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LIPID METABOLISM

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INTRODUCTION

By (the late) H. S. Raper*
The University of Manchester, Manchester, 13

In pursuing almost any project it is useful to pause and survey the ground covered as well as that immediately ahead. Thereby the research worker receives inspiration, his hopes are excited and his faith renewed. I take it that a Symposium such as this one, which has been organized by the Biochemical Society, has this purpose in view and it is my duty as Chairman of this opening session to give a brief introduction to our subject.

For me the subject of fat metabolism, if I may be allowed to call it by its old name, begins with the work of Lawes and Gilbert on the fattening of farm stock. You will remember that in 1853 they showed that in the pig, fat is produced from carbohydrate. This is one of the important biochemical syntheses which animals and plants are able to carry out to their great advantage. Although this discovery was published so long ago we are yet far from knowing the detailed steps by which this synthesis takes place. Most progress has been made in tracing the breakdown of carbohydrates to simpler substances from which the long-chain fatty acids could be built up. That a substance containing two carbon atoms is concerned, has long been suspected from the fact that almost all the naturally occurring fatty acids have an even number of carbon atoms in their molecule. Nearly fifty years ago Leathes suggested that this two-carbon substance might be acetaldehyde and evidence based on *in vitro* organic chemical reactions was put forward to support this view. If, however, there is anything I have learnt in what little I know of biochemistry, it is that a living cell does not as a rule utilize, in its syntheses, the familiar reactions of the organic chemist. Their nature is therefore best sought by studying living organisms themselves or catalysts derived from them. It is not surprising to me therefore that the greatest recent progress in the study of fat synthesis has come from investigations on the mammary gland. We are, I am sure, looking forward with eager anticipation to the communications on this subject by Drs Folley and Popják.

Another landmark in the study of fat metabolism was the publication by Munk in the years 1884–1901 of his work on fat absorption. Some

* Professor Raper had been invited to take the chair at the morning session of the Symposium. Before he died on 30 October 1951, he had prepared the above introduction which was read at the meeting by Dr H. J. Channon who acted as morning chairman.—R.T.W.

of his experiments were carried out in a patient with a lymphatic fistula from which chyle flowed, and therefore are referable directly to absorption in man. But others, and in particular those concerning the importance of bile in fat absorption, were done on dogs; Munk's work seemed to establish as proven :

(*a*) that fat was not absorbed without being first hydrolysed completely to fatty acid and glycerol, and

(*b*) that the fat found in the chyle during fat absorption had been re-synthesized from the absorbed fatty acids in the cells of the intestinal mucosa.

These views were fully accepted until about 1939, since when Frazer and his colleagues have published experiments which throw doubt on them. These indicate that fat might be absorbed in finely particulate form, that is without being first hydrolysed to fatty acid and glycerol, and that the fat in the chyle is not necessarily fat that has been synthesized in the intestinal mucosa, but is at least in part fat that has been absorbed unchanged from the lumen of the small intestine.

In addition Professor Frazer has produced evidence that when fatty acids are absorbed their immediate fate is different from that of absorbed glycerides.

The absorption of fats is to be dealt with today by Professor Frazer and Dr Cook so that the summary I have given may perhaps serve as an all too brief introduction to what they will have to say to us. An extension of this part of the subject is that dealing with the defects in fat absorption which will be considered by Dr French. It has long been known that a deficiency in the secretion of bile and of pancreatic juice will interfere with fat absorption, and this could be explained in terms of current physiological theory. But a failure to absorb fat which is either inborn or occurs in association with other diseases is well known and does not appear to be associated with disorders of secretion either of bile or pancreatic juice. During recent years progress has been made in trying to locate the causes of these hitherto unexplained defects and we therefore look forward to hearing from Dr French about this difficult subject.

