Some Recent Studies on Fat Digestion and Absorption

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FEW subjects in biochemistry have been the object of as much controversy for so long a time as the question of the degree of hydrolysis of fat in the intestine and the mechanism of its passage through the intestinal epithelium. Differences of opinion had already been the subject of polemics for many years when Pfluger in 1900 used a paper on the toxicity of horse meat (1) as an opportunity to elaborate on his saponification theory and vehemently to attack opposing opinions. Later in the same year he continued his diatribe (2). Since then the subject has been periodically reviewed (3, 4, 5, 6, 7, 8, 9, 10, 11) and much evidence collected purporting to support one theory or another.

Classically, the subject of fat absorption is studied and discussed in three phases. The first is the degree of hydrolysis of triglycerides in the lumen of the intestine. The second concerns the physical and chemical mechanisms of the passage of the various hydrolyzed or unhydrolyzed fragments through the outer border, the body, and the basal membrane of the mucosal columnar epithelial cells. The third involves the route of transport of absorbed lipide material to the general circulation. The present discussion will concern itself mainly with the first and second of these.

From the time the problem was first studied, it had been assumed that triglyceride molecules were either completely hydrolyzed to free glycerol and fatty acids or were not hydrolyzed at all. Thus Pfluger in 1900 (1, 2) and Verzar in 1936 (5) were adamant that all the fat must be split while Munk, Hammarsten, and Brucke (quoted by Verzar) were equally as insistent that it is not. That all physiologists were not convinced that the matter was all "white" or "black" is evidenced by the statement in a textbook by Foster in 1880 (12) that "in all probability saponification in the intestine is a subsidiary process, intending rather to facilitate the emulsion of neutral fats than to introduce soap as such into the blood." In spite of this willingness on the part of nonpartisans to compromise on the issue and to admit that some molecules may be hydrolyzed and some not, it did not seem to occur to anyone until recently that hydrolysis might stop at the di- or monoglyceride stage.

In 1935 Artom and Reale (13) reported a study of the action of pancreatic lipase in which the products of the reaction were fractionated into mono-, di-, and triglycerides by their relative solubilities in alcohol-water and acetone-water mixtures. They found that the products were mono- and diglycerides and not glycerol and fatty acids, as has been assumed by all workers up to that time. These authors later supported their conclusion by demonstrating that mixtures of fatty acids and glycerol were synthesized to lower glycerides by pancreatic lipase (14), an observation that recently has been shown by Borgström (15) to take place under physiological conditions.

Unfortunately the work of the Italian authors went unnoticed. A decade later Frazer and his coworkers, in support of their particulate theory of absorption (16), rediscovered the phenomenon and demonstrated that intestinal lipolysis produces some mono- and diglycerides which, with bile salts, produce emulsions of the unhydrolyzed fat of 0.5 μ or less (17). Frazer's position that partial hydrolysis produces emulsifying agents which prepare unhydrolyzed glycerides for absorption is reminiscent of Foster's statement 65 years earlier (12).

In 1947, Desnuelle, Naudet, and Rouzier (18) showed that when pancreatic extracts act on olive oil at pH 7 in the presence of bile salts, *in vitro*, formation of diglycerides was rapid, monoglycerides much slower, and free glycerol very slow and incomplete. In the following year the same authors showed that the proportion of 1- and 2-monoglycerides formed were in the ratio of 2:1 so that the enzyme has no specificity with respect to position. These results are in contrast to the recent report of Mattson, Benedict, Martin, and Beck (19), who found that *in vitro* digestion with pancreatic lipase yielded only 1-monoglyceride as measured by periodic acid analysis while the *in vivo* digestion produced the 2-isomer which partially rearranged so that the mixture, as analyzed, contained approximately equal proportions of the 1- and the 2-isomers. The longer the material remained in the animal, the larger the proportion of the 1-isomer.

IN 1950 Desnuelle, Naudet, and Constantin (20) reported that in the absence of calcium ions pancreatin and pancreatic juice hydrolyze triolein to diolein but, in the presence of these ions, produce monoolein. They found no evidence of free glycerol in either condition. They concluded that calcium ions activate lipases or the substrate by eliminating fatty acids from the interface. Frazer had previously pointed out (21) that *in vitro* pancreatic lipolysis is restricted because of the accumulation of the end-products at the oil/water interphase.

