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# THE ABSORPTION OF FAT IN THE INTESTINE

By G. E. Wilson, M.B.

George Brown Memorial Scholar in the University of Toronto.

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# ON THE ABSORPTION OF FAT IN THE INTESTINE.

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## I.—INTRODUCTION.

ONE of the most keenly discussed questions in physiology for the last forty years has been the manner in which the absorption of fat takes place in the intestine and the question is still, after so long a period of debate, as far from settlement as it was a generation ago. The basis of the discussion has been shifted somewhat recently, but the point of contention still is whether the fat passes into the epithelial cells in part as very fine particles in the form of a so-called emulsion and in part also in a dissolved condition, or only in a dissolved form as the products of hydrolysis, glycerine, fatty acids and soaps.* The former view had the support of Brücke, Kölliker, Donders, Eimer, von Basch, von Thanhofer, Grünhagen, Heidenhain and others while it was opposed by Pflüger, Cash, Ludwig, and Rindfleisch, and the number of its critics have increased in recent years with the result that the balance of opinion is inclined in favour of the other view.

What has told in favour of the alternative view is the advance in our knowledge of the action of digestive juices on the fats, which goes to show that fats are broken up into simpler compounds by the steapsin

* A complete account of the literature of this subject may be found in Oppel, Lehrbuch der Vergleichenden mikroskopischen Anatomie der Wirbelthiere, Zweiter Theil, Schlund und Darm, 1897; Kischensky, zur Frage über die Fettresorption im Darmrohr, Ziegler's Beiträge, Vol. 32, p. 197, 1902; Croner, Die neueren Ergebnisse der Frage von der Fettresorption, Biochemisches Centralbl, Vol. 3, p. 93, 1904.

of the pancreatic juice and to a certain extent also by the gastric juice, these simpler products, owing to their solubility in the chyle, diffusing as readily into the epithelium as do the peptones and the hydrolyzed carbohydrates. From this point of view the digestion and absorption of fat are not different fundamentally from the digestion and absorption of proteids or of carbohydrates, and therefore, the occurrence of a special process in the case of fat, such as does not obtain in the case of proteids and carbohydrates, was questioned and generally rejected.

In spite of the preponderance of observation and opinion against the view that fat is in part absorbed as fine particles in the form of an emulsion, there have not been wanting in recent years supporters of that view and amongst them may be counted Rosenthal,* Connstein,† Beneke,‡ Munk¶ and Rosenberg.** Included also is Kischensky,†† who has brought forward evidence which, in the opinion of the writer of this paper, meets the objection that no one has demonstrated the passage of fat particles through the striated border of the epithelial cells.

This observer employed in his technique a new micro-chemical reagent, scarlet red (Scharlach Rot), an azo colouring compound, introduced by Michaelis,‡‡ which is insoluble in water, acids, alkalies, slightly soluble in alcohol and readily soluble in chloroform and in fatty oils. Owing to its affinity for fats and fats alone, and in consequence to the intense red colour which it gives to the finest particles, this reagent is a very much more sensitive one than osmic acid which was previously regarded as the final test for fat in tissues.

Kischensky, by the use of this reagent, found very minute fat particles in the striated border of the villi in kittens, and that these particles, after passing through the border and reaching the underlying protoplasm, fuse to form larger, readily demonstrable droplets. He does not, however, regard this as the only manner in which fat enters the epithelial cells for he expressly states that the greater part of the fat is absorbed in the soluble form and that only a small portion goes through the striated border in the form of very fine particles. He further holds that in full grown cats the fat particles are transferred to the parenchyma by the cytoplasm of the

* Lehrbuch der allgemeine Physiologie, 1901.

† Ueber die Resorption und Assimilation du Fette, Die Med. Woche, No. 15, 1900.

‡ Die Fettresorption bei natürliche und künstliche Fettembolie und verwandten Zuständen. Beiträge zur Path. Anat. und zur allg. Path., Vol. 22, p. 343, 1897.

¶Zur Frage der Fettresorption, Centralbl. für Physiologie, Vol. 14, pp. 129 and 153.

** Zur Physiologie der Fettverdauung, Pflüger's Arch., Vol. 85, p. 152.

†† Zur Frage uber die Fettresorption im Darmrohr und den Transport des Fettes in andere Organe. Beiträge z. path Anat. und z. allg. Path., Vol. 32, p. 196, 1902.

‡‡Die indifferenten Farbstoffe als Fettfarbstoffe.—Deutsche Med. Wochenschrifft, 1901, p. 183, Also: Ueber Fettsfarbstoffe, Virchow's Arch., Vol. 164, p. 263, 1901.

cells, but in kittens the fat may pass not only through the cytoplasm, but also through the intercellular spaces to parenchyma.

The writer had, before Kischensky's paper came to his knowledge, begun the study of the absorption of fat and had, through Michaelis' observations, been led to employ scarlet red as a micro-chemical reagent for fat. With this and other means he carried out the work, and when it was completed, arrived at results which are, in many respects corroborative of those obtained by Kischensky, but were obtained from a larger number of vertebrate forms. These results are now published in the hope that they will serve in some degree to advance the solution of the question of the mode of absorption of fats.

## II.—METHODS OF STUDY.

During the first five months guinea pigs were almost exclusively used. Sometimes they were kept from all food for as many as four days before they were employed, while others were not so treated. They were fed on egg yolk diluted sufficiently with tap water to allow the mixture being drawn up into a pipette used for feeding, and fifteen to twenty cubic centimetres of this were given to each three times a day. At the outset of the investigation many of the animals were fed on diluted yolk to which one cubic centimetre of dialysed iron was added on each occasion. Others again were given only pure olive oil. Some were fed only once, and killed from three to six hours afterwards, others were fed one, two or three days, while a few were continued on this diet alone until death ensued. The rest of the animals employed were invariably killed with chloroform, the abdomen was at once opened, the duodenum exposed, and pieces about a centimetre and a half long, and one half a centimetre broad taken from different portions, were placed in Flemmings Fluid for about twelve hours. The composition of this fluid was:—

0. 1 per cent. chromic acid, 15 parts.

