The Absorption of Lipoid by the Intestinal Epithelium of the Mouse

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With one plate (fig. 1)

SUMMARY

During the absorption of fat by the intestinal epithelium of the mouse, spindle-shaped particles of lipoid material can be seen in the free border of the epithelial cells. The spindles, which lie with their axes at right angles to the surface of the free border, correspond in position with the spindle-shaped canals previously described by the author in various vertebrates.

INTRODUCTION

THE investigation described below was designed to discover, on cytological evidence, whether fatty food is absorbed by the intestine of the mouse in particulate form (e.g. as droplets), or in aqueous solution after hydrolysis. The intention was to find whether, during digestion, lipoid material could be seen actually *in* the free border of the epithelial cells of the intestine. It may be said at once that after fat has been eaten, particles of lipoid can be seen in the free border. Anyone can satisfy himself on this point by following the simple directions given in this and a previous paper (Baker, 1949).

The word *lipoid* will be used here (as in all the papers of my associates and myself) in the wide physical sense, to cover triglycerides and all other substances that occur in the tissues of plants and animals and are soluble in fat-solvents.

It was shown by Prewoznikoff (1876) and Will (1879) that lipoid can be demonstrated in the intestinal epithelial cells of vertebrates after feeding with soaps (or free fatty acids) and glycerol. Thereafter it was generally taken for granted that triglycerides were acted upon by lipase in the cavity of the intestine, absorbed in aqueous solution as soaps and glycerol, and reconstituted within the cells. This conclusion was supported by Krehl (1890), who made a large histological investigation of the absorption of triglycerides by the intestinal epithelia of various vertebrates, without ever witnessing the passage of particles of lipoid through the free border of the cells.

When the theory of absorption as soaps was later abandoned on account of the acid pH of the whole of the upper part of the small intestine, recourse was had to the old observations of Strecker (1848) and Latschinoff (1880) on

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the formation of compounds between bile acids and fatty acids. It was claimed that these water-soluble compounds were absorbed by the intestinal epithelial cells. Little notice was taken of the improbability of there being enough bile acids in the intestine to enter into molecular relations with all the fatty acid that might be contained in the triglycerides of a meal.

On account of the belief that lipoids were absorbed in water-soluble form and reconstituted within the epithelial cells of the intestine, few investigators searched for indications of their passage through the free border; but before there had been any full study of the histology of fat-absorption, von Basch (1870) had claimed to see droplets of lipoid in the free border of the intestine of rabbits and rats fed on fatty foods. He fixed pieces of the intestine in Müller's fluid and darkened the lipoid by the use of osmium tetroxide. He concluded that: 'Das Fett dringt in feinen Tröpfchen durch den Stäbchensaum hindurch in den Protoplasmakörper der Epithelzelle.' Nothing of the sort seems to have been seen again until Weiner (1928) studied the absorption of lipoid material by the intestine of the bat. He described its passage through the free border in particulate form, as 'Körnchen oder Stäbchen': these he coloured sometimes by Fischler's method (1904) and sometimes by sudan IV. He represented some of the lipoid particles in the form of spindles, lying in the free border at right angles to its surface. He relied upon Fischler's method as a positive indication that the particles in the free border consisted of fatty acid. Actually, as Kaufmann and Lehmann (1926) showed, Fischler's method is not specific: a wide variety of lipoid material can be stained by it.

Wotton and Zwemer (1939) fed fat coloured with sudan IV to cats, by stomach-tube; they fixed pieces of intestine in pyridine-formalin and cut frozen sections. They refer to 'Droplets' [of fat] . . . small enough to lie within the striations of the cuticular border', but apparently these were seen in the lumen of the intestine. They say that very small droplets of fat seem able to pass through the cuticular border, but they do not describe or illustrate this process. They figure a large droplet of fat in the lumen connected with a lipoid mass in the cytoplasm of an intestinal epithelial cell by a thin strand that appears to pass through the free border; they refer to this as a dumb-bell appearance.

In a series of very interesting and important papers Frazer and his associates (1942, 1943, 1946, 1947, 1948, &c.) now began to bring forward physiological and biochemical evidence that long-chain triglycerides are not wholly hydrolysed by lipase in the intestine. They concluded that much of the fatty food must be absorbed in the form of minute droplets of triglyceride, but they did not detect the passage of these through the free border.