To me a third landmark in the subject of our symposium was the theory of the β-oxidation of fatty acids put forward by Knoop in 1905. At the time, the idea that β, and not α-oxidation, occurred was so revolutionary and opposed to orthodox chemical experience that Knoop once told me it delayed for some time his promotion as a teacher. However, within a few years, Dakin was able to show that β-oxidation of fatty acids occurred readily *in vitro* when their ammonium salts were warmed with hydrogen peroxide. It was this together with the well contrived *in vivo* experiments which Dakin carried out on the interrelationship in the oxidation process between β-hydroxy, β-keto

and α-β unsaturated fatty acids and their phenyl derivatives that finally established Knoop's hypothesis. Although this hypothesis was largely built up on the behaviour in the body of phenyl derivatives of fatty acids, it was soon shown by Embden and Schutz, using the liver perfusion method, that it applied to the simpler normal fatty acids as well. Quastel and Wheatly were later able to show that β-oxidation is brought about by thin slices of guinea pig liver. This gave greater control of experimental conditions than that obtained in perfusion of the whole liver and consequently a more detailed analysis of the β-oxidation process could be made. Up to this point, no enzyme preparation had been made that would being about β-oxidation. Living tissues had always been essential. The position was thus different from that which had evolved in the study of carbohydrate metabolism. Here a variety of enzymes and coenzymes concerned in the process of glycolysis had been separately studied with the result that the breakdown of carbohydrates could be depicted in considerable detail. It seems probable that our comparative failure to follow the oxidative breakdown of long-chain fatty acids step by step has been due to our inability to isolate enzymes which would bring this about. However, we are glad to have with us today Dr Lehninger who will be able to tell us of the progress that has been made in overcoming this particular difficulty.

The final contribution to this symposium is on the ' Essential Fatty Acids '. That there are essential fatty acids, at least as far as the rat is concerned, was first shown by Burr, Burr & Miller (1932). I remember that what surprised me most when I first heard of this discovery was not that linoleic acid was an important nutritive necessity but that this fatty acid could not be synthesized by the rat, though other fatty acids are apparently made without difficulty. This problem will be solved no doubt when we know a good deal more about the synthesis of fatty acid in nature than we do now. The other problem as to why linoleic acid is so important in nutrition will I hope be dealt with by Dr Sinclair whom we welcome here today.

The communications to be made at this symposium do not exhaust the problems of lipid metabolism. I have in mind two others at least which are full of interest. One is the formation, location and function of adipose tissue. Experiments with fatty acids containing tracers have shown that the fat in this tissue is continually being deposited and replaced, but we have little knowledge as to how this is controlled and why some localities favour its formation and others do not.

The other subject missing from our list is that of a fat metabolism in the germinating seedling. Here before our eyes we know that fats are being broken down and carbohydrates synthesized. But biochemistry has so far taught us very little about how this occurs.

There is obviously here a great field for enquiry if methods can be devised for opening it up. The problems here are not only met with in the plant. It now seems clear that the conversion of fat into carbohydrate in animals is a daily occurrence but as in the plant we have no certain knowledge as to how it takes place.

These and possibly other aspects of lipid metabolism must be deferred for another symposium.

1. THE MECHANISM OF FAT ABSORPTION

By A. C. Frazer
Department of Pharmacology, University of Birmingham

The fundamental concepts of the Pflüger-Verzár or Lipolytic Hypothesis (Verzár & McDougall, 1936) were that fat absorption could not occur unless the glyceride molecule was completely hydrolysed to fatty acid and glycerol. Emulsification facilitated complete and rapid hydrolysis to glycerol and fatty acid, the latter forming water-soluble complexes with bile salts. The absorbed glycerol and fatty acid re-united in the intestinal cell to form phospholipid, which was an essential intermediate in the resynthesis of triglyceride. The adrenal cortex was a controlling factor in the phosphorylating mechanism. All the fatty material absorbed passed into the chyle as triglyceride.

Frazer (1940, 1946*a*, 1952) put forward an alternative Partition Hypothesis, based on experimental studies, which indicated that hydrolysis of long-chain fats was not complete; that intraluminar emulsification was an important step in glyceride absorption; that phospholipid was not an obligatory intermediate in fat absorption; that the adrenal cortex played no major specific role in the fat absorption mechanism; and that the chemical and physical characteristics of the ingested fat affected its digestion, absorption and distribution in the body.