In 1950 (22) and 1951 (23) Desnuelle and his coworkers described conditions in which they were able to force complete hydrolysis of one-third the triglyceride molecules in the substrate and convert the remaining two-thirds to partial glycerides. The outstanding feature of these conditions was the presence of a large aqueous phase rich in bile salts. More recently (24) Desnuelle and Constantin reported *in vivo* and *in vitro* studies in which the end-products of lipolysis were examined with micro techniques which permitted the determination of mono, di-, and triglycerides. They confirmed their earlier observation that calcium ions increased the proportion of monoglycerides (20) and found under their conditions that the intralumenary and *in vitro* hydrolysis produced the same proportions of hydrolytic products.

Borgström has made a study of the extent of pancreatic digestion of triglycerides *in vivo* and *in vitro*, with especial emphasis on synthetic activity of the lipase (15). Three substrates were used: a) corn oil containing C^{14} labeled palmitic acid; b) corn oil and oleic acid containing C^{14} labeled palmitic acid; and c) corn oil transesterified with C^{14} palmitic acid. A

study of the active acids in the mixtures of glycerides and free fatty acids after various periods of digestion led to the conclusions that *in vivo* there is approximately one-third each of free fatty acids, monoglyceride, and "glyceride residue" in the intestinal lipide mixture and that a partial resynthesis takes place during digestion. *In vitro* a mixture of bile and pancreatic juice obtained from cannulation of the bile duct of rats caused nearly 100% hydrolysis in 12 hours at 40°C., but after one hour only monoglyerides and triglycerides were obtained. Evidence of partial resynthesis during digestion was obtained. Borgström was of the opinion that the evident partial resynthesis "renders uncertain the calculations about the degree of hydrolysis of triglycerides in the intestinal lumen of the rat before absorption recently made by Favarger, Collet, and Cherbuliez (25) and by Reiser, Bryson, Carr, and Kuiken" (26). The objection is not valid. The calculations of the latter group are independent of whatever equilibrium may be established in the digestion mixture since they are a probability calculation of randomly distributed glycerides and concern only the relation between the material fed and the end-product in the lymph.[1]

The degree of hydrolysis of glycerides in the intestine has been of recent interest in the United States because of the use of monoglycerides in shortening and the concern of the Pure Food and Drug Administration with use of chemicals in foods. In an effort to demonstrate that monoglycerides are a normal product of digestion, Kuhrt, Welch, Blum, Perry, Weber, and Nasset reported analyses of lipides of the intestinal contents of two human subjects after the ingestion of a fat meal. They found 37.6% and 50%, respectively, of monoglycerides in the two samples (27).

THE above discussion has dealt with the long chain fatty acids only. Glycerides of water-soluble fatty acids are unquestionably hydrolyzed much more rapidly and completely. Thus Gidez and Karnavsky (28) reported that the rate of $C^{14}O_2$ excretion, and glycerol retention in the liver, were approximately the same after feeding C^{14} labeled glycerol as tributyrin or as free glycerol, while after triolein feeding excretion of $C^{14}O_2$ was low and retention of labeled glycerol in the liver lipides was high. One recent report to the contrary is the study by Schønheyder and Volqvartz of the action of liver and pancreatic esterases on water-soluble tripropionyl glycerol (29). These investigators interpreted their data to indicate that liver esterase hydrolyzes first a 1-propionyl group and then, with more difficulty, the 3-propionyl group, finally removing the 2-group. Pancreatic lipase however only produced 1,2-dipropionyl glycerol under the conditions used. These conditions were not physiological. The concentration of enzyme was very low, and the reaction was carried out at 22°C. The specificities of esterases and lipases have recently been very thoroughly reviewed (30). Goldman and Rayman (31) express the opinion that the apparent differences in the effects of lipases on glycerides is due to differences in degree of dispersion of the insoluble substrates, at least for any particular enzyme preparation.

