistence, or at least for a longer time following periods of fat absorption, than is the fat of the epithelial cells. This characteristic has already been mentioned in discussing the cœca.

Further studies ought to be made before advancing the point, yet one must mention here that no obvious lymph channels through which the fat is being removed have been observed. That is, no structures comparable to the mammalian lymphatic radicles of the mammalian intestine have been observed during these studies. This is not to be interpreted as an assumption that there are none, because the observations are insufficient in number to establish a point of this character. The fact must also be mentioned that no evidence of accumulation of stainable fat in the cavities of the blood vessels has been secured. In fact, fat droplets do not appear in any of the coagulated plasma nor in any of the free blood cells in so far as yet observed either in the intestinal blood vessels or those of other parts of the body.

Minute liposomes have been found in the endothelial linings of blood vessels and in the blood vessel walls. Such findings are shown in figures 3 and 6, plate XIII. The quantity of fat disposed in such places is small, but it was found to be present in fishes in which fat absorption was at its maximum, a fact that suggests but does not prove a relation to fat absorption.

ABSORPTION OF FAT BY THE SALMON STOMACH.

The fact of the absorption of fat by the epithelial lining of the stomach was first observed on the young salmon which had been experimentally fed with fat. Observations were not made on the adult feeding salmon in a way to determine whether or not gastric fat absorption occurred. The absorption of fat in the young was observed in both series, i. e., the specimens from Brookdale, Cal., and from the McCloud River at Baird, Cal. The young salmon in the McCloud River are feeding, but evidently on a source of food which is not particularly rich in fats. At any rate the specimens seined directly from the river and examined without further feeding showed only small amounts of fat in the epithelium of the stomach. In the series of four young fish no fat could be identified in one, a trace of fat only in one, and two contained obvious and easily identified fat droplets. These specimens were taken as typical of the average of those secured from the McCloud River, and were therefore considered as normals. The specimens that received fat as food by the method previously presented were examined in comparison with the normal series just given.

ABSORPTION AFTER FAT FEEDING.

The amount of fat taken up by the mucous lining membrane of the stomach is not anywhere near so great in amount as that shown by the mucosa of other portions of the alimentary tract in one and the same animal. However, this fat is in amount quite sufficient to form a very striking picture.

The microscopic evidence of fat absorption is largely limited to the superficial epithelium. At any rate, this tissue is most distinctly loaded with fat droplets, and the loading apparently occurs earlier than in deeper portions of the gastric mucosa. An examination of the epithelium of the stomach showed fat droplets present in practically every portion of that organ. The absorption takes place not only in the cardiac division, but also in the pyloric stomach.

The earliest indication of fat absorption is found in the appearance of fat droplets in the more superficial epithelium and in the distal ends of the cells. As time is allowed for the digestive and absorptive processes these cells become more fully loaded—in fact gorged—with fat, first in the outer limbs, then later the droplets appear nearer the bases of the cells. The glandular tissue of the gastric mucosa also shows the presence of fat droplets in the later stages of fat absorption. Apparently not only the superficial epithelium and the crypts even down to the neck cells, but also the glandular cells themselves are capable of taking up fat in quantities sufficient to produce the numerous droplets which the microscopic examination reveals. Since the structure of the gastric mucosa is characteristic and strikingly different in the two divisions of the salmon stomach, these regions will be discussed separately.

ABSORPTION IN THE CARDIAC STOMACH.

In the series of fish fed at Baird one had little or no fat in the stomach coat, while three showed the presence of fat in decided quantities. In those fish in which fat was present in the stomach it was in relatively large amounts as compared with the normals. That is to say, the amount of fat in the epithelium of the stomach in the fat-fed fish was larger in amount than in the fish coming directly from the river.

The amount of fat in process of absorption by the epithelium was greatest in fat-fed fishes nos. 88 and 91 of the McCloud River series. The fat was present in superficial epithelial cells of both the cardiac and the pyloric divisions of the stomach, but the amount in the cardiac division was very obviously less than in the pyloric division.

The fat in the cardiac stomach is distributed chiefly in the cylindrical cells of the superficial epithelium. It is in greatest quantity in those cells bordering freely on the cavity of the stomach. In a typical section through the cardiac region all that portion of the epithelium outside the nuclear zone and within the extreme outer clear zone will be studded with minute fat droplets. The fat droplets here vary much in size, but seldom reach more than 3 μ in diameter. The most of the fat is in such small divisions as to require an oil immersion lens to distinguish the individual droplets. (See fig. 4, pl. XIII.) In the outer clear zone or border I found fat in only one fish, and in this instance the droplets were extremely minute, i. e., liposomes.[a]

[a] The size of the fat droplets in the stomach shows every gradation from the larger size of 2 to 3 μ diameter down to a size that is discernable only with the highest magnification. In the salmon stomach, indeed in the salmon tissues in general, I am quite unable to distinguish any constant differences in appearance among these fats. There is no line to be drawn either as regards color, size, or contour. It is true that the color shade and the size vary greatly, but not in any way that does not admit of explanation without assuming any characteristic difference in the composition of the fat bodies stained. Under these conditions I use the term liposomes without reference to the kind of fat, only to designate the extremely small size of the droplets.

The basal parts of the cells have fat droplets, but rather smaller in size and not so numerous as in the outer limbs of the cells.