The object of this paper is to review the accumulated evidence of the last thirteen years, from which the relative merits of these two hypotheses may be judged. The evidence is presented in relation to three important steps which are known to be associated with the fat absorption mechanism—hydrolysis, emulsification and phospholipid formation.

HYDROLYSIS

Ingested triglycerides are hydrolysed by pancreatic lipase. The fundamental question is whether this hydrolysis is partial or complete. *In vitro* pancreatic lipolysis of long-chain glycerides is restricted and comes almost to a stop after 20–30% hydrolysis. This restriction can be shown to be due to the accumulation of end products at the oil/water interface rather than in the water phase (Frazer, 1948*a*). Restriction is lessened, therefore, by conditions that facilitate removal of long-chain fats from the oil into the water phase, such as an alkaline reaction and the presence of fatty acid receptors. The conditions normally prevailing in the lumen of the upper small intestine, however, are not likely to lessen restriction, since the reaction is acid and the available alkali largely reacts with gastric HCl. Liberated

long-chain fatty acids, therefore, tend to accumulate in the oil phase. The argument has been advanced that hydrolysis is more complete *in vivo* than *in vitro* because the fatty acids are removed by absorption in the former case. This, however, pre-supposes accumulation of fatty acids in the aqueous phase, which does not occur. The classical experiments of Levites (1906) showed a progressively increasing proportion of fatty acid in the intestinal contents of the dog, when these were sampled from above downwards. This evidence was said to indicate complete hydrolysis; it does not do that, but it does demonstrate that fatty acids are not rapidly removed from the intestinal lumen by absorption. The end products of hydrolysis of long-chain triglycerides were shown to be fatty acids and mono- and di-glycerides, rather than fatty acids and glycerol (Frazer & Sammons, 1945). This has been confirmed and amplified by Desnuelle, Naudet & Constantin (1950, 1951).

The extent of hydrolysis *in vivo* has been more difficult to assess. Experiments using suitably labelled molecules have been done by a number of workers during the last two years. All agree that hydrolysis of glycerides *in vivo* is not complete. Favarger, Collet & Cherbuliez (1951) showed that only a small proportion of deuterium-labelled glycerol was incorporated into glycerides recovered from the intestine of rats after feeding both glyceride and deuterated glycerol together. Favarger & Collet (1949) also demonstrated that deuterium-labelled palmitic acid or lauric acid was not incorporated into glycerides when fed with trielaidin to the extent that would have been expected if hydrolysis had been complete. They conclude that 90% of the fat absorbed in their experiments was absorbed as glycerides and that hydrolysis did not exceed 30%. Reiser, Dryson, Carr & Kuiken (1952) carried out experiments with ^{14}C-labelled glycerol which was incorporated into triglyceride, the fatty acids of which were principally conjugated linoleic acid. This labelled material was fed with a saturated unlabelled triglyceride, and the absorbed fatty material recovered from the chyle. The distribution of the labelled glycerol in this fatty material showed conclusively that complete hydrolysis had not occurred. They concluded that complete hydrolysis was not necessary for absorption and that the extent of hydrolysis was about 30–40%. The work of Bergström, Borgström, Carlsten & Rottenberg (1950) also supported the view that hydrolysis was incomplete. All this evidence shows that long-chain glycerides can be absorbed without complete hydrolysis, which is contrary to the most important of the fundamental concepts of the Pflüger-Verzár Hypothesis. If hydrolysis is only partial, there must be at least two forms of fatty material for absorption, water-soluble fatty acids and soaps and water-insoluble glycerides. Some of the implications of this partition of fatty material during absorption must now be considered.