The greater part of the thickness of the free border of the intestinal epithelium of vertebrates shows positive textural birefringence in relation to the direction at right-angles to the surface of the border (see Schmidt's (1943) investigation of the border in the tadpole). The most probable explanation of this fact is that the material of the border consists of or contains submicroscopic rodlets or fibres running perpendicularly to the surface of the border.

In the rat the existence of these submicroscopic rodlets has been proved by the use of the electron-microscope (Granger and Baker, 1950). Although these rodlets are below the limit of microscopical resolution in the rat, they can be glimpsed in the newt: thus, one can just get an impression of them, in surface view, in the section from which fig. 13 of an earlier paper was taken (Baker, 1942), though they cannot be seen in the photomicrograph.

The material constituted by the rodlets is hollowed out by canals. These run parallel to the rodlets, but are of a different order of size: they are easily visible with the light-microscope, especially if a 2μ section, cut perpendicularly to the plane of the border, is mounted in water. They have been shown to occur in the rabbit, starling, slow-worm, newt and frog (Baker, 1942), and in teleost fishes (Al-Hussaini, 1949). They are particularly easy to study in newts (*Triturus vulgaris* and *cristatus*), because in these animals they are very wide and far apart. The canals are narrow at the surface of the free border and at its opposite (attached) side, but are usually wider in the middle of the border, so that they are commonly somewhat spindle-shaped. In fixed preparations they are often closed at the two ends.

Beneath the layer of submicroscopic rodlets and microscopic canals there is a thin layer containing phospholipine. In my earlier paper I called this the 'granular' layer, because the phospholipine often shows a tendency to become aggregated in minute lumps. This layer is not made visible by the technique described in the present paper.

Beneath the granular layer lies the superficial part of the cytoplasm, which is here hyaline on account of the absence of mitochondria. I call this the 'clear zone'.

For a full account of the microscopic structure of the free border, and of the long controversy about it, see my 1942 paper. I pointed out there the possibility that particles of fat might enter the epithelial cells of the intestine through the spindle-shaped canals, but I did not study lipoid absorption. I I have done so now, and the present paper is the result.

Methods

The object was to follow cytologically the absorption of food in the small intestine of mice during the normal passage of a meal along the alimentary canal.

It was important to be able to cause the animals to eat at arranged times, so that they could be killed at known intervals thereafter. Being naturally nocturnal, a tame mouse that has been kept without food (or edible bedding or cage-material) for 10 hours previously will nearly always eat at dusk directly a palatable food is supplied to it. It should be left in the dark after the food has been supplied and not disturbed unnecessarily.

The foods used are listed below. Their names are given for easy reference, but are not to be regarded as exactly descriptive. For example, the carbohydrate food contains a small amount of protein; it receives its name because

carbohydrate greatly preponderates in it. Unmixed purified foods were not used, because they are not always eaten at once.

Carbohydrate food: crumb of white bread.
Protein food: boiled egg-white.
Lipoid-free food: crumb of white bread and boiled egg-white.
Lipoid food: butter and milk.
Oleic acid was supplied to mice as follows. The redistilled acid was shaken up with glycerol in the proportion of 1·24 : 1 by weight. The crumb of white bread was soaked in the emulsion and given to the animals; it was eaten at once.

The mice were at all times allowed to drink as much water as they wished.

Before the start of an experiment the mice were usually kept on the lipoid-free diet for two or three days. This reduced to a minimum the chance of error due to the retention in the stomach or intestine of lipoid material taken in at a previous meal. If the intention is merely to confirm that lipoid is absorbed in particulate form, this precaution is, of course, unnecessary.

The best period for the study of the absorption of lipoid in the upper part of the small intestine is from 1½ to 2¼ hours after food is supplied: 1¾ hour usually gives the best result. At this time the stomach is still distended, but the absorption of lipoid is proceeding rapidly in the intestine. At later stages the intestinal epithelial cells become loaded with large lipoid droplets.

When the proper period had elapsed, mice were usually killed with chloroform. Coal-gas presents no advantage: the appearance under the microscope is the same as when chloroform has been used.

