LIPID METABOLISM

BIOCHEMICAL SOCIETY SYMPOSIUM

A SYMPOSIUM on lipid metabolism arranged by the Biochemical Society was held on February 16 in the London School of Hygiene and Tropical Medicine. A large audience of members and visitors at both the morning and the afternoon sessions showed great interest in the subjects discussed; these were concerned largely with the absorption of dietary fat by animals and the synthesis of fat in the glands of lactating animals.

It had originally been intended that the late Prof. H. S. Raper should preside at the morning session. In consequence of his death last December, Dr. H. J. Channon took the chair; he opened his remarks with a personal tribute to Prof. Raper's worth as a friend and as a biochemist. Prof. Raper had prepared, before his death, an introduction to the symposium which was given to the meeting by Dr. Channon, and which took the form of a historical survey of fat metabolism, absorption and oxidative breakdown. Prof. Raper had expressed a marked preference for the term 'fat' instead of 'lipid', a point which seems appropriate when the metabolism of the long-chain compounds present in glycerides, phosphatides or cerebrosides is under discussion: the term 'lipid' as currently employed covers other materials (sterols, carotenoids, vitamins) which are soluble in fats or in fat-solvents, but which have no chemical relationship to the long-chain compounds which are the basis of true fats. After remarking that usually the living cell does not employ the synthetical methods of the organic chemist, Prof. Raper had commented on the renewed and accelerated interest of the past few years in the synthesis and metabolism of fats in living organisms. The ideas of fifty years ago of formation of fat from carbohydrate through an intermediate C_2 unit had received confirmation and clarification from the studies of Folley, Popjak and others. The older view that fat absorption proceeded by hydrolysis and resynthesis of glycerides in the intestinal mucosa had been modified since about 1939 in the light of the work of Frazer and his school. Since Knoop, followed by Dakin, had established in the early years of the century the occurrence of β-oxidation in the oxidative breakdown of fats *in vivo*, much work had revealed the complexities of the action: difficulty in tracing all the intermediate stages seemed to be due to inability to isolate the different enzymes specifically concerned. Prof. Raper had said he would have welcomed discussion of at least two topics not mentioned in the agenda, namely, the formation, location and function of adipose tissues in animals, and the fat metabolism of germinating seeds, of which little is known and a great deal awaits more complete explanation.

In the first communication to the symposium, Prof. A. C. Frazer gave a general review of his studies and conclusions on the particulate nature of fat absorption via the chyle and lymphatic system. In contrast to the older hypotheses of Pflüger and Verzär, which postulated complete hydrolysis and resynthesis of ingested glycerides in the small intestine, Frazer considers that three stages are involved in fat absorption: a partial hydrolysis of fat (some of which proceeds to fatty acids and glycerol, although this process is stepwise through the stages of di- and mono-glycerides, some of which remain present as such), emulsification of unhydrolysed fat (promoted not only by bile acids or fatty acid soaps but also largely by the partly hydrolysed monoglycerides), and absorption of the emulsion of dispersed glyceride particles through the intestinal wall into the lymphatic system. The free fatty acids or their soaps, on the other hand, pass by the portal route. Ease of complete fat hydrolysis depends on the nature of the fatty acids present—glycerides of short-chain acids (tributyrin) are more rapidly and more completely hydrolysed than those of long-chain acids (triolein). In discussion with Dr. G. D. Popjak, Prof. Frazer stated that short-chain acids (for example, butyric) are largely destroyed by oxidation for energy purposes, whereas free long-chain acids (for example, oleic) tend to accumulate in the liver. Prof. S. Bergström referred to his own work and that of others which has shown that 'labelled' fatty acids fed by stomach tube to rats appear in their depot fats as glycerides, and Prof. Frazer stressed the very great metabolic activity of the gut, for example, in ester-synthesis as well as hydrolysis, so that the experimental acids may well have been esterified to glycerides before passage through the chyle and eventually into the fat depots.

Dr. J. M. French, a colleague of Prof. Frazer, described work on conditions associated with defective fat absorption in human subjects, such as deficiency in pancreatic enzyme (which appears to inhibit the partial hydrolysis of fat necessary and antecedent to emulsification of neutral fat) or the disease termed sprue in which glyceride absorption is delayed, fatty acid production is abnormally large and leads to increased mucus secretion, and abnormally large proportions of fat are excreted. He directed attention to the difficulties of determining whether fæcal fat is derived from dietary fat, fat normally synthesized by the animal, or fat synthesized in the gut by intestinal microflora.