2. per cent. osmic acid, 4 parts.

One part glacial acetic acid was added to nineteen parts of the above when about to be used. The specimens were then washed in running water for half an hour, placed in fifty per cent. alcohol, after about twelve hours in seventy per cent. for twenty-four hours, then in ninety-five per cent. for another twenty-four hours, and finally into absolute alcohol for periods varying from twelve to seventy-two hours. When it was considered necessary the absolute alcohol was changed and fresh added.

Some of the specimens were then passed through chloroform into paraffin while others were infiltrated with celloidin before being imbedded in paraffin. Owing, however, to the presence of chromic acid in the fixing agent the proteids are so coagulated that not enough is left unchanged in the sections to cause the latter to adhere, so that in most cases a small amount of collodion and oil of cloves had to be employed for attaching the section to the slide. In a few instances the sections were made adherent with albumen and glycerine.

The preparations were treated after the iron-alum hæmatoxylin method, followed by eosin staining. Numerous other stains were used but this method gave by far the best results so that it was the one chiefly used. With this stain the nuclei stand out as blue black, the cytoplasm takes the eosin, while the fat is black or, if not very abundant and seen with the diaphragm of the microscope well open, a brownish tinge is evident.

With the view of investigating more closely the striated border I experimented with other fat stains, and finally obtained what I required in scarlet red (Scharlach Rot). This stain is insoluble in water, but soluble in seventy to eighty per cent. alcohol. It is also soluble in soap solution, but insoluble in glycerine or chloral hydrate, five per cent In using it one must be careful to get a solution of proper concentration, else the results are very unsatisfactory. It requires to be shaken from time to time with seventy, or even eighty per cent. alcohol for three or four days and then filtered. Before using it should be tested on a little olive oil smeared on a slide, when, if good, it will give a deep red reaction to the globules in about one minute.

In my first experiments I used guinea pigs fed for about three days upon olive oil. They were then killed with chloroform about three hours after the last feeding, the abdomen opened, a piece of the duodenum was put into ten per cent. formalin for about forty-eight hours, then frozen with carbon dioxide spray, and sections made about six-seven microns thick. These were put into the scarlet red solution for about forty-five seconds, then transferred to tap water for a few seconds and mounted in glycerine, the edges of the cover slip being later smeared with melted paraffin.

## III.—STRUCTURE OF A VILLUS.

Before dealing with the subject proper I wish to refer briefly to the structure of certain cellular elements of a normal villus of the duodenum of a guinea-pig.

The epithelial cells covering the villus are columnar, their length being approximately three to four times their width at the outer edge. They lie upon a basement membrane in which there are no nuclei. The majority of histologists describe this membrane as containing nuclei, but I have never been able to satisfy myself that they are present. It is true that in many cases nuclei appear to be present, but on very careful examination one will invariably find that such a nucleus belongs to an adjacent capillary. It is an easy matter to demonstrate blood capillaries lying adjacent to the basement membrane, and in specimens where the red corpuscles do not stand out prominently it is not difficult to fall into error.

When the epithelial cells are at rest, no demonstrable space exists between adjacent cells, their edges being closely approximated from upper to lower end. During their active stage, however, as I shall point out further on, their lower ends become separated. This separation I believe to be due to the contraction of the lower end of the cell so that the length of the cell is increased. The reasons for this view I will give later on when discussing the extrusion of the fat from the epithelial cells.

The striated border is particularly interesting inasmuch as no one, heretofore, except Kischensky, has been able to demonstrate fat within it. In the literature of the subject it is usually represented as having a distinct and clearly defined outline both above and below. It is a simple matter to demonstrate the sharply defined lower margin of this striated border; in fact one can readily see it in teased out preparations, but the distal edge is not equally well defined. Heidenhain* has indeed called attention to the changeable character of the outer edge and Kischensky and others have noted the same fact. I have examined a great many specimens of epithelium from various animals, and as a result, find that the striae are unequal at their distal ends. Further, I have on many occasions seen striae terminating as rodets distally. And it is perhaps what one might regard as probable, for in the higher invertebrates the epithelial cells of the intestine are ciliated, and in the larvae of frogs, also the epithelium is ciliated, while that of the adult frog is only striated. Again as I shall show later on these striae are able to pick up particulate

*Pflüger's Arch. Vol. 43, Supplement, p. 91.

substance, thus adding further weight to the contention that the striae have free ends.

Within the parenchyma of the villus is to be found a variable amount of smooth muscle fibre together with connective tissue strands. These latter branch in various directions between the basement membranes of the epithelial layer and the wall of the lacteal, leaving anastomosing spaces of irregular size and shape between. Within these spaces and also in the connective tissue are to be seen four main types of cells,—the ordinary polymorphonuclear leucocyte, the lymphocyte, the "mastzell," which, however, does not stain with the ordinary dyes, but can be easily demonstrated with methylene blue and, lastly, cells, which I have termed large lymphoid cells, varying in size from about ten to twenty microns in diameter, and from a spherical to a more or less elliptical outline. Their nuclei are, as a rule, eccentrically placed, and are usually vesicular. The outline of these cells is generally clearly defined, and the protoplasm takes the eosin stain, appearing as finely granulated. As far at least as the guinea pig is concerned, I have never seen these large lymphoid cells within or between the epithelium covering the villus nor in the lacteal, in this respect differing from the other leucocytes. They are, however, actually phagocytic as will be shown later on. They are precisely similar to the cells described by Macallum as containing iron when salts of that element are being absorbed from the intestinal cavity.*

The number of leucocytes in the villus is subject to great variation. The majority, however, during fat absorption, at least, are of the large lymphoid character and are especially numerous at the tip of the villus, a point of considerable importance when one remembers that most of the fat enters at that place.