Directly the mouse was dead, a piece of small intestine about ¾ cm. long was cut out and dropped into the fixative. The region from 4 to 12 cm. from the pylorus was generally used. After the lapse of 5 minutes, when the outer muscles had hardened sufficiently to cause the piece of tissue to retain its form, it was opened from one end to the other to allow free access of the fixative to the villi. The sudan black method, which I have described in detail elsewhere (Baker, 1949), was nearly always used. In this method short fixation in neutral formaldehyde-saline is followed by soaking in a mixture of formaldehyde and potassium dichromate solution; there is subsequent treatment with hot potassium dichromate. Sections cut on the freezing microtome are coloured with sudan black and carmalum, and mounted in Farrants's medium. The instructions given in the earlier paper were followed, except that the period in dichromate-formaldehyde was lengthened and that in cold dichromate shortened, to make it unnecessary to work at inconvenient times in the night. Sections must be thin (6μ) for this particular work. Such sections are easily cut if the instructions given in my earlier paper are followed.

Other methods are mentioned in the description of results.

Since lipoid is absorbed into the free border in extremely small particles, it was necessary to work with the highest useful powers of the microscope and to use what Dempster (1944) has well called 'controlled' illumination. A

Chance-Watson No. 4 light-filter was used in taking photomicrographs of sections coloured by sudan black.

Results

If a mouse is kept on the lipoid-free diet for two or three days and then killed after remaining for 10 hours without food, the free border of the epithelial cells of the intestine is nearly always devoid of any droplet or other particle of sudan-positive material (fig. 1A). Exceptionally, on prolonged search, one may find one of the lipoid 'spindles' that will be described below. Beneath the free border there is a clear zone; the cytoplasm here is hyaline on account of the absence of mitochondria. Beneath this again there is the mitochondrial region, which appears granular, and then the region of the lipochondria (Golgi bodies), which take the sudan black very deeply. Next comes the nucleus, and beyond that the basal group of mitochondria. The sudan black technique is not intended to show the mitochondria and they are not well represented.

Almost exactly the same appearance is given after feeding on carbohydrate (fig. 1B) or protein (fig. 1C) food.

When a mouse has been fed on lipoid food, sudanophil material is seen in the cavity of the intestine and also in the free border. Absorption of fat starts at the tips of the villi, and large droplets will often be seen within the cytoplasm of the cells in this region before any of it has been absorbed by the sides of the villi.

The lipoid material that lies in the cavity of the intestine and adheres to the surface of the villi and thus appears in microscopical sections, is in the form of spherical or subspherical droplets, averaging a little over $0{\cdot}8\mu$ in diameter (mean of 20 measurements, $0{\cdot}82\mu$). It happens by chance that the droplets seen in the cavity of the intestine in fig. 1, D and E, are smaller than the average.

The lipoid that lies within the free border is mostly in the form of particles of the shape of ellipsoids or spindles (fig. 1, D and E). The average length of these bodies is $0{\cdot}62\mu$ and their breadth at the widest point $0{\cdot}42\mu$ (means of 25 measurements in each direction). If one makes the assumption (which must be approximately true) that the particles are prolate ellipsoids of revolution with major axes 0·62 and $0{\cdot}42\mu$, the volume of each is 0·057 cubic μ; a sphere having this volume would be approximately $0{\cdot}48\mu$ in diameter. Thus the ellipsoids or spindles are smaller, on the average, than the lipoid droplets lying in the cavity of the intestine. A statistical analysis of the difference in volume between the droplets in the cavity on the one hand and the particles in the free border on the other, is not given here, because it might make readers attribute too great accuracy to the measurements on which the means are based. Such very small objects cannot be measured accurately. One can see that the droplets in the cavity of the intestine are bigger, in general, than the spindles, and one can only say that the means given represent an approximation to their size. It is clear that one spindle cannot usually represent one

whole lipoid spheroid that was formerly free in the cavity of the intestine, though it may well represent a part of it.

The spindles can only be properly studied when the section passes through a villus in such a way that the free border does not appear to move when one focuses up and down. If such movement occurs, one may make the mistake of supposing particles to be in the free border, when in fact they are outside it.