In certain regions the fat is present in the cylindrical cells of the lining walls down in the deeper folds of the crypts, but in other regions it is entirely absent. I have seen the fat in these cells down as deep into the crypts as the region into which the deepest gastric gland tubes open. In every case there is a very noticeable difference in the amount of fat present in the deep-lying epithelium and the more superficial—always in favor of the greater quantity in the superficial.

The tubes of the gastric glands open into the sides and bottoms of the crypts. There is a quick transition from the superficial epithelium to the gland cell type at the point where the mouth of the gland opens. It is not often that a section passes longitudinally through the mouth of a gland. This is largely due to the fact that the glands are somewhat convoluted in shape, rarely straight and tubular as in the gastric glands of most mammals. A number of gland tubes usually open into each crypt. Some of these are very short, and are only a few cells in length, while others extend quite down to the basement membrane. Occasionally a single tube may be as straight and direct as in the mammalia, but the majority are irregular. The transition in the epithelium from the superficial to the glandular type is sudden and sharp. In medium magnification the superficial epithelium looks darker because of the intense stain (i. e., hæmatoxylin), while the gland cells are more clear and granular.

The gastric glands proper, the differentiated cells of the secreting tubules, seem never to carry fat in other than the finest division. The gland tubes often show a distinct reddish shade of color when stained with scarlet red. In the gastric glands of at least one fish definite droplets were present quite large enough to be conclusively identified as fat of the usual kind and appearance. These droplets appeared to lie near the bases of the cells, and, taken with the numerous finest liposomes present, formed a delicate net-like mosaic. The liposomes were present in greatest numbers in this specimen, no. 88. It seems to me that in this instance the liposomes bear a definite relation to the increased amount of fat present in the cylindrical cells and are to be regarded as absorption fat.

In preparations of the gastric mucosa of young salmon no. 46, fixed in Flemming's solution, the osmic acid has stained the fat droplets a brownish black, which brings them in sharp contrast with the surrounding tissues. Figure 1 of a previous brief publication concerning these facts [a] shows the superficial epithelial cells of the gastric stomach containing the absorption fat. This black stain in the ends of the cells forms a dense black mass, but it is granular in character. At any rate, where the black masses are broken up granules are seen when examined under the oil immersion. Cross sections of the necks of the crypts present rings of black granular masses around the lumen. These masses are the blackened ends of the cells. Where the section cuts the crypt through the opening of the gastric gland it is noted that the black masses become progressively smaller in the deeper portion of the crypt and are absent from the surface of the secreting gland cells. The cell bodies of the superficial epithelium are stained the dark brown of the osmic fixative. Sections across the cell just beneath the blackened ends present numerous clear areas. These areas are spherical and very small, though

[a] Greene, op. cit.

they vary in size. The cells have all the appearance of cells in sections cut through the pyloric cœca of this fish where large quantities of fat are known to have been present, but is of course now dissolved out by the oils used in imbedding the material in paraffin. In the cells of the superficial epithelium of the stomach the clear areas are smaller and do not form so large a proportion of the body of the cell as in the epithelium of the pyloric cœca.

Also, through the superficial epithelium one finds black round globules of relatively small size in the middle of the body of the cell. These black dots correspond to the areas above the nucleus which scarlet red shows to contain fat. (Fig. 4.) In the base of the cells, especially in the cells of the outer folds of the epithelium, the same black granules are present. Undoubtedly all these black granules are due to fat stained with osmic acid. The neck cells of the crypts do not contain the black granules in the main body. The black staining in salmon no. 45 is limited to the ends of the cells.

ABSORPTION IN THE PYLORIC STOMACH.

The difference in structure of the pyloric stomach mucosa has already been described in a previous paper presenting the normal structure of the alimentary tract.[a] This division of the stomach is a much more active region for fat absorption than is the cardiac division. The main portion of the pyloric division shows more numerous fat droplets in the cylindrical cells than is shown by the cardiac epithelium in an experimentally fed animal.

In the region near the pyloric valve the fat fills the more superficial epithelial cells to a maximum. The amount more nearly approaches that in the intestinal mucosa, although the fat droplets never reach the relatively large size of those of the cells of the latter region. A reference to figures 1, plate XII, and 4, plate XIII, will reveal the comparative amounts of fat in different gastric epithelial regions. The crypts of this portion of the pyloric stomach are more open and the fat is more often found in the lining cells of their walls, even down to the bottoms of the crypts. Yet, in the most heavily fat-loaded preparations there are always some crypts that show no fat while others may be quite red with the stained droplets.

In the pyloric stomach, where the epithelial cells are morphologically intermediate in character between the gastric type and that of the intestine, one can not but make the inference that the absorptive power is also intermediate in degree. Yet the epithelial cells of the free surface of the mucous fold are distinctly gastric in character, as has already been described. The cells of the free surfaces are most loaded with fat, as figure 1, plate XII, shows. Another point of comparison is found in the fact that the fat droplets in the epithelium of the extreme caudal end of the pyloric stomach are very much smaller in size than the droplets of the cells of the intestinal epithelium just on the other or intestinal side of the pyloric valve. Sections of the pyloric stomach have been prepared in which the first whorl of cœca lying close around the wall of this part of the stomach were also cut. The epithelial coats of the cœca were in every instance filled with very large fat droplets. The fat droplets were four or five times larger in diameter than the droplets in the neighboring pyloric gastric epithelium.