It is noteworthy too that in the guinea pig very few leucocytes of any description are found either within or between the epithelium covering the villus, and this is true whether active fat absorption is going on or not, a fact of very great moment in relation to one of the older theories of fat absorption. In the same animal, too, one very seldom sees a leucocyte in the lacteal vessel.

The occurrence of lymphatics, with well defined walls within the parenchyma of the villus is still in doubt. I have on several occasions tried to inject such, using soluble Prussian Blue dissolved in gelatin, but failed to demonstrate them. The difficulties, however, are great owing to the valve at the base of the villus. I am convinced, nevertheless, that

*On the Absorption of Iron in the Animal Body, Journal of Physiology, Vol. 16, p. 268 1893.

they are not present. That there are lymph spaces, however, I feel certain, and there is quite conclusive evidence to show that the spaces between the epithelial cells of at least their basal thirds, are in direct communication with these intraparenchymatous lymph spaces, because one can frequently demonstrate streams of fat going direct from these spaces in the epithelium to the wall of the lacteal. (Figs 2, 5, and 8).

## IV.—FAT IN THE EPITHELIUM.

That the fat enters the villus directly through the epithelial cells can no longer be questioned. When a guinea pig is fed on fat containing food for a day or two and killed about four or five hours after the last feeding, and the preparations stained as above outlined, the picture is conclusive. Fat droplets of various sizes are seen throughout the cells. Although there is no uniformity regarding the size of these drops the largest are usually seen towards the distal (free) end, (Figs. 1 and 14). Above the level of the nuclei the fat is often intercellular, (Fig. 7). At the level of the nuclei and below them it may be intracellular (Figs. 4 and 9), or intercellular (Fig. 5), but below the nuclei most of the fat is between the cells. I do not wish to convey the idea that the nuclear zone is a hard and fast line of demarcation, but there is a difference in the distribution of the fat in the cell above and below it. I am aware that Heidenhain claims that the fat only goes into these intercellular spaces when in excess. Von Basch holds that it is present only in a sort of backwash. The former explains its presence between the cells by the contraction due to the fixing agent, whereby the fat is squeezed out of the parenchyma into the intercellular spaces. This one cannot accept, for preparations from frogs fed with olive oil for a day or two and killed about three hours after the last feeding are decisive. The intestines of these fixed with 10 per cent. formalin for twenty-four to forty-eight hours, sectioned on the freezing microtome and the sections stained in scarlet red and mounted in glycerine show these intercellular spaces to be filled with fat. If, however, before they were treated with the fat stain they were placed in ether for about half a minute, these spaces which before were red are now light, the fat having dissolved out. If one saw fat in these spaces only in shrunken sections Heidenhain's explanation would appear the more probable one, but it is such a constant feature in well preserved sections that one must endeavour to account for it in some other manner. I believe therefore, that the fat is to a large extent excreted by the cell activity into these intercellular spaces. Watney believes that the fat is taken up wholly between the cells. Against this may be placed

the fact that in properly prepared sections the most striking thing in the field is the great amount of fat inside the epithelial cells.

Bruyne claims that there is connective tissue between the epithelial cells, containing lymphatics so that leucocytes can penetrate between the cells and pick up fat from the intestinal contents. He is of the opinion that all the fat lying between the cells has been deposited there by these leucocytes. So constantly have I found the fat between the cells that I cannot accept this explanation. If leucocytes are instrumental in placing the fat there one would expect to see them in abundance between the epithelium, but in the guinea pig at least their occurrence there is a rarity.

Not uncommonly one sees more or less irregular circular spaces just beneath the striated border which are clear and free. They are found after treatment with 10 per cent. formalin, saturated bichloride of mercury, or absolute alcohol, and even with Flemming Fluid if the osmic acid is not of the usual strength. They are due to fat globules having dissolved out leaving vacuoles behind. In some cases one can see these spaces containing just a small amount of fat, in others the spaces are filled while the next cell has a vacuole, (Fig. 8). Adjacent cells do not necessarily take up an equal amount of fat. Very frequently one sees several adjacent cells loaded with fat while others near them are free from it.

The nuclei of the epithelial cells never contain fat. It is true that a section will at times apparently show fat in the nucleus, but it is due to the way in which the section is cut, the fat in the cytoplasm then appearing under or above the nucleus.

Concerning the presence of fat in the striated border special reference is made below.

## V.—FAT IN THE PARENCHYMA.

When a guinea pig is fed fat in excess the adenoid tissue of the villus is black with it. (Figs. 2 and 12). It lies in the spaces between the leucocytes and the fat streams communicate freely in all directions. In some instances, especially when the fat is not abundant, minute drops of fat are visible and these frequently present the appearance of a row of minute beads, the series being directed generally toward the lacteal. (Fig. 8). In others the droplets have coalesced into large masses of fat, which take the shape of the spaces through which the fat is passing, (Fig. 5). It is frequently possible to trace these fat streams proceeding directly from the basal end of the epithelial cells, (Fig. 8), but much more com-

monly do we find them connected with the interepithelial spaces, (Figs. 5, 6 and especially Fig. 8). Again one can demonstrate fat within the lower third of the epithelial cells and reaching almost to the basement membrane and yet not going directly towards the lacteal, but, instead, being excreted into the interepithelial space and pressed into the parenchyma as a fine stream, (Fig. 5). In the same drawing one can see the fat within the epithelium streaming towards the interepithelial space. For these reasons I feel justified in stating that most of the fat is excreted into these interepithelial spaces which apparently are in direct communication with the large, fat-containing space of the parenchyma. Within the fat streams, especially at the tip of the villi, numerous leucocytes, more or less laden with fat, are to be seen. (Fig. 1). At the sides of the lacteal, however, where the leucocytes are much less numerous one can frequently trace fat streams from the basement membrane to the lacteal without the intervention of leucocytes, a point which adds considerable weight to the contention that the leucocytes play a minor role in the transference of the fat through the adenoid tissue of the villus. Often too the only leucocyte intervening is a polymorphonuclear one which, so far as I can make out, never takes up fat, (Fig. 2).

