Lipid Transport

H. C. TIDWELL, PH.D.*

THE first mechanism in the transport of lipids necessarily involves the passage of the lipid across the intestinal barrier. A better understanding of the exact nature of the forms required for intestinal absorption of glycerides should give further insight into their transport across all membranes in the body.

TRANSPORT ACROSS THE MUCOSA

The generally accepted theory of the mechanism of fat absorption has varied markedly during the last few years. The "partition hypothesis" of Frazer[1] gained acceptance for a time. According to this hypothesis, most of the triglycerides are absorbed as such, then pass through the chyle into the systemic circulation, while a smaller, hydrolyzed fraction goes directly into the portal blood. It now appears to be well established by Bloom et al.[2] that most if not all of the absorbed lipid enters the circulation by way of the lymphatics.[3] Reiser[4] and Dowse[5] and their associates have reported that only trace amounts of triglyceride are absorbed as such. Numerous investigators have suggested that up to 50 per cent of the ingested fat is completely hydrolyzed before absorption. A partial hydrolysis of the remainder of the triglyceride would necessitate the absorption of at least two-thirds of the ingested glyceride as free fatty acids.

Recent studies from our laboratory[6] support the hypothesis that most of the fatty acids of triglycerides are absorbed, not as glycerides, but as unesterified fatty acids. Evidence for this conclusion can be summarized in four categories:

(1) Ingested fatty acids are at least as rapidly absorbed as fed triglycerides if only the amounts actually available for absorption are considered. In support of this, Borgström[7] contends that fatty acids are selectively absorbed when small amounts are fed with a fat.

(2) The percentage of fatty acids in the intestine during fat absorption bears a closer relationship to the rate of lipid absorption than does the degree of emulsification.

(3) The rate of fat absorption was not promoted by the presence of the mixture of fatty acids, monoglycerides and triglycerides, which Frazer found to be ideal for promoting emulsification.

(4) Fat supplements, which accelerate the action of the lipolytic enzymes, are associated with marked increases in the rate of absorption of an ingested fat. Supplements such as histidine and ethionine increased the rate of enzymatic hydrolysis and absorption, while glycine and methionine did not.[6] Hence any effect of methionine on absorption cannot be the result of an increased rate of hydrolysis of the fat.

Whether phospholipids play an important role in the absorption and conversion of fatty acids to glycerides is not known. Studies on phosphorous turnover in the intestinal wall have indicated that the phospholipids cannot have a major role in the absorption of fats.[8] This may not be true if only one form of phospholipid is actively involved, or if phosphoryl choline should be transferred intact instead of breaking down to phosphate and choline. Nevertheless, choline and possibly methionine were found to hasten the absorptive process and this result might be associated with an increased formation of phospholipid. This has been questioned by others when results were obtained under conditions different from ours. How-

From the Department of Biochemistry, University of Texas Southwestern Medical School, Dallas, Texas.

* Professor and Chairman.

Presented at the First Symposium of the Lipotropic Research Foundation, April 13, 1958, Philadelphia, Pennsylvania.

ever, phospholipids may have some part in the absorption and transport of fatty acids across the intestinal barrier. Eventually, the fatty acids enter the chyle and appear in the blood as chylomicrons, consisting principally of triglycerides in association with small amounts of phospholipid, cholesterol and protein.

TRANSPORT IN THE BLOOD

Two different theories have been advanced regarding the mechanism of transport of neutral fat in the blood. One group of investigators believes that the β-lipoproteins are involved in the transport of the fatty acids derived from the chylomicrons of the blood, while another group assigns the major role in this process to albumin.

Korn[9] has produced evidence that the triglycerides in the chyle may be associated with small amounts of protein (probably β-lipoprotein) as chylomicrons. The triglycerides of the chylomicrons and the low density β-lipoproteins may be acted upon by lipoprotein lipase to yield free fatty acids and possibly high density β-lipoproteins. This process frees the lipoproteins which then can form more complexes with fat. Hence, the lipoproteins may serve as intermediates in the conversion of the triglycerides of the chylomicrons into complexes which can be acted upon by lipoprotein lipase.