[a] Greene, op. cit.

ABSORPTION FAT IN THE TUNICA PROPRIA OF THE STOMACH.

The tunica propria of the stomach is very complex in its convolutions because of the fact that its net supports the irregularly shaped gastric glands. Varying quantities of fat droplets are found in the tunica propria of the young salmon during the time of absorption of fat. Often it happens that the connective tissue immediately beneath the superficial epithelium is perfectly free of fat droplets, in fact, is always relatively free of fat droplets. But during the active stage of absorption in the fat-fed specimens occasional minute fat droplets are to be found, as shown in figure 1, plate XII. In the later stages the fat seems to accumulate in the tunica propria and is removed only after long periods of time. In certain of the younger specimens observed the fat was still present at a time at which the epithelial cells were approximately free of fat droplets. In these late absorption stages the tunica propria fat is chiefly limited to that portion which lies just within the stratum compactum. The droplets are small in size and greater in number between the bases of the deep gastric glands and the inner border of the stratum compactum.

It would seem that the connective tissue of the tunica propria, like that in the intestine and pyloric cœca, holds on to its fat with great persistence. Stating the fact in other words, the lipolytic process whereby the fat is removed from this region to other parts of the body must proceed very, very slowly. It has seemed to the writer that this connective tissue region serves as a temporary storage of absorption fat, also that the process of dissociation and removal from the region is markedly influenced by the presence of the stratum compactum. In the paper on the normal structure of the alimentary tract emphasis was placed on the observation that the stratum compactum is a continuous membrane. Only at points where it is punctured by blood vessels entering into the deeper structures within the stratum is it punctured by other tissues. This mechanical structural feature would throw upon the organs concerned the physiological necessity of disposing of the fat by two possible channels. The first of these is the vascular channel. In order that the fat may be taken up by the capillaries within the tunica it must first be dissociated and diffused into the vascular channels. The second possibility is that the fat may pass through the substance of the stratum compactum. Here dissociation must also take place and be followed by diffusion through the relatively thick and dense substance of the stratum. Since blood vessels of the stomach do not form capillary nets in the statum granulosum immediately external to the stratum compactum it follows that the fat diffusion must be carried through this coat, i. e., the stratum granulosum, into the submucosa and muscular coats before it could be taken up by the circulatory system and washed away into the general regions of the body. In both instances the fat distributing process is comparatively slow, hence one may expect the removal of the absorbed fat from the tunica propria to be sharply retarded. These points of view coincide with the facts of observation as measured against the time which has elapsed from the moment of feeding and absorption to the time of the preparation of the tissue for examination.

THEORETICAL CONSIDERATIONS.

The observations detailed in the preceding pages made on adult normal feeding salmon and on younger specimens under artificial and experimental feeding of fats show beyond doubt that fat is absorbed by all portions of the alimentary tract. The food of the salmon, which is representative of the carnivorous fishes, is made up of living organisms. These are wholly marine forms during adult life and are represented by the crustacean, molluscan, and piscatorial forms. All these classes of animals possess a high percentage of fat in their tissues, particularly the fishes, which form so large a portion of the salmon foods. Fats, therefore, form a large percentage of the normal food substance for the king salmon. The importance of this food material needs no further emphasis. The question at issue in this paper, therefore, is that of the ability of the salmon to digest and absorb the fatty elements so rich in quantity in its foods.

It is of vital significance that the fats are digested and absorbed in all the great divisions of the alimentary canal. It is true that fat digestion as such has not been followed in this series of experiments, but much collateral evidence has been obtained, and certain experiments not reported have shown something of the digestive process. Of all the observations the most important would seem to be the establishment of the fat-absorbing function of the pyloric cœca on the one hand and, on the other, the fact that fat is absorbed in the stomach.

As regards the pyloric cœca, the function of these organs has previously been deduced rather than proven by scientific experiment. Cuvier, at the beginning of the nineteenth century, considered the cœca as pancreas. At a still earlier date the general theoretical view was advanced that the cœca had to do with absorption. In more recent times statements have been advanced that the cœca are concerned with digestion and absorption. Of course, in any division of the alimentary tract it is a safe assumption that the function has to do either with digestion or absorption of some one or more of the food principles.

So far as I can find, no one has, previous to my experiments, attempted to demonstrate the relation of the pyloric cœca to fat digestion and fat absorption. The preceding observations establish beyond further doubt that the pyloric cœca are primarily fat absorbers. Incidental observations indicate that fat digestion may and does take place in these organs as shown further in my first publication of facts from this investigation.[a]

The second important observation, that of the fat-absorbing power of the stomach, is also of great physiological significance. As was indicated in the introductory discussion of the literature, the fact that fats are digested and absorbed in the stomach has been established previously by work on mammals. Strange to say, this work has been largely overlooked or for one reason or another questioned, so that the full acceptance of fat digestion and absorption by the stomach has not even yet been granted. Van Herwerden first showed fat absorption by the stomach in fishes. Following the publication of my preliminary report,[b] Weiss[c] published a brief report on experiments showing the absorption of fats by the stomach of the snake. Emphasis was laid on the fact that the fat absorption takes place more readily in the young than in the adults.

[a] Greene, op. cit. [b] Idem, op. cit. [c] Weiss, op. cit.