I have already stated that I regard these fat-containing spaces as connected with the central lacteal on the one hand, and with the interepithelial spaces on the other. In any event one can, with the greatest ease, demonstrate fat streams going direct from these interepithelial spaces through the parenchyma to the wall of the lacteal, (Fig. 8).

## VI.—TRANSFER OF FAT THROUGH THE PARENCHYMA.

There have been various views put forward as to how the fat is carried through the adenoid tissue. Von Basch* believes that the connective tissue strands are channeled and that the fat proceeds through these openings. As Heidenhain† has pointed out, such coarse strands of connective tissue are not to be seen unless produced by shrinking and bad technique so that Von Basch's theory has no basis of fact to support it. The contention of Zawarykin‡ that the leucocytes send out pseudopodia and take the fat from the epithelial cells and later discharge it against the lacteal wall cannot be substantiated. I have carefully examined many hundreds of sections and have seen nothing which could be so interpreted. The question now arises: what part do the leucocytes take in the transfer of

*Sitzungsber. der Wiener Akad., Math. Nat. Cl, Vol. 62, p. 1, 1870.
†*Op. cit.*
‡Pflüger's Archiv, Vol. 31, p. 231, 1883.

the parenchymatous fat? The answer to this question involves a determination of the part played by the leucocytes in the absorption of fat from the intestinal contents.

If the leucocytes are wholly or to a large extent responsible for the transfer of fat one would expect to see a vast increase in their number when a guinea pig has been fed pure olive oil. Such an increase is not found. When a guinea pig, ordinarily fed, is killed, the sections show about the same number of leucocytes, at least their increase is not at all proportionate if they are so necessary for the transference. It is true that the majority of the leucocytes present are of the large lymphoid variety, but the same is true when iron is being absorbed. Then when the adenoid tissue is laden with fat one would expect to see every leucocyte in the central portions of the parenchyma, at least, more or less filled with fat, while those leucocytes near the basement membrane, and the lacteal wall should show many variations according as they were laden or uncharged with fat. On the contrary no such variation can be demonstrated, and the centrally placed cells contain no more fat than those near the chyle vessel or the basement membrane, (Fig. 12). Moreover, when so much fat is to be transferred one would expect to see these leucocytes discharging it near or against the lacteal wall, but I have never been able to demonstrate such a condition. Furthermore, if the leucocytes are so active in carrying fat one would expect them to be laden with their burden in some direct ratio to the abundance of fatty material. No such ratio exists. In some sections these cells are surrounded with fat, yet the lymphoid cells contain none (Fig. 8). Finally fat streams can be traced from the basement membrane to the wall of the lacteal with no leucocytes intervening (Fig. 11). As already remarked there are four kinds of leucocytes in the villus, but one only is concerned in this, namely the epithelioid cell, for in the guinea pig fat does not occur in the lymphocyte or the polymorphonuclear cells (Figs. 2).

In the demonstration of the presence of fat one must not rely wholly on the result given with osmic acid, for as Heidenhain has well said: "Everything that stains black with osmic acid is not fat." In preparation from guinea pigs fed on the ordinary diet, fixed with Flemming Fluid, and stained with iron alum and eosin, dark granules are found in these large lymphoid cells, which have the appearance of fat and for some time I considered them as such. Later I noticed that in similar specimens fixed with absolute alcohol, and stained with hæmalum and eosin, these granules were present and had the same dark or black appearance. Evidently then such granules could not have been fat since the alcohol agent would dissolve all the fat out of the sections. These granules were evidently particles

of inorganic salts, probably iron, as Macallum has conclusively shown that these cells pick up that element. One can, however, demonstrate that they also contain fat. If preparations from animals fed with fat made with Flemmings Fluid be placed in ether for twenty-four to thirty-six hours, the granules, which before appeared deep black, become colourless, having slowly dissolved in the fluid. Further, if the preparation is made with ten per cent. formalin for twenty-four to forty-eight hours, and the preparations sectioned with freezing microtome and stained with scarlet red, for forty-five seconds the fat granules appear bright red. If, however, before staining they are placed in ether for twenty seconds the red granules disappear, this showing that the cells do take up fat.

As already pointed out it was Zawarykin who first brought forward the view that leucocytes were the sole means by which fat was taken up from the intestinal contents. He claimed that after picking up the fat they returned to the chyle vessels. Schäfer,* while admitting that fat droplets are present in the epithelial cells when the amount of fat in the chyme is relatively large, holds that the leucocytes are under ordinary conditions the only structures capable of taking fat from the lumen of the bowel. He says too that "no fat particles are, as a rule, found between the epithelium and the central lacteal, save such as are embedded in lymphoid corpuscles." In the guinea pig at least, and, according to Heidenhain, in puppies also, it is a rare thing to find a leucocyte of any description either within or between the epithelium. As remarked, the large lymphoid cell is the only leucocyte which picks up fat, and I have never observed such a cell either within or between the epithelium, nor have I ever seen such a cell in the central lacteal. I have on several occasions seen a polymorphonuclear leucocyte in the epithelium with fat almost surrounding it, yet containing none itself (Fig. 6). More frequently one is able to see lymphocytes within the epithelium, but always free from fat (Fig. 8). Leucocytes are present in numbers in the epithelium in young kittens, but whether they take up fat I cannot say, since I have not studied their action in this respect, but even if they do contain fat, their number is altogether inadequate to accomplish very much, a fact which tells against the view that they transfer fat through the parenchyma to the lacteal. Schäfer found that in spring frogs, which had been fed fat the epithelial cells showed considerable fat, while November frogs showed very little fat absorption, and then none was present in the epithelium, the whole being taken up by the leucocytes. He concludes from these experiments that under ordinary circumstances the leucocytes take up all the fat, while the epithelial cells act as storehouses when the fat is in