Albrink et al.[10] have found the major part of the lipid in the blood after a fat meal in the chylomicrons, which are largely neutral fat with small amounts of phospholipid and cholesterol. During a fasting period, most of the lipid is in soluble form having a much higher proportion of cholesterol and phospholipid than neutral fat. These investigators have presented evidence to support the thesis that the labile, rapidly removable, neutral fat is transported by the more stable lipoprotein complex, which consists basically of cholesterol, phospholipid and protein. Swank and Fellman,[11] have found that an injection of a sizable amount of chyle is followed by a large increase in plasma β-globulins, and later by increases in α-globulins. This supports the aforementioned theory. Similarly, Jones et al.[12] have presented evidence to suggest that low density lipids are involved in the transport of fatty acids.

An opposing theory is favored by Bergström,[13] Havel[14] and their co-workers. Earlier, Bergström[13] had found a rapid turnover of absorbed fatty acids, as much as 1 mg. per minute over a four-hour period, even when the absorbed labeled lipid amounted to a maximum of only 15 per cent of the total plasma lipids. In other words, the absorbed triglycerides were not mixed with the existing plasma lipids, which were mainly in lipoprotein combinations. Havel et al.[14] found 97 per cent of the absorbed activity from ingested labeled fat in the triglyceride of the chylomicrons in the chyle, which contained little if any unesterified fatty acid. After injecting labeled chylomicrons, little activity was found in the α- or β-lipoproteins. The percentage of the unesterified fatty acid increased as the chylomicron content decreased. The unesterified fatty acids had a half-life of about two minutes, while that of the chylomicrons was about twenty minutes. Their data do not support the concept that other lipoproteins are involved in the removal of the lipid of the chylomicrons from the plasma. The site of lipolysis of the triglyceride is not known.

Rodbell[15] has recently obtained additional evidence that, starting with chylomicrons, lipoproteins rich in triglycerides are not gradually transformed from lower into higher density lipoproteins by the successive removal of triglyceride. If the transformation were due only to loss of lipid, the protein would be the same in both low and high density lipoproteins. He found that the various types of lipoproteins did not contain a common protein as judged by content of some amino acids. However, this would not exclude the possibility that the unesterified fatty acid may combine with certain of the blood protein fractions for fatty acid transport.

We found that the activity associated with the super layer, after centrifuging out the chylomicrons, had increased from about 30 to 60 per cent of the total blood activity at five hours as compared with one and a half hours after feeding a labeled fat.[16] A trichloroacetic acid precipitation of the proteins revealed that

only about half of the activity of the precipitate could be extracted with fat solvents even after treatment with alkali. At this longer interval, a considerable part of the remaining activity was associated with a protein fraction whose nature is yet to be determined.

Dole,[17] Gordon[18] and co-workers suggested that the metabolically active form of fat in the body is the unesterified fatty acids. It also may be true that this is the major form involved in the transfer of most of the fat across all cell membranes.[19]

The exact role of lecithin in lipid transport is as yet unspecified and that of cholesterol is still to be determined. If the lipoproteins do aid in the transport, it is possible that the fundamental role of these two lipids may be associated with structure.

Much of the conflicting evidence reported can probably be explained by the use of different kinds of experimental animals under quite different experimental conditions. Many of these studies reported were made and conclusions drawn without due regard to species differences which now appears quite evident in some cases.

SUMMARY

Available evidence suggests that most of the fatty acids of ingested triglycerides are absorbed as unesterified fatty acids and not as glycerides. Some phospholipid fraction may be involved in the transport of the fatty acids into the mucosa or their conversion into triglycerides. After release into the blood largely as triglycerides in chylomicron form, the fatty acids are transported at least partly with the aid of the blood lipoproteins. It is possible that the unesterified fatty acids released from these lipoproteins are the most metabolically active form of fat and that they are the major form involved in the transport of fat across cell membranes.