In fact, Weiss states that in the young cat the stomach has the power to absorb fat, but this power is lost after a few months. Experiments carried out in this laboratory[a] indicate that the ordinary laboratory mammals—the rat, the cat, and the dog—possess the power to absorb fats not only in the young but in the adult.

It would seem, therefore, that the process of fat absorption in the stomach does take place with somewhat greater ease and facility in the young than in adults, but we are convinced that it is a function of the stomach which is retained throughout life and not lost at an early stage of development, as claimed by Weiss.

The process by which fats are taken up by the mucosa of the alimentary canal is quite naturally brought in question. The histological method used here does not follow the digestive processes. But there are certain facts under constant observation which indicate the nature of the absorptive process. The introductory quotation from Wells sets forth in terse and concise terms our current views of the mechanism by which fats are absorbed. Not only that, these views apply to the mechanism of fat transference in the body in general. Our general conception is that lipases are produced in the body and that through a process of dissociation the fats are split into easily diffusible forms. This dissociation takes place in digestion. In the resulting diffusible form the fats can readily enter the superficial border of the epithelial coat. The laws of lipolysis, as formulated by Kastle and Loewenhart,[b] readily account for the resynthesis of fats when once the fat cleavage products are present and in sufficient concentration within the walls of the cells. That this is the process in the salmon is indicated by two proven facts—first, the fact that fat droplets are never found exactly in the striated borders of the superficial epithelial cells of any portion of the alimentary tract of the salmon; the second fact is that these cells in the height of absorption are loaded with fat droplets of such size and numbers as to gorge the bodies of the cells. In fact, numerous histological pictures indicate that the cell boundaries are under internal tension or pressure. Figure 11, plate XV, as also a number of the other figures presented here, gives one a conception of the physical condition of the cell when loaded with fat. This condition can be explained by two links in the chain of evidence assumed by our present theories of fat mobilization. The first of these is the fact that during rapid digestion of fats, say in the cavity of a pyloric cœcum, the fatty acids and the glycerin will diffuse through the free wall of the columnar epithelial cells at a very rapid rate. Synthesis within the cell will convert these fat cleavage products into the relatively inert fat molecules which accumulate in ever increasing quantities. This removal of the cleavage products maintains an osmotic condition favorable for further and continued diffusion into the cell, thus producing a distinct pressure in an already mechanically overdistended tissue.

Emphasis can be laid on this process as an explanation of the enormous loading of the fats, as shown especially in figures 4, 5, and 8; also in lesser degree in a majority of the figures presented. In many instances the fat droplets within the cells are so large as to occupy the full diameter of the cell, and so numerous as to load the entire outer end of the cell from surface border to nucleus. When an epithelial cell is thus loaded with fat the fat is of mechanical necessity arranged in the regular beaded rows that give the diagrammatic appearance which is often presented by the figures.

[a] Greene and Skaer, op. cit.

[b] Kastle and Loewenhart: Concerning lipase, the fat-splitting enzyme, and the reversibility of its action. American Chemical Journal, vol. XXIV, 1900, p. 491.

SUMMARY.

In summarizing the results presented in the preceding pages the salient facts may be mentioned as follows:

1. Fats are absorbed through the columnar epithelium of all portions of the alimentary tract of the king salmon.

2. The primary function of the numerous pyloric cœca is that of fat absorption. Probably the larger portion of the fats of the food of the salmon are absorbed by way of these organs.

3. The intestine is a region of active fat absorption. The power of the intestinal epithelium to take up fat is similar to that of the pyloric cœca.

4. The salmon stomach is also a fat-absorbing organ. Fat is absorbed by both the cardiac and the pyloric types of columnar epithelium.

5. The microscopic indications are that the fats pass through the outer portions of the columnar epithelial cells in a dissociated form and that resynthesis takes place within the cell, thus accounting for the numerous large fat droplets present in the cells during active fat absorption.

DESCRIPTION OF FIGURES.

The following list of figures was drawn for me by Mr. George T. Kline, biological artist of the University of Missouri. It is difficult, especially in low magnification figures, to represent the exact amount of fat in the plain of a cross section. But the relative amount is represented and by the aid of a camera lucida. In the figures of high magnification the exact size and location of every droplet has been followed with the greatest care. Figures 5 and 6 represent preparations in which the fat was stained by osmic acid. All other figures are from sections prepared from frozen section stained with scarlet red.

Plate XII.

Fig. 1.—Showing fat absorption by the epithelium of the pyloric portion of the salmon stomach. This fish was a young specimen from the McCloud River, Baird, Cal. It was fed olive oil 20 hours before preparation. The superficial epithelium is crowded with fat. Other portions of the same section show even a greater loading, extending down to the cells of the bottoms of the crypts. Traces of fat liposomes are noticed in the lymph vessels in the folds. Fat-fed salmon no. 88. Camera lucida outlines. Magnification, Leitz ocular 1, objective 7.

Fig. 2.—Transverse section showing fat absorption in the posterior loop of the intestine in a fat-fed salmon from Brookdale, Cal. This young specimen had been fed 18 hours previous to killing. Fat is crowded into the cylindrical epithelial cells, and has passed in considerable quantity into the spaces of the tunica propria. The folding of the intestinal mucous epithelium is relatively simple in young salmon of this age. Brookdale salmon no. 45. Camera lucida outlines. Magnification, Leitz ocular 3, objective 3 with the lower lens removed.

Plate XIII.