* Pflüger's Arch. Vol. 37, p. 395, and Inter. Monatschr. für Anat. und Hist. Vol. 2, 1885, p. 6.

excessive amount. The presence of fat within the cells of a spring frog may be explained in an altogether different manner. If one kills such a frog, fixes a portion of the intestine in 10 per cent. formalin for twenty-four to forty-eight hours, then places it in 5 per cent. chloral hydrate over night, and afterwards in scarlet red solution en masse for another eight to twelve hours, washing in water for a few seconds and teasing the material in glycerine furnishes a preparation which is conclusive. The whole epithelial cell is more or less filled with fat droplets like those found in the epithelium of a fat-fed guinea pig. One never, however, sees the fat in the nucleus or in the striated border in such an instance. The same is true of a starving Necturus (Fig. 17). Moreover the presence of fat droplets during the starving period is quite general as the cytoplasm of the liver cells is more or less filled with them while their nuclei give no evidence of their presence. Fat is likewise abundant in the epithe ium of the renal tubules.

From this it may be concluded that the fat which Schäfer found in the intestinal epithelium of the spring frog was, in great part, that which is normally present under such conditions. I have not had an opportunity of making observations on November frogs, but feel certain that if such were fed olive oil there would be no difficulty whatever in demonstrating fat in the epithelium. Schäfer used lard in his investigation, which on account of its comparatively high melting point is unsuited to cold blooded animals. As I shall show further on, when winter frogs are fed olive oil for several days it is possible to demonstrate minute fat drops in the striated border (Fig. 16).

## VII.—PASSAGE OF FAT INTO THE LACTEAL.

Heidenhain offers no explanation as to how the fat goes through the lacteal wall, but says that the fat does not attain a finely divided condition until it gets to the central chyle vessel. This is supported by my own observations. That the fat does not necessarily remain as very minute droplets is equally certain (Figs. 2 and 10) for one can frequently see large globules forming in the lacteal from the minute fat droplets as they have penetrated the wall. Fig. 8 illustrates the manner in which the fat penetrates the lacteal wall. The representation is from the side of a villus and one can see minute fat droplets on either side of the wall and in some instances delicate streams of fat going through. In many cases these minute droplets after entering coalesce into larger fat droplets or spherules. It may then be concluded that the fat penetrates the lacteal wall as very minute droplets of particles through numerous stomata in its

surface, these latter being especially numerous at the tip. As to the forces at work causing the fat to go towards the lacteal one can only offer conjectures as to their character. Weymouth Reid* has shown that the epithelial cells of the intestine will absorb fluids when the osmotic pressure is negative and the hydraulic pressure less upon the lumen side so that in this case the vital activity of the cells must play the most important, if not the whole part. Certainly osmotic pressure cannot have anything to do with the transfer of fat through the lacteal since it depends for its results upon the number of dissolved molecules which in this instance is nil. The epithelial cells, by their contractions, can remove the fat from within them into channels which communicate with the lacteal; the contraction of the muscles about the chyle vessel which is supplied with a valve at its base empties the lacteal and the resulting dilation causes a relatively lower pressure so that the fat is forced to go in the direction of least resistance, probably by the stimulus received from the epithelial contraction. It is generally conceded that the proteid material normally goes by way of the blood vessels, but when fed along with excess of fat it frequently enters the chyle vessel with the fat. Fig. 11 shows that physical condition cannot be overlooked in considering the causes of fat transference.

Bruch† claimed to have demonstrated that the capillaries took up fat. He described certain vessels as half filled with fat, the other half showing the normal blood reaction. Heidenhain has criticized Bruch's work and concludes that his technique was very faulty in that the whole section showed up more or less black owing to the fixing agent not having been washed out before placing in alcohol. I have never been able at any time to satisfy myself that any fat ever entered the blood capillaries, probably because, as Heidenhain suggests, the fat in the parenchyma is not in a sufficiently divided condition to enter them. Fat, undoubtedly, in other parts of the body, leaves the capillaries for the tissues, but it must be as very minute particles indeed, and if it can leave the capillary its passage in the opposite direction should not be an impossibility, but from experiments upon the percentages of fat in the portal vessels as compared with such vessels as the carotids and femorals the probability of fat entering the capillaries of the villi is negative.

As to the part supposed by some to be played by leucocytes as agents in transferring fat to the lacteal it may be stated that in the guinea pig, evidence of such an activity, except as a rare feature, cannot be found, and in fact the presence of leucocytes within the lacteal is a rare occur-

* Phil. Trans. Roy. Soc., Vol. 192B, 1900, p. 211.
† Zeit. für wiss. Zool., Vol. 4, 1853, p. 288.

rence, one having to examine many specimens before seeing an example, such as is represented in Fig. 11. The leucocyte, which is of the polymorphonuclear variety is surrounded by fat, which forms a fine meshwork, and yet contains none itself. It is perfectly formed and shows no signs whatever of disintegration. In all the preparations from the intestine of the guinea pig I have not seen half a dozen leucocytes in the chyle vessel, nor have I seen a leucocyte penetrating the wall, facts which should tell against this view. I have, moreover, never seen large lymphoid leucocytes in the lacteal,and these are the cells which, undoubtedly, pick up fat. Then too the fewness of leucocytes relative to the amount of fat to be transferred must always cast grave doubt as to the efficiency of their action in this respect. Finally, as I have already pointed out, one can frequently trace the fat as minute streams going through the parenchyma into the central vessel, and this without the intervention of leucocytes of any description. (Figs. 8 and 10).