REFERENCES

1. Frazer, A. C. The absorption of triglyceride fat from the intestine. *Physiol. Rev.*, 26: 103, 1946.
2. Bloom, B., Chaikoff, I. L., Reinhardt, W. O., Entenman, C. and Dauben, W. G. Quantitative significance of lymphatic pathway in transport of absorbed fatty acids. *J. Biol. Chem.*, 184: 1, 1950.
3. Borgström, B. On the mechanism of the intestinal fat absorption. *Acta chem. scandinav.*, 5: 643, 1951.
4. Reiser, R. and Diekert, J. W. Intestinal absorption of unhydrolyzed tripalmitin. *Proc. Soc. Exper. Biol. & Med.*, 92: 649, 1956.
5. Dowse, C. M., Saunders, J. A. and Schofield, B. The composition of lipid from jejunal contents of the dog after a fatty meal. *J. Physiol.*, 134: 515, 1956.
6. Tidwell, H. C. Absorption of free and combined fatty acids. *Proc. Soc. Exper. Biol. & Med.*, 98: 12, 1958.
7. Borgström, B. On mechanism of intestinal fat absorption; metabolism of lipids. *Acta physiol. scandinav.*, 25: 291, 1952.
8. Zilversmit, D. B., Chaikoff, I. L. and Entenman, C. Are phospholipids obligatory participants in fat transport across intestinal wall? *J. Biol. Chem.*, 172: 637, 1948.
9. Korn, E. D. Clearing factor, a heparin-activated lipoprotein lipase. I. Substrate specificity and activation of coconut oil. *J. Biol. Chem.*, 215: 15, 1955.
10. Albrink, M. J., Glenn, W. W. L., Peters, J. P. and Man, E. B. Transport of lipids in chyle. *J. Clin. Invest.*, 34: 1467, 1955.
11. Swank, R. L. and Fellman, J. H. Plasma proteins and fat transport in dogs. *Am. J. Physiol.*, 192: 318, 1958.
12. Jones, J. B., Gofman, J. W., Lindgren, F. T., Lyon, T. P., Graham, D. M., Strisower, B. and Nichols, A. V. Lipoproteins in atherosclerosis. *Am. J. Med.*, 11: 358, 1951.
13. Bergström, S., Borgström, B. and Rottenberg, M. Intestinal absorption and distribution of fatty acids and glycerides in rat; metabolism of lipids. *Acta physiol. scandinav.*, 25: 120, 1952.
14. Havel, R. J. and Fredrickson, D. S. The metabolism of chylomicra. I. The removal of palmitic acid-1-C^{14} labeled chylomicra from dog plasma. *J. Clin. Invest.*, 35: 1025, 1956.
15. Rodbell, M. N-terminal amino acid and lipid composition of lipoproteins from chyle and plasma. *Science*, 127: 701, 1958.
16. Burr, W. W., Jr., McPherson, J. C. and Tidwell, H. C. Chylomicron metabolism: alterations of absorbed labeled lipid. *Am. J. Physiol.*, 195: 769, 1958.
17. Dole, V. P. The significance of nonesterified fatty acids in plasma. *Arch. Int. Med.*, 101: 1005, 1958.
18. Gordon, R. S., Jr. and Cherkes, A. Unesterified fatty acid in human blood plasma. II. The transport function of unesterified fatty acid. *J. Clin. Invest.*, 35: 206, 1956; 36: 810, 1957.
19. Fredrickson, D. S. and Gordon, R. S. Transport of fatty acids. *Physiol. Rev.*, 38: 585, 1958.

DISCUSSION

DR. W. STANLEY HARTROFT (*St. Louis, Missouri*): What has occurred in the last two or three years as a result of study with the electron microscope is the realization that the phenomenon of pinocytosis, which has been recognized for a long time and studied extensively in protozoa and amoeba, is apparently capable of being performed by all the cells of the body, even in mammals.

Using this method, the study of fats is going to be easy because the usual form of fixation for electron microscopy is osmium, which preserves the fat and makes it black.

We are becoming interested in the possibility that this phenomenon of pinocytosis may occur in the liver under normal conditions, and we wonder whether or not it is altered in choline deficiency. Our own investigations with the electron microscope are aimed in this direction but we have nothing to report at this state.