Fig. 3.—Showing fat absorption in a transverse section through the intestine of fat-fed salmon no. 45. This figure represents with larger magnification one of the folds shown in figure 2. The general outlines of the figure are drawn with camera lucida. The fat of the epithelial cells was laid in primarily from this section, but in part from a comparative study of other sections. The fat-bearing portion of the epithelium between the two goblet cells to the left was torn in the section, and this portion is all recon-

structed from the study of similar folds. The effort was made to present an accurate picture of the relative amount and distribution of the fat in the cells. The rather regular beaded arrangement of fat is shown in sections of the same fish, fixed in Flemming, in which the fat droplets are stained black in figures 5 and 6. It is also shown in material fixed in corrosive sublimate, where the fat has been dissolved out, leaving fat vacuoles. The inner ends of the epithelial cells contain only slight quantities of fat. No fat is ever found in the outer borders of the mucous cells.

The tunica propria is filled with a medium load of fat, the fat being caught in the spaces of the tissue and in the connective tissue cells. This fat is all laid in with the camera lucida. No fat is present in the stratum granulosum, either in the cells or in the supporting connective tissue. Traces of fat are present in the connective tissue surrounding the blood vessels, and also in the vessel endothelial cells. A few fat droplets are also present in the cells of the muscular coats, especially in the muscularis longitudinalis. Magnification, Leitz ocular 1, objective 7.

FIG. 4.—A high magnification of a section through the superficial fold of cylindrical epithelium of the cardiac portion of the stomach showing fat absorption in an early stage of the process. The fat is largely limited to the outer or most superficial zone of the cylindrical cells, but small amounts are present in the basal zone. This salmon had been fed olive oil by the method of rectal injection, the oil passing through the intestine and forward into the stomach. Brookdale young salmon, no. 46. Camera lucida outlines. Magnification, Leitz ocular 1, objective 7.

FIG. 5.—A section of a group of epithelial cells of the pyloric intestine fixed in Flemming's solution to show fat absorption. Young salmon no. 45 from Brookdale, Cal., which had been fed fat artificially. The amount of fat is not so great as present in the section of the caudal length of the intestine shown in figure 4. The same beaded arrangement of fat droplets is shown, but more smaller droplets are present in the ends of the cells—a fact showing either an earlier stage or a slower rate of absorption. Camera lucida drawing. Magnification, Leitz ocular 1, objective 7.

FIG. 6.—Showing fat in the transverse section of a fold of the pyloric cœcum of young salmon no. 45, the same fish as in figure 2. The salmon was previously fed olive oil by rectal injection and the tissue fixed in Flemming's solution. This section presents a typical picture of fat absorption in the pyloric cœca when the process is at its height. It is splendidly fixed, sharply stained, and is reproduced under camera lucida with the greatest possible care. Note the fine division of the fat droplets shown in the outer margin of the cylindrical epithelial cells, also the relatively small amount of fat of the zone within the nucleus. The tunica propria contains an excessive quantity of fat, the boundary limit of which is sharply marked by the broad band of the stratum compactum. In this specimen an occasional minute liposome is present in the connective tissue of the stratum granulosum as well as in the muscular coats, a fact that is very seldom shown. Camera lucida outlines. Magnification, Leitz ocular 1, objective 7.

PLATE XIV.

FIG. 7.—Showing fat absorption in the pyloric cœcum 18 hours after fat feeding. Young salmon no. 45. In this specimen fat is crowded in the superficial epithelium, also in the tunica propria. The details of histological structure are largely omitted in order the better to emphasize the great amount of fat present. The droplets in the tunica propria are especially numerous in this particular fish. The stratum compactum forms a sharp outer limit to the fat-bearing zone of the tunica propria. Fat-fed young salmon no. 45. Magnification, Leitz ocular 2, objective 3. ×45.

FIG. 8.—Showing fat absorption in the pyloric cœcum 42 hours after fat feeding. Young salmon no. 46, the same fish from which figure 1 was taken. The fat is largely removed from the superficial epithelium, except in the tips of the folds, but is supercrowded in the tunica propria. Camera lucida outlines. Magnification, Leitz ocular 2, objective 3.

FIG. 9.—Showing fat absorption in the pyloric cœcum of fat-fed young salmon no. 88, from the McCloud River, Baird, Cal. The structural detail is shown in only one-half the figure. Fat is rather evenly distributed throughout all portions of the cylindrical epithelium and is present in medium amount in the tunica propria. There are a few small droplets in the outer muscular coat. Time of absorption, 20 hours. Magnification, Leitz ocular 1, objective 4.

FIG. 10.—Showing fat absorption in the pyloric cœcum of a young McCloud River salmon no. 91, after 70 hours of absorption. This specimen shows the epithelial cells unusually crowded with fat. The fat has not yet reached the tunica propria, although the time for possible absorption is longer than in no. 88 of the same experimental series. Camera lucida outlines. Magnification, Leitz ocular 2, objective 4.

PLATE XV.

FIG. 11.—Showing fat absorption in the superficial epithelium of the free margin of a mucous fold of the pyloric cœcum of young salmon no. 46. This figure represents the maximal loading of fat. Many of the cells are so gorged with fat that their surface outlines are projecting as though under a high internal osmotic pressure. Camera lucida outlines. Magnification, Leitz ocular 2, objective 1/12.