Tributyrin was more rapidly and completely hydrolysed than triolein. This was demonstrated *in vitro* provided that the volume of the water phase was not unduly restricted, and *in vivo* by the use of ^{14}C-labelled glycerol incorporated into tributyrin and triolein (Karnovsky & Gidez, 1951). If tributyrin was fed, no fine emulsification was observed in the intestinal lumen, in contrast to triolein. The intestinal cells showed no stainable particles during tributyrin absorption, but were full of Sudanophil globules after triolein. Most of the triolein, but no tributyrin, could be recovered from the chyle (Davis, 1930). Absorbed long-chain fats were deposited in the fat depots, as shown by Schoenheimer & Rittenberg (1936) with labelled materials and by Frazer (1943 *a*) with stained fats. Tributyrin was not deposited in fat depots (Hughes & Wimmer, 1935)—an observation borne out by the extensive studies on the composition of fat depots by Hilditch (1947). Furthermore, double adrenalectomy depressed the absorption of triolein in the rat (Verzár & Laszt, 1935 ; Barnes, Miller & Burr, 1941 ; Frazer, 1948 *b*), but it did not affect the absorption of tributyrin (Bavetta & Deuel, 1942). It is clear, therefore, that there are striking differences between the absorption of triolein and tributyrin. The differences observed are compatible with the view that tributyrin is completely hydrolysed and absorbed entirely as water-soluble compounds, whereas triolein is not completely hydrolysed and its absorption is more closely related to the mechanism of absorption of the water-insoluble fat fraction.

One of our earliest observations, which began to raise some doubt about the occurrence of complete hydrolysis, was the finding that olive oil and oleic acid behaved differently during absorption. In these experiments no emulsification of the fatty acid was observed in the intestinal lumen, but the glyceride was finely emulsified ; the intestinal cells during glyceride and fatty acid absorption showed different staining characteristics ; the glyceride absorption was associated with milky lacteals, fat laden chyle, systematic hyperlipaemia and deposition in the fat depots, but during fatty acid absorption there was little change in the appearance of the chyle, no systemic hyperlipaemia and much less deposition in the depots. Definite increase of stained fatty material occurred in the liver with fatty acid feeding, but not after similar moderate doses of glycerides. This was a histological observation, however, and its quantitative significance is difficult to assess (Frazer, 1943 *a*). Under certain circumstances fatty acids may become finely emulsified in the intestinal lumen, and, if this occurs, there may be no demonstrable differences between fatty acid and glyceride absorption. This may be the explanation of the observations of Tidwell (1950).

In addition to these differences during absorption, differences in sequelae were also demonstrated. Thus, fatty acids, but not glycerides,

caused a marked delay in gastric emptying time ; differences could be demonstrated between glycerides and fatty acids with regard to inhibition of gastric secretion ; glycerides did not cause increased mucus secretion or the radiographic flocculation pattern in human subjects, which could be readily induced with small doses of their constituent fatty acids (Frazer, French & Thompson, 1949). Finally, if pancreatic lipolysis was completely inhibited by sodium cetyl sulphate, fat absorption was not significantly altered. The addition of extra lipase to ingested fat caused depression of the systemic hyperlipaemia and other changes which suggested that hydrolysis was not normally complete (Frazer, 1943 *b*). Clearly all these observations could not be reconciled with the view that every molecule of glyceride had to be broken down to its constituent fatty acids before absorption could occur.

Olive oil and oleic acid were used in these experiments, since they were easy to handle in controlled feeding studies, both being fluid at body temperature. From the beginning it was clear that absorption of oleic acid differed from the absorption of long-chain saturated fatty acids. Stearic acid and palmitic acid fed alone were poorly absorbed, whereas oleic acid absorption exceeded 90%. The addition of olive oil to stearic or palmitic acid increased their absorption to 90% or more. As a result of these and other experiments, we considered that the apparent selective rejection of long-chain fatty acids in the stools in the sprue syndrome, about which Dr French will speak later, might be due, at least in part, to delay and depression of glyceride absorption, with a consequent increase of fatty acid absorption. Due to the decreased absorption of carrier glyceride, the absorption of long-chain saturated fatty acids might thus become defective (Frazer, 1947). It would be anticipated on the basis of the Partition Hypothesis that long-chain saturated fatty acids fed in a suitable glyceride vehicle would be absorbed and recovered from the chyle. This has been shown to occur using ^{14}C-labelled stearic, palmitic and penta-decanoic acids (Bloom, Chaikoff, Reinhardt, Entenman & Dauben, 1950) ; Chaikoff, Bloom, Stevens, Reinhardt & Dauben, 1951 ; Bergström *et al.*, 1950 ; and Borgström, 1951). In some cases the observations have been quite erroneously interpreted by the authors as being contrary to the Partition Hypothesis. In more recent studies, Bloom, Chaikoff & Reinhardt (1951) have shown that ^{14}C-labelled fatty acids of shorter chain length than myristic acid do not pass so completely by the lymphatic route, only 15–55% of lauric acid and 5–15% of decanoic acid being recovered from the chyle. Since absorption had occurred, it was concluded by these authors that these shorter chain fatty acids passed into the body by the portal vein. These various observations abundantly confirm the general concepts of the Partition Hypothesis that hydrolysis is not complete, that more than one form of fat is presented for absorption, and that chain length, solubility