DR. TIDWELL: Dr. Frazer raised the question as to whether all fat has to be split into fatty acids before absorption. I do not think one can say with certainty that this is so, but some of our recent studies offer considerable evidence that would suggest that fatty acid is the form most likely to be absorbed by the intestine or to escape from the vascular bed.

For example, we found that despite general reports, the fatty acids were not more slowly absorbed than triglycerides. When fed as such, the fatty acids were retained in the stomach longer than triglycerides, as were the monoglycerides; whereas, if only the lipid in the intestine is considered as available for absorption, the fatty acids are removed just as rapidly, if not faster, than the triglycerides.

When the lipids were available in a form to best promote emulsification according to Frazer, there was no increase in the rate of absorption. We compared the effect of emulsification with that of an increase in the percentage of free fatty acids. There seemed to be a much closer relationship to the amount of free fatty acids than to the degree of emulsification. Feeding substances which promoted the rate of hydrolysis *in vitro* also markedly hastened the rate of absorption of the fat.

An investigator in our laboratory, who has been studying *in vitro* absorption, was unable to obtain any absorption into the mucosal cells of fat from emulsions of triglycerides under conditions at which hydrolysis could not readily take place. When a complex of albumin and free fatty acids were employed, he obtained a ready passage of these free fatty acids across the barrier. Undoubtedly, traces of glyceride may get into the cell as such, but available evidence suggests that the major part of the glycerides has to be split and enters in the form of free fatty acids.

DR. HARTROFT: Incidentally, Dr. Frazer described cilia as hollow, but this view has not been confirmed by electron microscopy.

DR. CAMILLO ARTOM (*Winston-Salem, North Carolina*): Dr. Tidwell, could absorption of fatty acids be to a large extent in the form of monoglycerides?

DR. TIDWELL: I do not think so. Of course, there is a possibility that limited amounts of all three glycerides may be absorbed. If at least half of the ingested triglyceride is completely hydrolyzed, at least two-thirds of the total amount of fatty acids will be present as free fatty acids. Undoubtedly, some of the glycerides do get across as such but the evidence is rather convincing that the major part of the lipid does not get across in the form of glycerides, either mono- or triglyceride.

DR. HARTROFT: I was glad that Dr. Cornatzer considered the nucleus in his studies. Some years ago Dr. Wilgram and I observed that stainable fat could be demonstrated in the hepatic nuclei of choline-deficient animals. It is not peculiar to the choline-deficient type of fatty liver, however. Recently, my associate, Dr. Grisham, has been measuring hepatic nucleus-nucleolar ratios and nuclear-cytoplasmic ratios in various stages of choline deficiency. He has found fat in the nuclei of these liver cells after less than two weeks of choline deficiency. Of course, we knew it appeared later when cirrhosis developed, but the fact that it occurred so early was startling.

Polyploidy also appeared at an early stage. We thought it might be a mechanical effect of an excess of fat in the cytoplasm but it also clearly occurs in cells having the least amount of fat.

Am I right in stating that you found phospholipid synthesis was stimulated in the nucleus?

DR. W. E. CORNATZER (*Grand Forks, North Dakota*): By giving a single dose of the mixture of CoA, CoA choline and AMP, greater than occurs in the nucleus and in the mitochondria, this effect was achieved.

DR. HARTROFT: But choline itself cannot produce this effect; other constituents must be added?

DR. CORNATZER: Yes. We have analyzed the lipid content in liver necrosis induced by bromobenzene and have found that there is a decrease in the lecithin phosphorus in the nucleus.

DR. HARTROFT: Is this in the necrotic nuclei or in the non-necrotic nuclei?

DR. CORNATZER: This is in the necrotic nuclei.

DR. HARTROFT: How do you separate them?

DR. CORNATZER: We separate them by a centrifugation method. The control subjects receive the corn oil and the subjects with necrosis receive corn oil plus the halogenated agent.

DR. HARTROFT: But in the animals that received the toxins, you have a mixture of necrotic and non-necrotic nuclei?