FIG. 12.—Showing fat absorption in a portion of two adjacent folds of pyloric cylindrical epithelium from a normally feeding adult salmon from the fishing banks of Monterey Bay. The clear marginal zone is well shown in this figure, also the characteristic finely divided liposomic fat immediately beneath it. This zone shades off into one of larger droplets lying just external to the nuclear layer. Note the comparatively small amount of fat in the inner zone of the epithelium and in the thin layer of the tunica propria. The fat droplets are most carefully laid in from camera lucida outlines. Salmon number 22. Magnification, Leitz ocular 1, objective 1/12.

FIG. 13.—Showing fat absorption in a normally feeding adult salmon, no. 28, Monterey Bay, Cal. This figure represents a later stage of absorption than the proceeding. It shows a loading of the inner ends of the cells with finely divided liposomes and a similar charge of fat in the adjacent tunica propria. The fat has passed the outer zone. This stage of fat absorption was rather rarely observed. Camera lucida outlines. Magnification, Leitz ocular 1, objective 1/12.

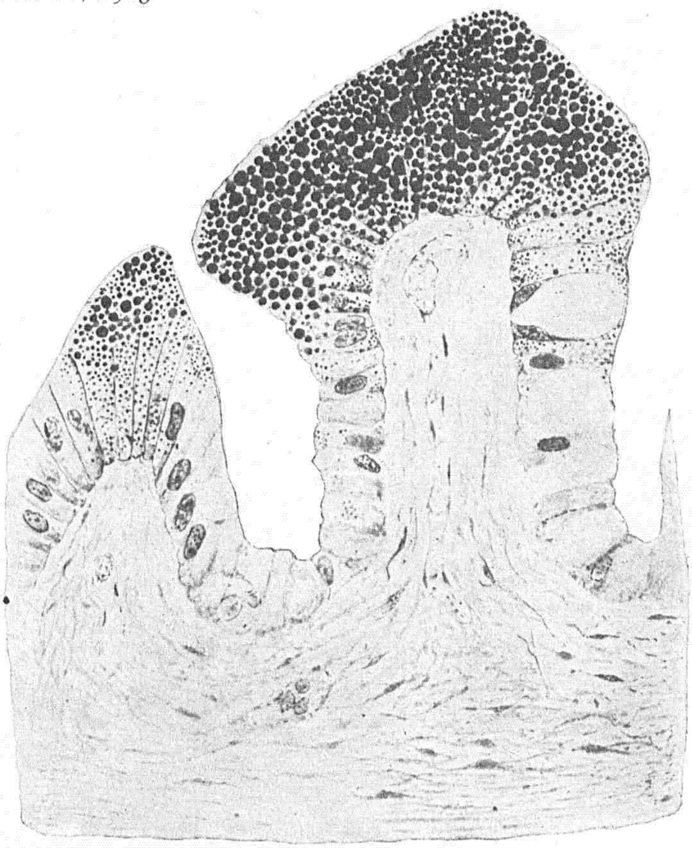


FIG. 1.

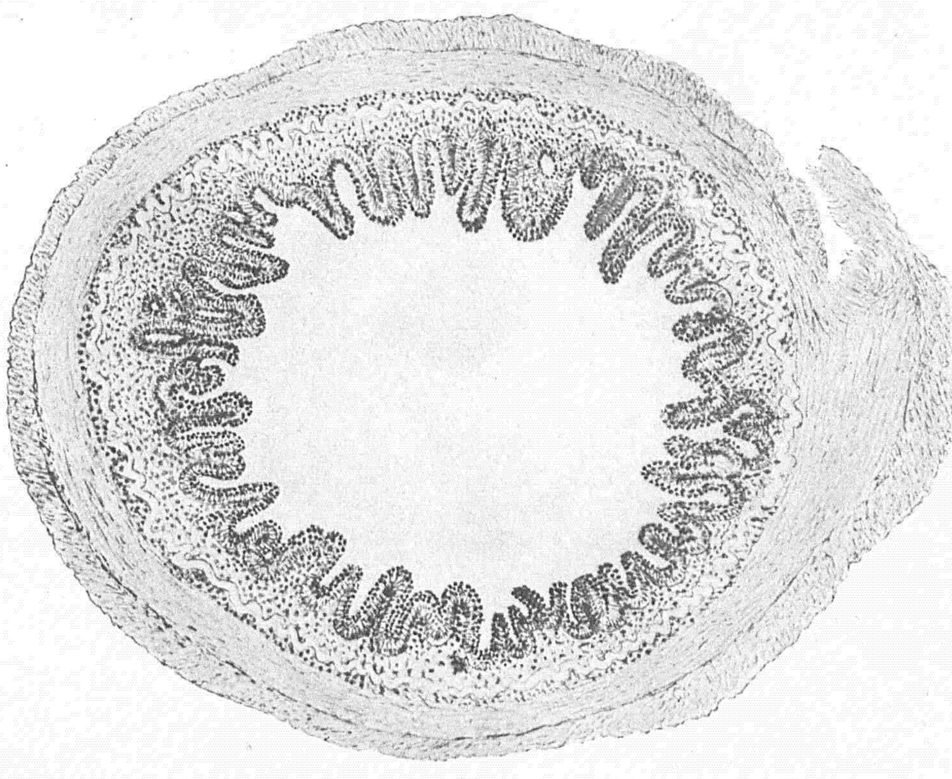
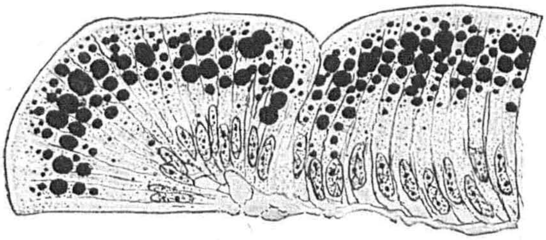


FIG. 2.