Any conclusions concerning the form in which fats are absorbed into the intestinal mucosa, based on analyses of the products of lipolytic hydrolysis *in vivo* or *in vitro*, are subject to serious criticism because of possible alternative interpretations. It can always be argued that some product of digestion: fatty acids, monoglycerides, diglycerides, or triglycerides, are constantly being removed from the digestion *milieu*. Whatever the composition of the intestinal contents at any time, the material absorbed can still be either only free fatty acids, only mono-, di-, or triglycerides, or any possible combination of the four. This is true even though, as Frazer argues (10), the lipide products of hydrolysis accumulate in the oil phase rather than the water phase. Solubility in the oil phase does not preclude selective absorption.

It is becoming increasingly clear that enzymatic hydrolysis, and especially pancreatic hydrolysis, of fats takes place readily at the 1-position of a triglyceride, less readily at the 2- or 3-position of the resulting 2,3-diglyceride, and only under special conditions can remove the last fatty acid from the residual 1-monoglyceride. Furthermore the 2-monoglyceride is readily converted to the more stable 1-monoglyceride. The degree of emulsification and the presence of calcium ions, which remove liberated fatty acids from the interface, modify the rate and probably the degree of the hydrolysis.

It cannot be overemphasized that the above facts concerning the hydrolysis of glycerides prove nothing about the degree of hydrolysis necessary for absorption and do not demonstrate which molecular species pass into the intestinal epithelium.

In 1949 Favarger and Collet published a very significant paper (32). These authors fed rats the following three types of mixtures: a) glycerides mixed with deuterium labeled glycerol; b) trielaidin mixed with free elaidic acid and deuterium labeled palmitic acid and; c) trielaidin mixed with labeled lauric acid. The intestinal wall and blood were examined for the markers. It was assumed that if hydrolysis took place, the hydrolyzed glycerol and fatty acid would mix with the free molecules and be randomly utilized in glyceride resynthesis. The degree of incorporation of the labeled free glycerol and free fatty acids into the resynthesized fat was therefore taken as the measure of the degree of hydrolysis. Since very little labeled glycerol was incorporated, it was concluded that over 90% of fat must be absorbed as tri-, di-, and monoglycerides and that absorption of triglycerides was certain since hydrolysis of acids was never greater than one-third.

Unfortunately Favarger and Collet's assumption was wrong. The failure of these authors to observe the incorporation of labeled free glycerol into glycerides was not due to the lack of hydrolysis, as they originally interpreted it, but was due to the fact that free glycerol and glycerol hydrolyzed from fat are not utilized in glyceride resynthesis during absorption. Thus Reiser, Bryson, Carr, and Kuiken (26)

[1] Two new papers by Borgstrom on this subject have appeared in Arch. Biochem. Biophy. *49*, 268 (1954) and in Biochemica et Biophysica Acta *13*, 491 (1954), since this review was written. In the first Borgstrom reported that labeled free fatty acid was incorporated into monoglyceride in the lumen of the intestine, forming di- and triglycerides, thus demonstrating that the reaction is a true synthesis and not simple interesterification. (Free glycerol, he emphasized, is not esterified). Upon feeding 1 ml. of unlabeled monoolein he recovered approximately 8, 12, 20, and 25 mg. of free fatty acid from the intestine in 4 trials. There was no control. He concluded, however, that "Fed monoglycerides are significantly hydrolyzed in the lumen of the small intestine before absorption."

In the second paper it was concluded that "hydrolysis of glycerides by pancreatic juice enzyme proceeds *via* the 1,2-diglyceride to both 1- and 2-monoglycerides, the hydrolysis to the 2-monoglycerides prevailing." This is in confirmation of Mattson *et al.* (19). Borgstrom also demonstrated that Ca ions accelerate the rate of hydrolysis coincidental with a decrease in the rate of triglyceride resynthesis.

fed rats conjugated trilinolin, in which the glycerol was labeled with C^{14}, and examined the lipides of the thoracic duct lymph for the labeled glycerol. The C^{14} activity of the lymph triglycerides had but 60% of the activity of the glycerol fed.[2] The experiment was repeated with the modification that the labeled unsaturated triglyceride was mixed with non-labeled saturated triglycerides. The saturated and unsaturated glycerides of the thoracic duct lymph were then examined for the labeled glycerol and conjugated linoleic acid. A probability study showed that the 60% labeled glycerol that had not disappeared had been absorbed as monoglycerides and been randomly resynthesized to triglycerides with the hydrolyzed fatty acids. It is evident that none of the 40% hydrolyzed glycerol, within the limits of error, was reutilized for glyceride synthesis.