DR. CORNATZER: That is true. You are thinking of a dose of halogenated compound that Kutz, Weiser and Poplar used to induce massive necrosis. We have used that dose, and we probably still have some normal cells although I do not know the number. We have not separated them, but the dose used produces massive necrosis throughout the liver.

DR. G. WILGRAM (*Toronto, Canada*): I was

interested in the results obtained by Dr. Artom when the animals on a diet low in methionine were fed methionine and choline, and the specific activity of the labeled fatty acids was registered in the liver in the *in vitro* tests. When choline was added to this diet, it did not result in the complete removal of fat from the liver. However, we presume this would have occurred if large doses of methionine had been added to the diet, showing that although choline under conditions similar to yours has some effect, it does not compare with the effect achieved by administering large doses of methionine.

DR. CORNATZER: When choline is given by stomach tube, there is always some destruction of the choline by bacteria to trimethylamine. Once it reaches the liver cell there is still some destruction of the choline by choline oxidation *per se* as compared to methionine.

Methionine can go into active methionine, and Stetten has shown that the monomethyl group of choline comes only from methionine. The other methyl groups are not from methionine.

DR. WILGRAM: The point that I am considering is somewhat different. Does choline have the same effect as methionine on a low choline diet in increasing fatty acid oxidation? Dr. Artom's figures lead me to believe it has not.

DR. ARTOM: I believe the figures show no significant difference. Considering that the results are somewhat variable from one experiment to another, the two figures represent the same effect. I cannot see any difference.

DR. M. CHENOWETH (*Midland, Michigan*): I think that the combination of methionine and diethanolamine might be considered here. This combination would circumvent the excretion of choline, as some recent work on diethanolamine has shown.

DR. CORNATZER: Studies made some time ago by Dr. Artom and myself demonstrated the effect of diethanolamine as compared to choline. Diethanolamine on an equal molar basis stimulates more than choline, and the choline given by stomach tube is partially destroyed by choline oxidase, whereas choline oxidase has no effect on diethanolamine. Only one methyl group is required to convert diethanolamine to choline, and that methyl group, according to Stekol, apparently comes from methionine—available methionine in the liver cell, or active methionine.

DR. HARTROFT: Of course, if the choline was given in sufficient amounts, the effect should be obtained. Dr. Wilgram, did it matter how much choline you added?

DR. WILGRAM: No.

DR. CORNATZER: Was the choline given orally?

DR. WILGRAM: Yes, it was.

DR. COLIN C. LUCAS (*Toronto, Canada*): It has been proved that there is destruction of choline. However, I think that the amount of destruction either varies in different laboratories or is, perhaps, related to the basal diets that are used. In our work, we have obtained dramatic lipotropic effects from minute amounts of choline chloride, 0.02, 0.03, and 0.04 per cent.

Too much cannot be destroyed or there would be no effect. With 0.08 per cent, we obtained close to maximal effect. That is a minute amount of choline chloride, so maybe 2 to 5 or even 10 to 20 per cent is destroyed. I am not sure why Propert should find so much destroyed. Certainly, there is no evidence that our results are anything like that and, there is one paper (I do not recall the authors) that records minimal destruction.

DR. HARTROFT: Yes, there is another one besides that. I think that the importance of destruction has been overemphasized. However, it may occur in some laboratories, on some basal diets, or with some strange type of rats.

DR. ARTOM: The intestinal flora are probably involved.

DR. LUCAS: This is what I mean. With a certain basal type of diet intestinal flora may be active in destroying choline, but that is not inevitable.

DR. CHENOWETH: I question the absorption of choline. Has anyone ever determined blood levels of choline following single large oral doses to see whether it plateaus, whether absorption can be pushed past a certain blood level?

DR. ARTOM: When labeled choline is given, you can find quickly, after about an hour, that approximately 80 per cent is already converted into phospholipids. That happens when not too much choline is given. Of course, when larger doses of choline are given there is a decreasing rate of incorporation, but beginning with a reasonably low dose it is quite readily absorbed.

I think that the experiments of Stekol do arrive approximately at the same conclusion—that a large proportion of choline is quickly incorporated into phospholipids.