Dr. R. P. Cook had meanwhile given a general account of the lipid components of fæces, in comparison with the level of absorption of ingested fats of different kinds by various animal species. He suggested that one of the functions of the lipids in the intestine, as well as the skin, is to act as a conditioning agent. Attention was directed to the excretion of lower fatty acids (acetic to hexoic, largely butyric), which is marked in the dog but less so in rats or human subjects. An interesting feature of Dr. Cook's observations related to the fate of cholesterol and other steroids in animals, an issue which was commented upon in discussion by Prof. R. A. Morton, who inquired to what extent particulate absorption of carotenoids or sterols present in solution in glyceride fat goes on, a matter of some importance in connexion with suggested relations of cholesterol to arteriosclerosis and of stigmasterol as an anti-syphilitic factor. The chairman (Dr. Channon) suggested that, since the absorption of dietary glycerides had been the subject of study for many years prior to the progress latterly associated with Prof. Frazer's name, it might require some considerable further time before the even more difficult picture of sterol absorption became clarified.

In the afternoon session (with Prof. T. P. Hilditch in the chair) Dr. Popjak summed up his recent work

on the synthesis of lipids from acetate and from glucose containing radioactive carbon. From acetate in which the carboxyl carbon was the isotopic carbon-14, the lactating mammary gland builds up saturated fatty acids in which alternate carbon atoms are radioactive, showing that the saturated acid chains are formed by stepwise lengthening from a shorter acid at the carboxyl end by addition of an acetate group. Glucose had been shown to be as good a source as acetate for the production of both fatty acids and cholesterol, and to be readily broken down (in experiments on rabbits) to acetate. Probably the conversion of glucose to fat involves breakdown of the former to pyruvate, which is decarboxylated to acetate (acetaldehyde). In reply to a question, Dr. Popjak afterwards added that the experiments showed that 30–70 per cent of the lower acids in milk fats were synthesized from acetate (or from glucose via acetate) in the mammary gland; in an experiment with lactating rabbits, 30–40 per cent of the short-chain acids in the milk fat were derived from carbon-14 glucose which had been administered.

Dr. S. J. Folley discussed the synthesis of milk fats by ruminants, in the blood of which significant amounts of acetic acid are present. Experiments with isotopic acetic acid ($CH_3.^{14}COOH$) have shown conclusively that acetate is a major source of carbon for fatty-acid formation in the udder, and that the milk short-chain acids arise not by degradation of oleoglycerides (as postulated by Hilditch) but are intermediates in the formation of long-chain acids by stepwise chain elongation. Dr. Folley summarized in equal degree the evidence based on the pattern of glyceride structure in milk fats (which strongly suggests close relationship between these, other body fats and blood glycerides) and concluded with the suggestion that milk fat may have a dual origin—part synthesized in the udder from small molecules, part entering the gland from the blood (and perhaps there undergoing partial chain-shortening). This contribution provoked a vigorous discussion. Prof. Hilditch pointed to a possible reconciliation of the apparently divergent views as to ruminant milk fat formation. From the glyceride structure angle it seems to follow that preformed oleo-glycerides are transformed into short-chain glycerides. This, however, does not necessarily involve chemical degradation of the oleo-group; the same glyceride pattern would result if oleo-groups were *replaced* by butyro-, etc., groups by the process of acyl interchange. The latter process is well known to take place readily *in vitro* and may well proceed in the living tissue—indeed, the selective character of mixed glyceride configuration in plants and animals strongly suggests its operation. Dr. Folley welcomed the suggestion, since it appeared to be in harmony with both sides of the experimental facts which had to be taken into account in a satisfactory explanation. The specific presence in ruminant and some other milk fats of traces of 9-decenoic and 9-dodecenoic acid (apparently derived from oleo-glycerides by degradation) is nevertheless not explained by the acyl interchange hypothesis.

Some discussion also took place as to whether evidence based on ruminant fats is necessarily applicable to all animal fats. While Dr. Popják inclined to the view that the mechanism of milk-fat synthesis is probably basically similar to that of all other fats in the animal, Prof. H. D. Kay remarked upon the essential differences in the blood of ruminants and of other animals: that of the latter contains very little acetic acid, whereas ruminant blood contains as much as 12 mgm. per cent of acetic acid but is lower in glucose content. The conditions of blood supply are therefore quite different. Prof. Hilditch felt that, in view of the many animal species whose milk fats are almost identical with their body fats, ruminants should still be considered a special case, probably differing from the majority of species.