In confirmation of the above Bernhard, Wagner, and Ritzel (33) fed deuterium-labeled glycerol, free or as triacetin (the latter is readily completely hydrolyzed by pancreatic lipase) and, upon finding only traces of deuterium in the lymphatic fat, concluded that "fed glycerol evidently does not participate in reesterification of the fats in the intestine." These authors also fed triolein, deuterium-labeled in the glycerol portion, and a fat labeled in both the glycerol and the fatty acid moieties. On the basis of the deuterium content of the neutral fats of the lymph, they calculated that only 24% to 53% of the fats were completely hydrolyzed to glycerol during absorption. No effort was made to determine whether the "unhydrolyzed" fraction remained as triglyceride or was converted to di- or monoglyceride.

The non-participation of free glycerol in glyceride resynthesis has also been confirmed by Favarger, Collet, and Cherbuliez (25), who, in checking their earlier work (32), fed labeled glycerol with fat and found insignificant proportions in the absorbed fat. Their earlier conclusions (32), based on the evidence of labeled acids and not on the faulty assumption, are quite valid with respect to degree of hydrolysis and also indicate that approximately one-third ingested fat is hydrolyzed to free glycerol.

VERY recently, Buensod, Favarger, and Collet (34) examined the lipides of the intestinal wall after the ingestion of deuterium-labeled glycerol and glycerol phosphate with free fatty acids and fat. Neither the glycerides nor phospholipides contained significant amounts of deuterium, and it was again concluded that if any free glycerol is formed during fat digestion, neither it nor glycerol phosphate, which may be formed from it, participate in the resynthesis of fat.

These observations raise the question as to whether glycerol, *per se,* is the immediate precursor of glyceride glycerol. In an effort to obtain some information on this problem Reiser and Williams fed 1-palmitoxy-3-hydroxyacetone labeled in the ketone and fatty acid moieties (35). It appeared in the lymph as triglyceride with but little loss of C^{14} activity in the glycerol. The possibility is thus presented that dihydroxyacetone instead of glycerol may esterify with fatty acids as the first step of triglyceride resynthesis.

Labeled monopalmitin has also been fed and found in the lymph as triglyceride with little loss of C^{14} glycerol activity (35). The simplest mechanism to account for this conversion is as follows:

$$3\ GA \longrightarrow GA_3 + 2G.$$

It had previously been shown that labeled lymph triglyceride glycerol consistently has about 40% less activity than that fed (26). The assumption at that time was that any hydrolysis during digestion and absorption takes place in the intestinal lumen. However the regularity of the changes after both monoglyceride and triglyceride ingestion, and the significant differences between the two suggest that an intracellular mechanism regulates the hydrolysis. This concept, and the observations reviewed above that lipases attack monoglycerides with difficulty, led to the suggestion that digestion of triglycerides in the lumen of the intestine proceeds only to the monoglyceride stage and that the replacement of labeled glycerol with "cold" glycerol somehow takes place during triglyceride resynthesis (35).

It has been repeatedly shown that ingested free fatty acids are esterified with glycerol, or its precursor, and appear in the lymph as triglycerides. During the absorption of a mixture of monoglycerides and free fatty acids therefore it is to be expected that there will be competition between the monoglycerides and glycerol, or its precursor, for the available fatty acids, thus resulting in the loss of some original glycerol and its replacement with new.

Studies have been made in this laboratory to test this hypothesis by comparing the glycerol activity of ingested triglyceride, mucosa triglyceride and phospholipide, and lymph triglyceride and phospholipide (38). The surprising observation was made that there was one-third less activity in the mucosa and lymph phospholipide glycerol than in that of the triglycerides. This observation is strongly suggestive that the mucosa and lymph phosphatides are not precursors of chyle fat but, conversely, are more likely formed from it.