## VIII.—FAT IN THE STRIATED BORDER.

And now I come to the discussion of the most interesting part of my investigation. With the exception of Kischensky, all observers are agreed that fat has not been found in the striated border of the epithelium. From an histological standpoint, this fact has been used as a proof that fat enters the cells in some soluble form, for example, as a soap, and is later reconverted into a neutral fat through some action of the epithelium, all observers, further, regarding the fat present in the epithelium as in a neutral condition. As to the fat in the intestinal cavity a great deal of attention has been given to determine whether it is present as a soap or an emulsion, but into a discussion of this question I do not propose to enter, and it suffices to say that there is abundant experimental evidence to show that both are present. In fact, to have a permanent emulsion, one must have present an alkali and some free fatty acid or, in other words, a soap, and as Heidenhain has pointed out, it is a common occurrence to see fat droplets of various sizes lying in the lumen. Indeed, it was the presence of such minute droplets in immediate contact with the striated border, together with the view I had taken regarding the structure of the striae as enunciated in the first part of this paper, that led me to think the fat was taken up in some particulate form and not alone in solution.

In this connection, too, it is of interest to note that von Thanhofer* described a to and fro movement of the striae in the duodenum of winter frogs whose nerve roots had been severed. He claimed to have seen red

* Pflüger's Arch., Vol. 8, p. 406.

blood corpuscles and desquamated epithelium carried along by this movement and even saw fat droplets lashed into the interior of the epithelial cells. These phenomena he watched for three quarters of an hour. He could not, however, confirm these results upon spring and summer frogs, but did so once more on winter frogs. Fortunatow*, likewise, on two occasions, claims to have seen a motion like that of cilia in the striae. One asks why the same should not be observed in spring and summer frogs and, with Heidenhain, what the cutting of the nerve roots has to do with it, yet I think the view is not by any means so fantastical as later investigators suppose. Schäfer rejects it on the ground that small particles of carmine are not picked up by the epithelium. The physical properties of such particles are very different from those of very minute fat droplets and consequently one should not expect to find the same result in both cases. The occurrence of fat particles in the striated border as Kischensky's and my own observations show can only he explained as a result of the physical activity on the part of the striated border.

The most striking evidence regarding the occurrence of fat particles in the striated border is to be found in preparations treated with scarlet red, as described under Methods of Study.

When such sections are examined with an oil-immersion lens the appearance found is like that represented in Fig. 13. Large and small drops are seen within the cells while very minute, but nevertheless, quite distinct globules are seen in the striated border. Their distribution is not uniform. In some parts of the specimen no such globules are present, in others they are few, while other portions again show numerous beadlets, such variations depending, doubtless, upon the different periods of activity on the part of the cells. Often the globules are irregularly scattered throughout the border, but one can frequently see rows of beadlets running parallel to the striæ and occasionally a line of fat running the whole length of the same and having the appearance of an elongated fat droplet. Having satisfied myself that fat droplets could be demonstrated in the striated border of guinea pigs I made observations, with the aid of scarlet red, on the intestinal epithelium of frogs, cats, rabbits, dogs and Necturi (lake lizards). The frogs and lizards were in the starving condition, and the material obtained from them was placed in five per cent. chloral hydrate solution over night and teased the next morning. There is considerable variation in the striæ in all these animals, that of the rabbit being the coarsest, and in consequence best suited for demonstrating the border fat. To the first rabbit I gave 20 c.c. olive

* Pflüger's Arch., Vol. 14, p. 288.

oil without previously depriving it of food and killed it about twenty hours later. A piece of the duodenum was put into five per cent. chloral hydrate over night, then placed in the scarlet red solution for about twelve hours, removed therefrom to water for a few seconds, then teased in glycerine and mounted, and the edge of the slip being subsequently smeared with paraffin. The result was quite remarkable, (Fig. 18). In some instances the droplets were irregularly scattered throughout the border, in others they were arranged in beadlets running parallel with the striæ, in others again a string of beadlets is seen running transversely to the plane of the bodies, and at the very tips of the striæ, as though the beadlets were just entering the cell, while in still others the same elongated form of the fat droplets, as described in the guinea pig, is evident. The same technique was carried out with the dog, kitten, frog and Necturus and in each instance I was able to demonstrate fat particles in the striated border (Figs. 13, 18 and 19).

The question naturally arises: Why cannot these fat particles be demonstrated with osmic acid? That it fails in paraffin sections I believe can be accounted for by the slight solvent action of the alcohols used. While it is true that alcohol is not an active solvent for fat since you can put fat-fed specimens which have been fixed in formalin and cut with the carbon dioxide freezing method into seventy per cent. alcohol for an hour, then into ninety-five per cent. for fifteen minutes, all without any appreciable difference in the fat reaction when afterwards stained with scarlet red, yet when long continued it might readily affect that which is most accessible. It must be borne in mind, also, that osmic acid is not as delicate, or as sensitive a stain, for fat as scarlet red.

During the last few days of my investigation I directed my attention to the demonstration of fat droplets in the border by means of osmic acid. A rabbit was fed 20 c.c. olive oil, without being previously starved, and killed three and a half hours later. Pieces of the duodenum were put into five per cent. chloral hydrate over night and then into one per cent. aqueous osmic acid solution and small portions teased out from time to time in glycerine. The osmic acid penetrates very slowly under such circumstances so that not until about the fourth day do you get any marked reaction. Fig. 21 shows the appearance of the cells after four days in the osmic solution. Here too one can see fat beadlets in the striated border, and when compared with Fig. 19 the similarity is striking.