Time had become very short for the reception of the final two communications, which were of the same high order of interest as those in the preceding part of the symposium. Dr. A. L. Lehninger (University of Chicago) gave a most valuable summary of recent work on the enzymatic oxidation of *n*-octanoic acid. The isotope experiments of Weinhouse and his associates indicated that acetoacetate is produced in the oxidation by an essentially random condensation of acetyl residues. Recent refinements of the procedure suggest that more than one molecular species of active acetyl may be formed by β-oxidation of the original acid chain, and that these may differ in ability to serve as precursors of the 'head' and 'tail' of the acetoacetate molecule. Isolated rat liver mitochondria (the only active constituent of rat liver), when used as the oxidase system for saturated fatty acids, require for activity magnesium ions, orthophosphate, adenosine triphosphate, an optimum concentration of electrolytes or of certain non-electrolytes such as sucrose, and an oxidizable 'priming' metabolite, which may be a Krebs citric acid cycle intermediate. The presence of the latter is, however, not essential, for reduced cozymase activates the oxidation equally well. The fatty-acid oxidase system attacks oleic and other natural unsaturated acids and also their *trans*-(elaidic) forms, but requires the presence of a free carboxyl group—long-chain alcohols, aldehydes, amides, etc., are not oxidized. The recent work of Barker and his colleagues on fatty acid synthesis and oxidation in cell-free extracts of *Clostridium kluyveri* indicates the utility of this organism for oxidative studies; a detailed mechanism which accounts for all the recent findings in connexion with conversion of butyric acid to acetoacetate by this oxidase system, and involving the presumption of an intermediate complex of coenzyme-*A* with butyric acid, has been worked out recently by Kennedy and Barker.

Dr. H. M. Sinclair made some important additions to knowledge of the 'essential' fatty acids (linoleic, etc.) especially in regard to their relation with pyridoxine. He showed that the pathological condition in the skin of rats associated with deficiency of linoleic or other essential fatty acid is almost identical with that found in deficiency of pyridoxine; deficiency of either nutrient produces the effect in young rats, but more apparently if both nutrients are deficient. He suggested, as a working hypothesis, that there is failure of growth in fatty-acid deficiency because phosphatides and therefore cell membranes cannot be formed, whereas in pyridoxine deficiency the cause is interference with protein metabolism. Further, in fatty-acid deficiency histamine is liberated from cells more readily than normally, whereas in pyridoxine deficiency there is deficiency of histaminase and therefore inadequate destruction of histamine. He made the further observation that deficiency of essential fatty acids is not fatal to adult rats, although their growth ceases after a certain point, and that the water retention of the tissues may be controlled to a large extent by the presence of these highly unsaturated acids.

In summing-up the proceedings, Prof. Hilditch directed attention to the degree to which, during the day, attention had been focused on the metabolism of *saturated* acids, especially perhaps butyric, and emphasized that the probably different metabolism of *unsaturated* acids (mainly oleic and linoleic), which are by far the most abundant in Nature, merits much more specific attention than it has yet received from biochemists and physiologists.

It will be seen that the symposium covered a wide field. It was one of the most interesting yet held by the Biochemical Society, as may be gathered from the fact that, although it lasted an hour beyond its scheduled time, almost all the large audience remained until it terminated.

OVERALL DENTAL DIMENSIONS OF HOMINOIDS

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OUR attention has recently been directed to the fact that there was a systematic error in the estimates we have provided of the standard deviations of the distribution of individual dimensions of the teeth of apes[1], all our figures being approximately $\sqrt{2}$ times the actual values[2]. The standard errors of the means are correct as published. In the interval since the publication of these earlier observations, we have considerably extended our basic data; but it is nevertheless desirable that we amend certain comparisons[3] between the overall dental dimensions of various fossil hominoids and those of existing apes and men that were based on our incorrect estimates.