properties and other characteristics of the ingested fat may play an important part in determining the mechanism and route by which absorption will occur, and the fate of the absorbed material.

EMULSIFICATION

Fat is finely emulsified in the intestinal lumen to a particle size of less than $0{\cdot}5\mu$. The only system that has been shown to produce this fine emulsification under the conditions that prevail in the upper small intestine is the triple combination of fatty acid, lower glyceride and bile salt (Frazer, Schulman & Stewart, 1944). The fatty acids and lower glycerides are formed during pancreatic lipolysis (Frazer & Sammons, 1945). In pancreatic enzyme deficiency or biliary obstruction, lack of fine emulsification of ingested fat could be demonstrated by intubation. The addition of the appropriate missing factor re-established fine dispersion (Frazer, 1948*b*). If hydrolysis is partial, water-insoluble glycerides are presented for absorption and the possibility that fine emulsification may be an important step in the absorption of this fraction has to be considered.

Glycerides could be demonstrated in particulate form in the intestinal lumen, in the intestinal cells, in the chyle and in the systemic blood during absorption. If fine emulsification did not occur in the intestinal lumen, as in pancreatic enzyme deficiency, glyceride particles were no longer observed in the cells, in the chyle, or in the systemic blood. If a finely dispersed emulsion was introduced into the duodenum in such a case the normal systemic hyperlipaemia was re-established (Anderson, Frazer & French, 1952).

In long-term experiments Channon & Devine (1934) showed considerable absorption of hydrocarbons and this has been confirmed with labelled material (Stetten, 1943). Paraffins were not absorbed, however, when fed alone in acute experiments. This might be due to the fact that fatty acids and lower glycerides, essential for fine emulsification, were not formed. That failure of absorption was related to emulsification was further supported by the observation that finely dispersed emulsions of paraffin introduced into the duodenum could be shown to pass into the intestinal cells (Frazer, Schulman & Stewart, 1944). Particle size and charge were of critical importance. Lundbaek & Maaloe (1947), however, were unable to repeat this observation. Shoshkes, Geyer & Stare (1950) have shown that fine emulsification increased the absorption of paraffin by 23% as compared with an increase of 26% with corn oil similarly treated. In a recent series of experiments Daniel, Frazer, French & Sammons (1951) have shown that paraffin was freely absorbed if it was mixed with a sufficient quantity of carrier glyceride. Absorption was demonstrated in both short and long-term experiments and the absorbed paraffin was recovered from the chyle. Mead, Bennett,

Decker & Schoenberg (1951) have also shown the importance of carrier glyceride in the absorption of long chain-saturated fatty acid esters. Another series of studies were carried out on castor oil, which was poorly emulsified in the intestinal lumen. When fed alone little absorption occurred and the oil passed down into the lower bowel and caused catharsis. When fed with carrier glyceride, such as olive oil, free absorption, but no catharsis, was found (Frazer, French & Sammons, 1949). These observations indicate that fine emulsification is an important step in the absorption of the water-insoluble fat fraction. It may be that fine emulsification is the selective mechanism by which unhydrolysable materials are normally excluded from the intestinal cells. Partial hydrolysis provides necessary components for the emulsifying system and the finely dispersed glycerides may enter the fine canals in the outer border of the intestinal cell which have been described by Baker (1942). In a more recent publication, Baker (1951) has shown that these fine canals are filled with lipid-staining material during the absorption of fats, but not during the absorption of carbohydrates or proteins.