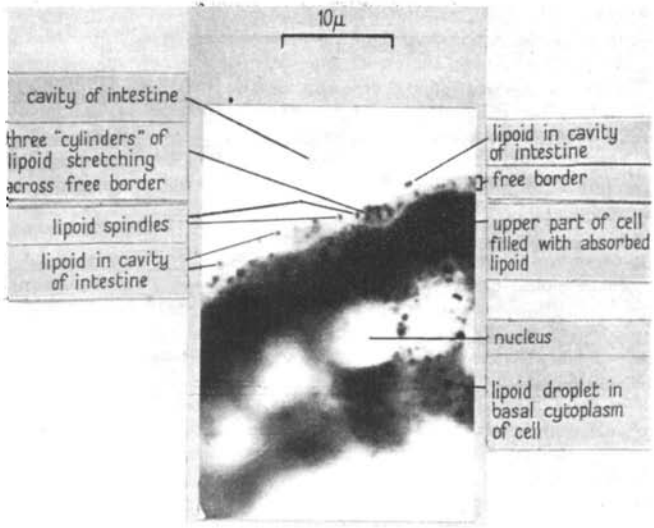

FIG. 2. Photomicrograph of intestinal epithelial cells of a mouse after lipoid feeding, to show 'cylinders' of lipoid stretching across the free border. (Gelatine section coloured by the sudan black technique.)

The photomicrograph was taken at very high magnification so as to make the labelling easier.

The lipoid particles in the free border have not always the shape of an ellipsoid or a spindle. Sometimes they are approximately cylindrical, and they then often extend right across the free border from its outer to its inner side (fig. 2). When this is so, their outer ends sometimes make contact with lipoid droplets lying in the cavity of the intestine.

In my previous paper on the free border (1942) I mentioned that the spindle-shaped canals do not usually extend the whole way across the free border in fixed preparations, though they appear to do so during life. The position and shape of the canals corresponds closely with that of the lipoid spindles. I do not know any fact making it unlikely that the spindles represent lipoid material taken into the canals of the free border from the cavity of the intestine.

It is very rare to find lipoid particles in the clear zone beneath the free border, though I have occasionally seen them there and can demonstrate them to others.

Within the cytoplasm of the epithelial cell the lipoid material generally appears in the form of spherical sudanophil droplets. The largest have a non-sudanophil vacuole within them; this is perhaps an artificial appearance. The non-vacuolate droplets that are definitely not mitochondria (because too large) measure up to more than 5μ in diameter; the mean of 30 measurements was 2·43μ. These droplets in the cytoplasm give a pink reaction with nile blue, and one can therefore exclude the possibility that they consist of fatty acid (see Cain (1947) for a full consideration of the reactions of nile blue).

During fat-absorption one occasionally finds in the upper part of the cell, between the clear zone and the region of the lipochondria, a number of sudanophil particles of approximately the size and shape of the spindles of the free border, but usually somewhat curved so as to resemble short bananas.

If triglycerides were wholly hydrolysed in the intestine, and the resultant fatty acids dissolved in water, before absorption, by saponification or combination with bile-acid, it is unlikely that one could demonstrate the presence of fatty acid by the use of sudan black: at the most one could only expect a pale grey colour extending diffusely over the section. Instead one sees particles of lipoid, intensely coloured, in perfectly definite sites. It is almost necessary to assume that the spindles that are seen in the free border after lipoid feeding consist *either* of undigested fatty esters (triglycerides, &c.), *or* of fatty acids produced by the digestion of such esters, *or* of both mixed together. Unfortunately the spindles are so small that only very intense colouring-agents, such as sudan black, will show them. Nile blue, which would decide the question if it coloured lipoids strongly enough, leaves them untouched.

Since a direct histochemical approach to this problem did not seem possible in the present state of knowledge, it was decided to get an answer to this question: Can fatty acid be absorbed in particulate form, and, if so, does it then appear in the free border in the form of spindles? To determine this, mice were fed for two days on the non-lipoid diet, and then on the oleic-acid food described on p. 82. Since the cavities of their intestines would then be devoid of fatty esters, any sudanophil material seen in the free border would be likely to consist of fatty acid.