The error in these estimates is $\sqrt{2}$ only when the two members of every pair of teeth measured were present in the same skull. It is smaller when some skulls contained only one of each pair of teeth measured. In order to reduce the additional computation involved, and because we shall later be publishing new comparisons based on our larger series of observations and a wider range of statistical techniques, we have employed the factor $\sqrt{2}$ to correct all our previous estimates of the standard deviation of the dimensions of the ape teeth. In the case of the human material, a much larger percentage of skulls contributed only one member of certain pairs of teeth, and the precise correction factors were therefore used. We have in consequence slightly biased our revised comparisons with the ape teeth, in so far as we may now be indicating a few more significant differences between the fossil teeth and those of living apes than actually exist. A random check, using precise correction factors throughout, indicates that the new figures given in the accompanying table are an adequate summary of the results to which comparisons based on our previously published measurements should have led. The figures shown in brackets are the incorrect figures given in our earlier paper[3]. Apart from comparisons with the fossil hominid *Pithecanthropus* (= *Sinanthropus pekinensis*), no new data have been brought into the revised comparisons. The table shows the number of fossil teeth which differ in one or more (overall) dimensions or indices from both the male

Overall Dimensional Correspondences between Hominoid Teeth

"M.D." refers to deciduous teeth; otherwise the permanent dentition is implied

Fossil species	No. of teeth compared	No. of teeth which differ in one or more dimensions from the: Chimpanzee	Gorilla	Orang-utan	Ancient Egyptian	Australian	*Pithecanthropus*	No. of teeth compared	No. of teeth which differ in one or more indices from the: Chimpanzee	Gorilla	Orang-utan	Ancient Egyptian	Australian	*Pithecanthropus*	No. of teeth compared	No. of teeth which differ in one or more indices or dimensions from the: Chimpanzee	Gorilla	Orang-utan	Ancient Egyptian	Australian	*Pithecanthropus*
Australopithecus africanus	2	(2) 2	(0) 0	(0) 0	(2) 2	(2) 2	0	2	(0) 0	(0) 1	(0) 0	(0) 0	(0) 0	0	2	(2) 2	(0) 1	(0) 0	(2) 2	(2) 2	0
Australopithecus africanus M.D.	6	(4) 5	(3) 6	(1) 2	—	—	—	5	(1) 4	(3) 4	(2) 3	—	—	—	5	(4) 5	(3) 5	(2) 4	—	—	—
Australopithecus prometheus	8	(6) 8	(0) 2	(0) 0	(8) 8	(6) 6	4	4	(0) 0	(0) 0	(0) 0	(0) 0	(0) 0	0	4	(2) 4	(0) 2	(0) 0	(4) 4	(2) 2	1
Australopithecus prometheus M.D.	2	(2) 2	(0) 0	(0) 0	—	—	—	—	— —	— —	— —	—	—	—	—	— —	— —	— —	—	—	—
Plesianthropus transvaalensis	21	(16) 19	(2) 5	(0) 3	(20) 20	(14) 16	14	18	(0) 0	(0) 2	(0) 0	(4) 4	(3) 4	3	18	(15) 16	(2) 5	(0) 2	(18) 18	(13) 15	12
Paranthropus robustus	10	(10) 10	(1) 1	(0) 1	(10) 10	(9) 10	7	10	(0) 1	(0) 3	(0) 0	(0) 0	(0) 2	0	10	(10) 10	(1) 4	(0) 1	(10) 10	(9) 10	7
Paranthropus robustus M.D.	4	(2) 3	(1) 1	(1) 2	—	—	—	3	(0) 1	(2) 2	(1) 1	—	—	—	3	(2) 3	(2) 2	(1) 1	—	—	—
Proconsul africanus	55	(5) 12	(37) 46	(23) 38	(33) 36	(22) 35	25	23	(3) 6	(1) 6	(3) 4	(11) 16	(9) 13	13	23	(4) 8	(16) 21	(11) 16	(18) 19	(11) 15	17
Meganthropus palæojavanicus	3	(3) 3	(1) 1	(0) 0	(3) 3	(3) 3	2	2	(0) 0	(0) 0	(0) 0	(0) 0	(0) 0	0	2	(2) 2	(1) 1	(0) 0	(2) 2	(2) 2	2
Gigantopithecus blacki	3	(3) 3	(2) 3	(3) 3	(3) 3	(3) 3	3	2	(2) 2	(0) 1	(0) 1	(2) 2	(2) 2	2	2	(2) 2	(2) 2	(2) 2	(2) 2	(2) 2	2
Eoanthropus dawsoni	2	(0) 2	(1) 2	(0) 0	(2) 2	(0) 1	0	2	(0) 0	(0) 0	(0) 0	(2) 2	(2) 2	0	2	(0) 2	(1) 2	(0) 0	(2) 2	(2) 2	0