These observed changes in which the reconstituted glyceride glycerol has 60% of the original glycerol activity, and the phospholipide 40% may be represented by the following equations in which "G" represents labeled glycerol, "gb" unlabeled glycerol precursor, "A" fatty acid, and "PB" a phosphorylated base:

Digestion: $5\ G\ A_3 \longrightarrow 5\ G\ A + 10A$

Triglyceride resynthesis:
$5\ G\ A + 2gb + 10A \longrightarrow 3\ G\ A_3 + 2gA_3 + 2\ G$

Phospholipide synthesis:
$2\ G\ A_3 + gb + PB \longrightarrow 3\ G\ A_2\ PB$

The above hypothesis has the attractive feature that it resolves most of the past controversies. The postulate of hydrolysis in the lumen to monoglycerides only conforms to the demonstrated facts of hydrolysis, as discussed above. The postulate of absorption of monoglycerides satisfies the convincing evidence that complete hydrolysis need not occur.[3]

THE concept of competition between absorbed monoglycerides and endogenous glycerol, or its precursor, for absorbed fatty acids explains the changes

[2] The loss of glycerol activity, as used in this paper, refers to the reduction in activity of labeled glycerol as compared to the reduction in the activity of the fatty acid, according to the following:

$$L = 100 - Af/Ar \times Gr/Gf \times 100$$

in which L = % loss of glycerol activity (or % dilution of labeled glycerol); Af = activity of fatty acid fed; Ar = activity of fatty acid recovered; Gf = activity of glycerol fed; Gr = activity of glycerol recovered. This calculation permits one to make allowance for dilution with other fat on the assumption that no labeled fatty acid is metabolized before its appearance in the lymph.

[3] It is not meant that absolutely no glycerol is released in the lumen during digestion. That a few percent of ingested fat may be completely hydrolyzed is likely.

in labeled glycerol activity experimentally observed. The postulate that not glycerol but a precursor, such as dihydroxyacetone (26), is involved in triglyceride and phospholipide resynthesis explains the failure of numerous observers to note the incorporation of more than traces of labeled free glycerol into glycerides when fed with fat or free fatty acids (25, 32, 33, 34, 39). Only that fraction of ingested glycerol which forms the ketone derivative would be available for glyceride synthesis. This concept also explains the failure of glycerol, hydrolyzed from fat during digestion and absorption, to be reutilized (26, 33, 34).

The formation of phospholipide as a product of resynthesized triglycerides resolves the conflict between the observation which clearly demonstrates the incorporation of ingested fatty acids and the rate of turnover studies with P^{32} which indicate that the phospholipides cannot be intermediate in triglyceride synthesis (37).

Finally, the apparent contradiction between the observations that intralumenary lipolysis proceeds only to the monoglyceride stage and the clear-cut evidence that glycerol is lost and not reused during absorption, is resolved.

It is not meant to infer that the results point unequivocally to the above mechanism. For example, all the phospholipides except sphingomyelin were analyzed as a unit, including any phosphatidic acid. Had they been partitioned, it is possible that all the labeled glycerol would have been found in one or the other and could have the same activity as in the triglycerides. This would mean that triglycerides are formed from phospholipides since that conversion involves no dilution of glycerol.

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Soybean "Lecithin" and Its Fractions as Metal-Inactivating Agents[1]

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"LECITHIN" has been used as an antioxidant in edible oils more or less widely ever since its antioxygenic properties were first described by Bollmann in 1923 (2). The phosphatides have since that time been extensively investigated both as a primary antioxidant, as a synergist, and as a metal inactivator in antioxidant mixtures (6, 7, 8, 10, 12, 14, 18, 19). Although there is much evidence showing a positive improvement in the oxidative stability of fats and oils upon phosphatide addition and although lecithin has been approved by the Food and Drug Administration for use in fats, there also have been questions raised as to its protective value (9). The concentration of lecithin employed in studying the oxidative stability of vitamins and edible oils has been varied from its saturation level, about 5% (3, 8), to levels of 0.02% (4). Within these wide concentration ranges, variations in results are to be expected. Insofar as color and flavor characteristics of soybean lecithin are concerned, results obtained are dependent upon the concentrations employed (1, 4). Above 0.02%, adverse color and flavor characteristics limit the use of soybean lecithin.

As a result of studies on the composition of soybean lecithin, a number of fractions of size suitable for oil-stability evaluations have become available. It was believed that individual components of the mixture of phosphatides known as "soybean lecithin" might vary in their color, flavor, and stabilization

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