From the above considerations it is evident that fat can be and is absorbed in some particulate form. By means of scarlet red it is not

possible to differentiate a neutral fat from one of the fatty acids since it gives just as distinct a reaction with oleic and stearic acids as with neutral fats. Consequently one cannot say positively whether the droplets are fatty acids or neutral fat, although in all probability the latter. If, however, they are fatty acids, these are absorbed in particulate form and not in solution. The conclusion arrived at, therefore, from the study of the above results is that fat is taken up in particulate form by the striated border, either as neutral fat, or fatty acid,—in short as an emulsion.

Both J. Munk* and Will† have shown that the organism, probably the epithelial cells, can furnish the glycerine radicle necessary for the synthesis of neutral fat from fatty acids so that it is immaterial as to the ultimate result whether the fat is taken up in a neutral form or as a fatty acid.

Hofbauer‡ performed certain experiments to show that fat was taken up as an emulsion. Before feeding, the fat was stained with Alkanna Roth or Lackroth A. These are insoluble in water, but soluble in alcohol. On examination he found the fat in the villi stained. Pflüger, in criticizing Hofbauer's work points out that the stains used are soluble in bile, glycerin and soap so that the stain might readily be absorbed and colour the fat afterwards. Hence one cannot draw any positive conclusions from Hofbauer's researches. I repeated his experiments, using scarlet red, which has one advantage over Hofbauer's stains in that it is insoluble in glycerine. It is, however, soluble in soap solution. The fat in the villi was found to be coloured red. Nevertheless, when taken in conjunction with the demonstration of fat in the border it is a fact in support of the emulsion theory of fat absorption.

Henriques and Hansen¶ conclude from their experiments that all fat is absorbed as soap. They took equal parts paraffin and fat with a little fatty acid and added an aqueous solution of sodium carbonate. There results an emulsion in which the droplets contain an equal quantity of fat and paraffin intimately mixed. On feeding this they found that most of the fat was absorbed while the paraffin was in the excrement. Upon superficial consideration this seems to be a very strong objection to the emulsion theory. But what reason have they for supposing that their made up emulsion remains intact until it reaches the duodenum? Milk is an emulsion, but without being acted upon by the intestinal fluids

* Virchow's Arch., Vol. 80, p. 20 and Vol. 95, p. 431.
† Pflüger's Arch., Vol. 20, 1879, p. 255.
‡ Pflüger's Arch., Vol. 81, p. 263, Vol. 84, p. 619 and Zeit. f. Klin. Med., Vol. 47, p. 477.
¶ Centralbl. f. Physiol., 1900, p. 313.

would not be absorbed because the fat globules are not nearly minute enough. Without the action of the intestine upon their artificial emulsion neither would the fat be absorbed for the same reason. The very fact that they conclude it is absorbed as soap admits of disintegration of their emulsion so that their experiments are altogether negative, neither supporting nor favouring the emulsion theory.

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## EXPLANATION OF PLATES.

In the various Figures *ad*, signifies adenoid tissue; *b*, blood capillary; *c*, a polymorphonuclear leucocyte; *f* and *im*, columns and masses of fat between epithelial cells; *fd*, fat in lumen of lacteal; *fl*, fine streams of fat traversing wall of lacteal; *fs*, network of fine lines of fat in wall of lacteal; *ec*, large lymphoid cells.

FIGURES 1—12 and 14.—From villi of guinea-pigs fed with yolk and dialysed iron. Flemming's Fluid, Hæmatoxylin and Eosin.

FIGURES 3 and 8 are tangential sections of the epithelial cells of the villi.

FIGURE 13.—From villus of guinea-pig fed with yolk. Formalin ten per cent., four and a half days, Scarlet Red. Showing beadlets of fat in the striated border.

FIGURE 15.—From villus of guinea-pig fed with yolk, showing contents of central lacteal with some fat droplets.

FIGURE 16.—Intestinal epithelial cells of frog fed with olive oil, isolated, showing fat particles in striated border. Scarlet Red.

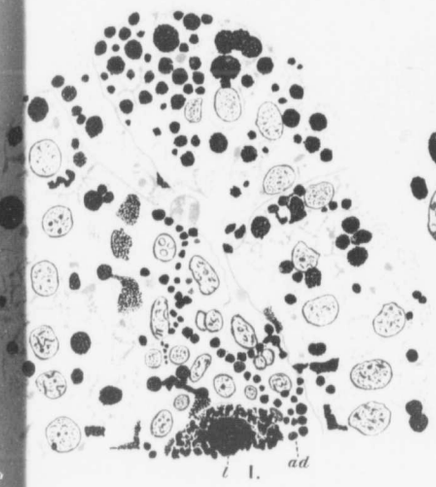
FIGURE 17.—Isolated epithelial cells of intestine of starving Necturus, showing droplets of fat in cytoplasm, but none in striated border. Formalin, Scarlet Red.

FIGURE 18.—Isolated epithelial cells of the intestine of a Necturus fed with olive oil, showing the distribution of fat in the striated border and in the cytoplasm. Formalin, Scarlet Red.

FIGURE 19. Epithelium, isolated, from duodenal villi of rabbit fed on olive oil, showing beadlets of fat in the striated border and in the cytoplasm. Formalin, Scarlet Red.

FIGURE 20.—Isolated epithelial cells from the intestinal villi of a dog fed on olive oil, showing fat in the striated border as villus in the cytoplasm. Chloral hydrate, Scarlet Red.

FIGURE 21.—Isolated epithelial cells of the intestinal villi of a rabbit fed on olive oil and killed three and a half hours afterward. Osmic acid for twenty-four hours.