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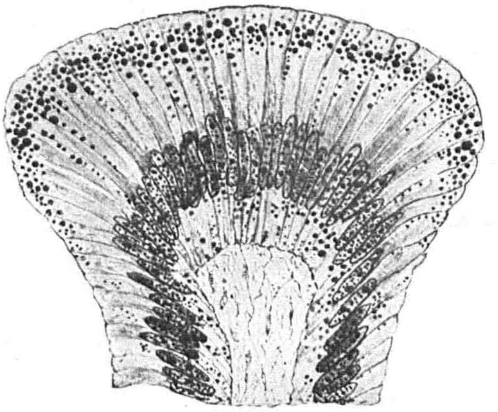

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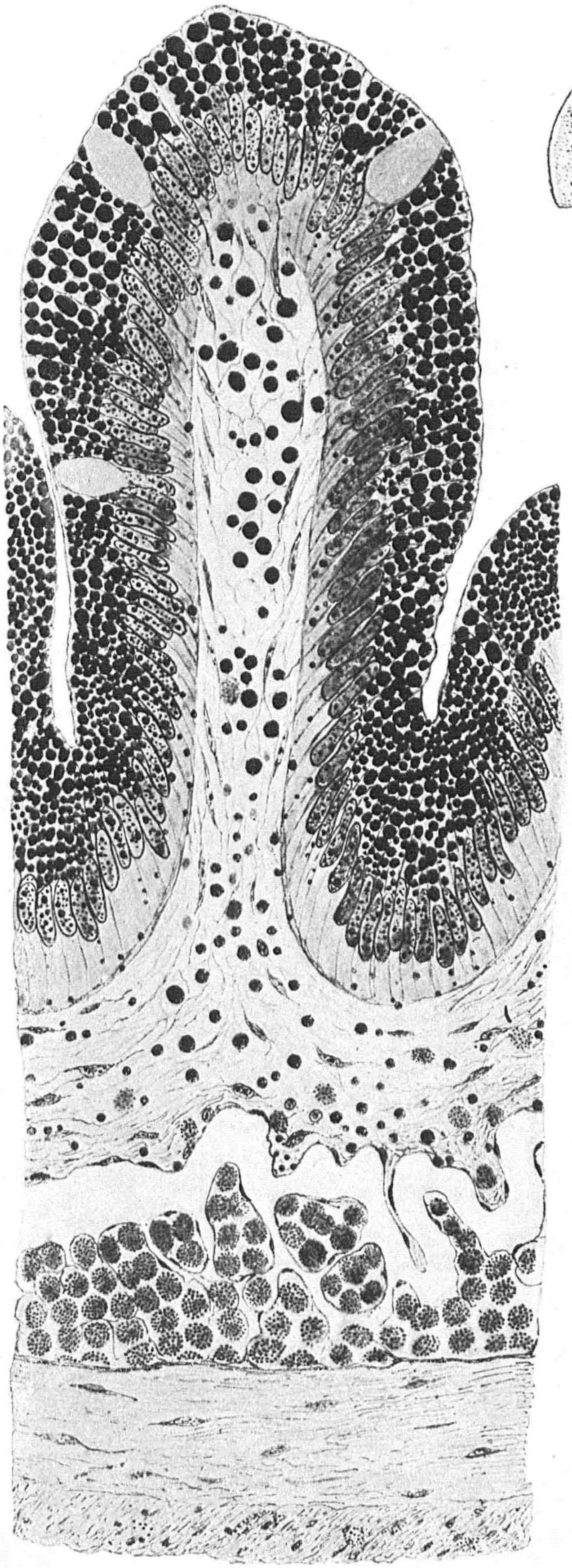
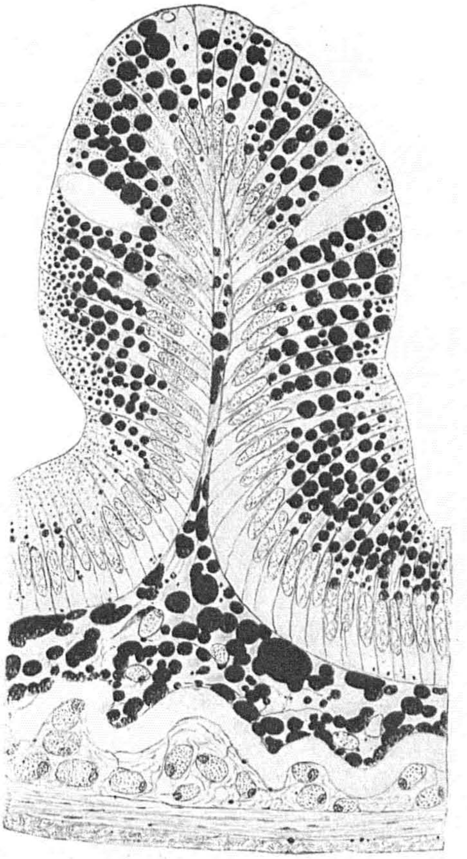


FIG. 3.