This experiment gave a definite answer. After fatty acid feeding, many sudanophil droplets are seen in the cavity of the intestine, attached to the surface of the free border; the droplets are even smaller than when the food contains triglycerides. Sudanophil particles are seen in the free border. They are fewer than when the food contains triglycerides, and they are not quite so intensely coloured by sudan black. Some of them have the characteristic shape of spindles; occasionally one is seen stretching right across the free border in the form of a cylinder; often they appear as ovoid bodies close to the inner side of the free border. The latter arrangement is not commonly seen after triglyceride feeding. Lipoid is definitely being absorbed, for fairly large

sudanophil droplets are seen in the cytoplasm of some of the epithelial cells, especially near the tips of some of the villi; but the amount absorbed appears to be much less than when the food contains triglyceride.

This experiment shows that fatty acid can be taken into the free border in particulate form, but it does not show that the sudanophil particles seen in the free border after triglyceride feeding consist of fatty acid. Spindles are not seen if formaldehyde-saline alone is used as fixative, with no subsequent treatment with potassium dichromate, nor yet if sodium iodate is substituted for potassium dichromate in the standard technique. I am at present trying to devise methods of revealing the chemical composition of the spindles that are seen when triglyceride is being digested.

Since von Basch (1870) used potassium dichromate in his fixative, it is quite probable that he actually saw the spindles, though the absence of a figure makes it impossible to be certain of this. Weiner's (1928) figures suggest strongly that he saw them, though it is surprising that he could colour them with sudan IV after simple formaldehyde fixation. I have not seen anything resembling the 'dumb-bell' appearance described by Wotton and Zwemer (1939).

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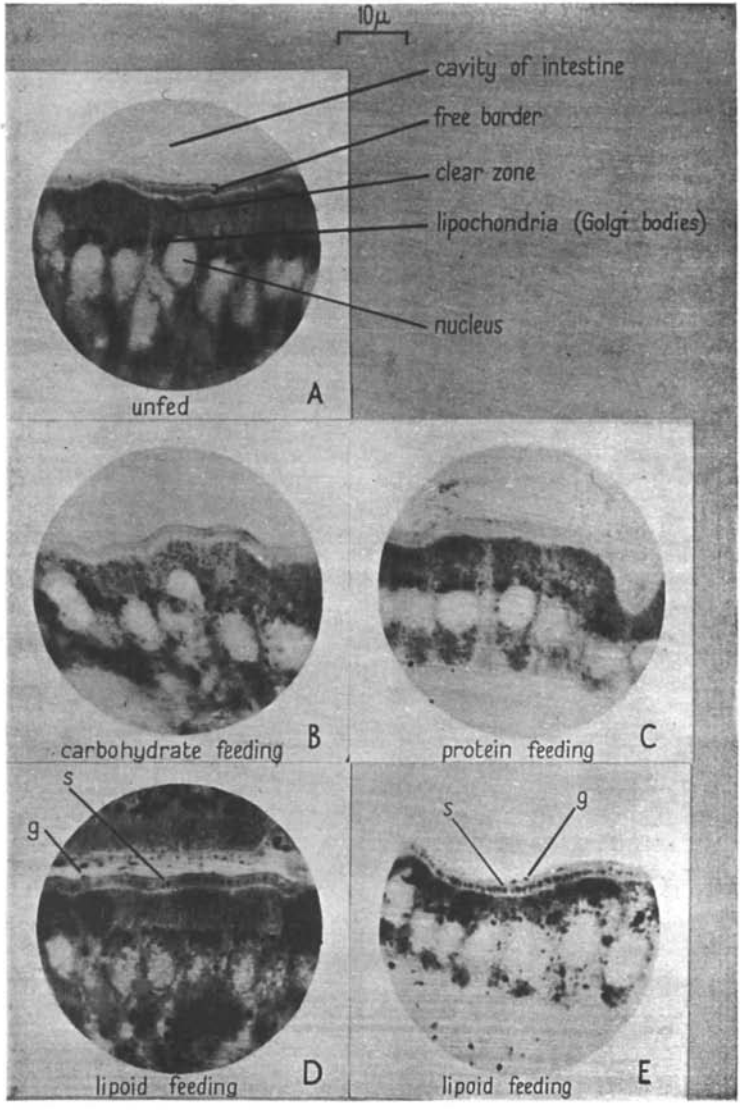

FIG. 1. Photomicrographs of the intestinal epithelium of the unfed mouse, and of mice fed in various ways. (Gelatine sections coloured by the sudan black technique.)
s, lipoid spindle in free border. *g*, lipoid globule in cavity of intestine.