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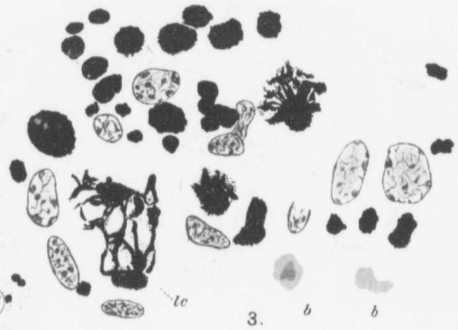

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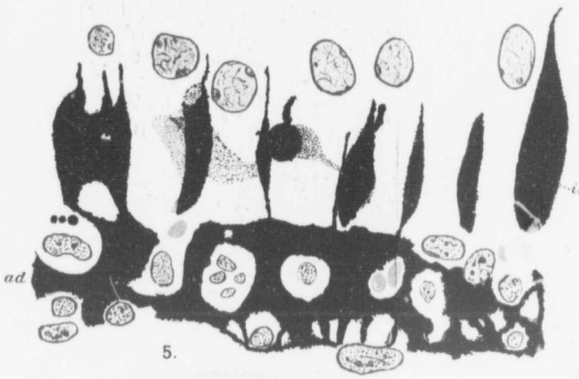

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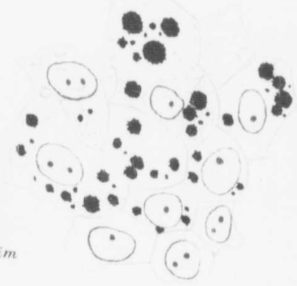
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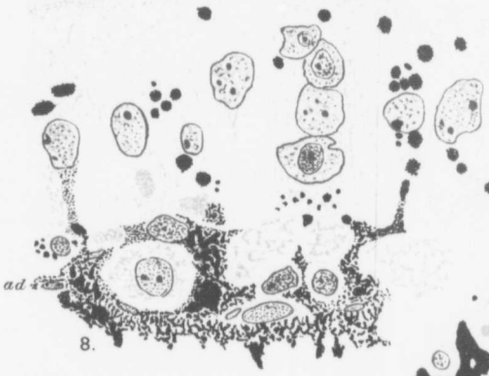

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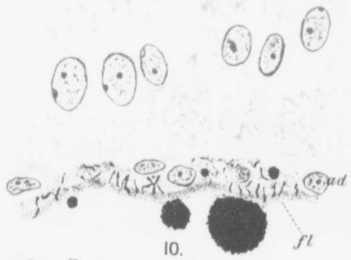

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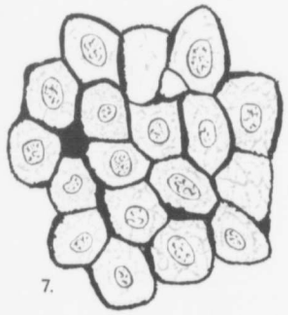
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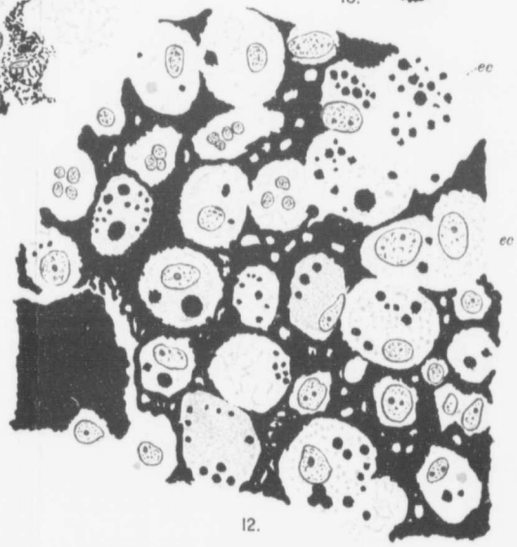

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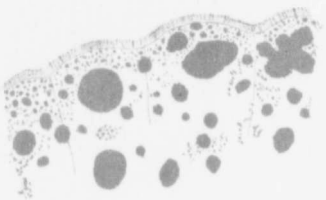

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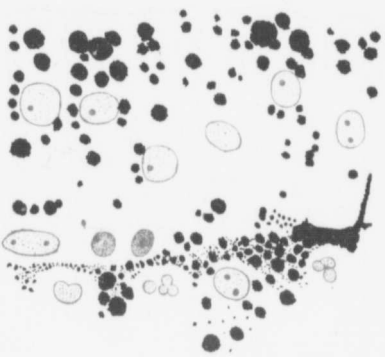

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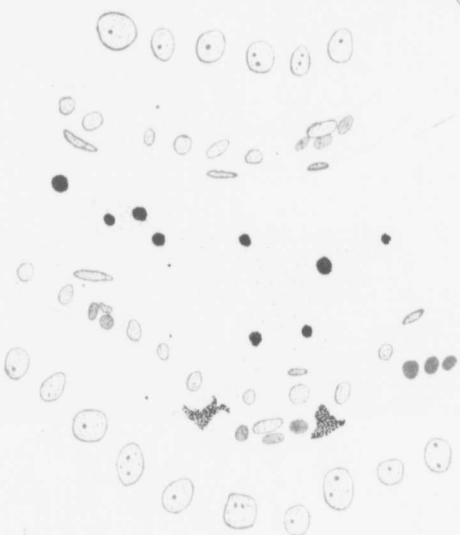

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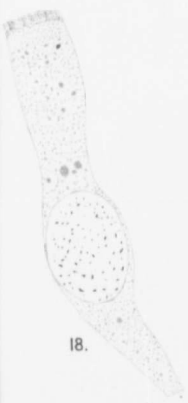

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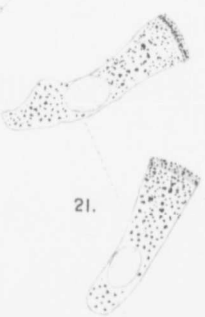

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