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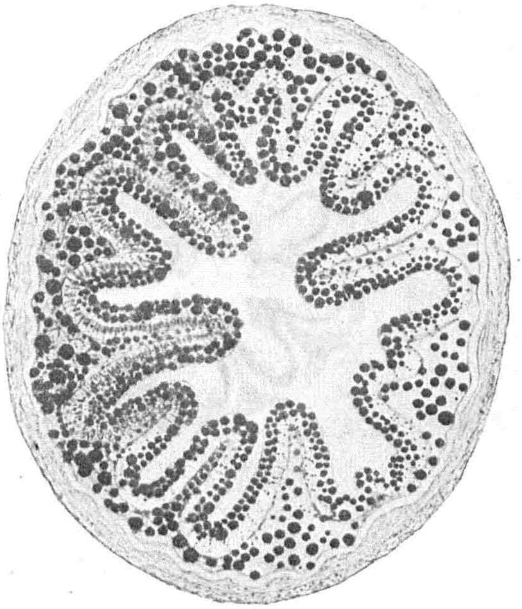

FIG. 7.

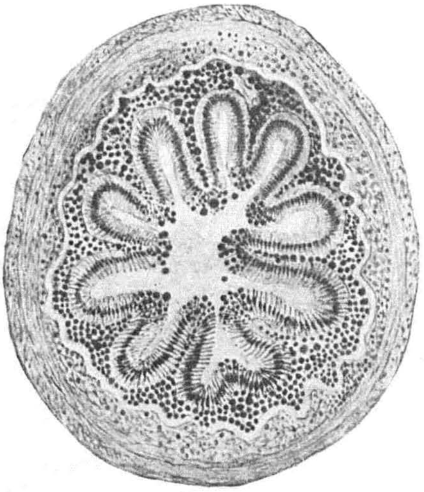

FIG. 8.

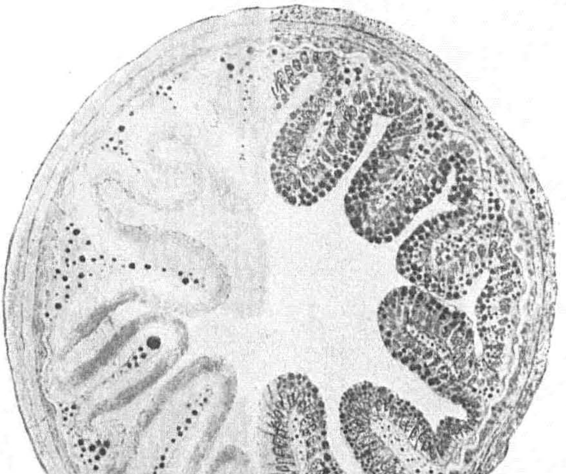

FIG. 9.

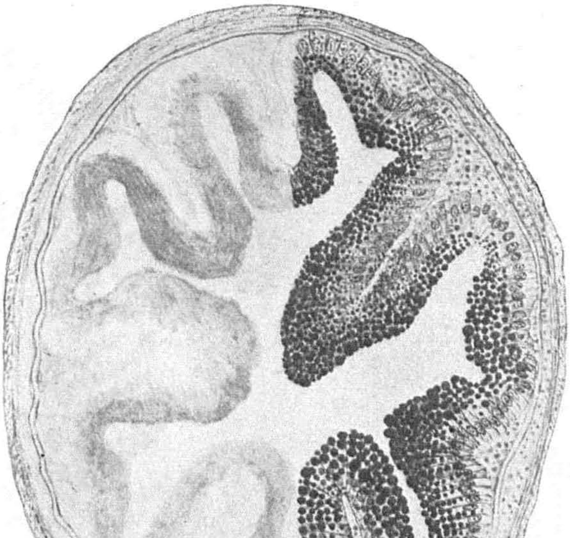

FIG. 10.

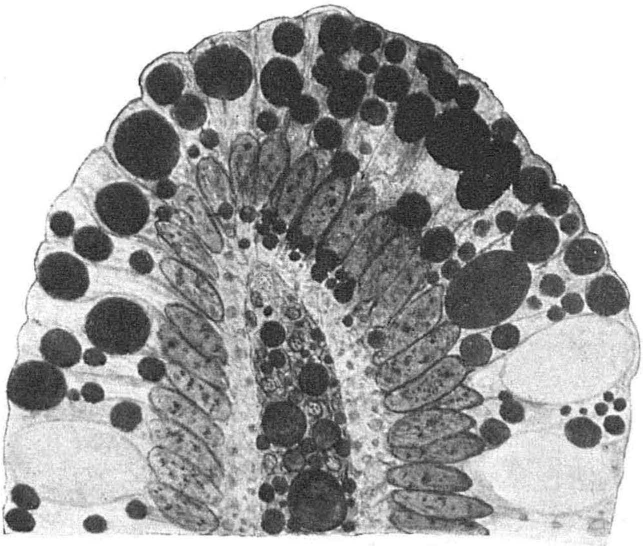

FIG. 11.

FIG. 12.

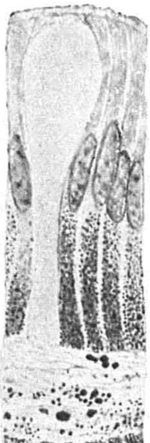

FIG. 13.