PHOSPHOLIPID FORMATION

There is no doubt that phospholipid is formed in the intestinal cell during fat absorption (Sinclair, 1936). It is also clear from this and subsequent work that absorbed fatty material becomes incorporated into these phospholipids. It was claimed that this phospholipid was an essential step in triglyceride resynthesis. Using radioactive ^{32}P, Zilversmit, Entenman & Chaikoff (1948) showed that the turnover of phospholipid in the intestine during fat absorption was so small, however, that phospholipid could not be an obligatory intermediate.

The adrenal cortex was thought to control the phosphorylating process in the intestinal cell, since double adrenalectomy caused faulty fat absorption in the rat (Verzár & Laszt, 1935). It was shown by Barnes, Miller & Burr (1941), however, that fat absorption greatly improved in adrenalectomized rats on adequate salt therapy. Later, Stillman, Entenman, Anderson & Chaikoff (1942) showed that phospholipid turnover in the intestine was not altered by double adrenalectomy. Particulate absorption of long-chain fats, but not the absorption of fatty acids or tributyrin, was depressed after double adrenalectomy. This effect may be due to concomitant changes in water and electrolyte absorption, rather than any more specific function of the adrenal cortex in the fat absorption mechanism.

The depressant effect of mono-iodoacetic acid intoxication on fat absorption was also said to be due to interference with phosphorylation (Verzár & Laszt, 1934). Schmidt-Nielsen (1946), however, could not demonstrate any change in phospholipid turnover in the intestine under these conditions. The alteration in fat absorption in mono-

iodoacetic acid intoxication was shown to be due to alterations in gastric and intestinal motility and not to interference with the cellular mechanism of absorption (Frazer, 1948 *b*).

Thus, much of the evidence which was thought to link phospholipid formation and fat absorption has not been substantiated. It can be shown, however, that the addition of glycerophosphate, lecithin, or choline to ingested fat causes a significant increase in the rate of fat absorption (Verzár & McDougall, 1936 ; Augur, Rollman & Deuel, 1947 ; Frazer, 1946 *b*, 1951 ; Tidwell, 1951). Whether this effect of these various substances is due to the same or related causes is unknown. At present it seems reasonable to conclude that the available evidence does not support the view that phospholipid is an essential intermediate, or that the adrenal cortex plays any specific part in fat absorption. Whether phospholipid formation may facilitate the passage of fat through the intestinal cell in some way must be left open.

SUMMARY OF THE MECHANISM OF FAT ABSORPTION

Intraluminar Phase. In the intestinal lumen the hydrolysis of long-chain fats is normally neither rapid nor complete. Short-chain fats may be more completely hydrolysed. Lower glycerides and fatty acids are formed, which, with bile salts, bring about the fine emulsification of the glyceride residue. This particulate fatty material may enter the fine canals in the outer border of the intestinal cells. Fatty acids that can be removed from the oil into the water phase may be absorbed in a state of molecular dispersion.

Cellular Phase. Fatty material can be demonstrated in the fine canals in the outer border of the intestinal cells. The fat passes into the cell, where considerable modification and resynthesis of triglycerides may occur. Phospholipid is formed, but it is not an obligatory intermediate, although it may facilitate the passage of fat through the cell.

Distributive Phase. Negatively charged particles do not pass through the blood capillary membrane from without inwards, but they can pass readily into lymphatics. Finely dispersed negatively charged particles of fat leaving the intestinal cells thus find their way into the lacteals and the chyle, and not into the portal blood. Under normal circumstances, therefore, most of the absorbed fatty material passes into the body by the lymphatic pathway. Short chain fatty acids, however, are not found in the chyle. They may be used for synthesis of other substances or metabolized, or they may pass into the body in the portal blood. Under the abnormal conditions of fatty acid ingestion or administration, or excessive hydrolysis, other distributional effects may be observed which may have little relevance to the normal fat absorption mechanism. In the light of the experimental evidence of the last thirteen years, it must be concluded that the Pflüger-Verzár Hypothesis is no longer tenable. The Partition

Hypothesis appears to be compatible with the main facts at present available.

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