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SERUM LIPIDS IN DIFFERENT PHASES OF CARBOHYDRATE METABOLISM‡

If the reader would integrate the intermediary metabolism of lipids with that of carbohydrates let him read, albeit in reverse direction, Dr. Peters' chapters on lipids and carbohydrate in Grollman's text,[62] the article on "The interrelations of foodstuffs in metabolism" in the 1951 Yale Journal of Biology and Medicine,[61] the chapter on lipids in the 1946 edition of *Quantitative clinical chemistry* by Peters and Van Slyke,[65] the 1941 Yale Journal article "A new frame for metabolism,"[60] and the chapter on lipoids in the 1931 edition of *Quantitative clinical chemistry.*[64] Certain landmarks, which Dr. Peters recognized before the era of isotope experiments, stand out: that fatty acids could be synthesized from carbohydrate or protein foodstuffs except for the essential, unsaturated fatty acids, that cholesterol could be synthesized endogenously and followed different routes in intermediary metabolism than triglycerides or phospholipids, that fat in adipose tissue was an invaluable source of fuel for energy metabolism.

The above source articles written throughout a span of 25 years give a forceful picture of interlocking relationships between the rôles of carbohydrate and fat in the physiological balance of storage and expenditure of bodily energy. From the stimulus of the above articles stemmed investigations on lipid fractions during recovery from diabetic acidosis and measurements of alimentary lipemia after a breakfast containing carbohydrate, proteins, and fat or after the same breakfast with supplementary ingestion of carbohydrate.

Diabetic acidosis affords a unique opportunity in which to examine rapid and extensive changes in serum lipid concentrations in man. Fatty acids of triglycerides increase excessively, sometimes to tenfold the normal maxi-

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‡ Editorial limitations have necessitated omission from the bibliography of many significant contributions. By inclusion of recent and review papers with extensive bibliographies appreciative recognition is extended to those investigators whose publications could not be cited individually.

The complete list of Dr. Peters' publications, which is in this issue, contains his articles on lipid metabolism which are not included in this bibliography.

mum, but during the first 24 hours of active treatment decrease to concentrations within or approaching the normal range.[5, 35, 55, 56, 65] Occasionally, serum cholesterol initially is as much as twice or more the concentration subsequently found to be characteristic for the individual. Phospholipids rise more than cholesterol but far less than triglycerides, if compared in a percentage relationship to their respective concentrations following convalescence. When the carbohydrate starvation and acidosis are clinically significant, the elevation of these three lipid fractions is far in excess of the degree of dehydration evaluated from changes in hematocrit or serum proteins.[55] The pattern of lipids given above has been found in usual instances of diabetic acidosis but hyperlipemias of extraordinary magnitude were observed in sporadic patients.[5] Characteristic abnormalities of the elevation of serum lipids in diabetic acidosis are the capricious distortion of interrelations in the lipid pattern and the load of triglycerides carried in the plasma.

Serum triglycerides of fatty acids thus attracted the focus of attention for the specific condition of diabetic acidosis in which carbohydrate metabolism is inseparable from that of lipids. In serum and in tissues the fraction of lipids which will be discussed involves these triglyceride fatty acids, calculated as the fatty acids not esterified with cholesterol or in phospholipids.[63, 65] In some of our earlier papers this triglyceride fraction was called "neutral fat" or "free fat." The latter name now is applied to that fraction of fatty acids, possibly an extremely active component in lipid turnover, which is unesterified in serum or plasma but seems to be bound to albumin and which by our calculations would be included in triglycerides.[12, 19, 29, 31, 68] By utilization of the term triglycerides the Committee on Lipid and Lipoprotein Nomenclature of the American Society for the Study of Arteriosclerosis has made no attempt to distinguish the nonesterified fatty acids or fatty acids of di- or monoglycerides from triglycerides.[16]

Triglyceride fatty acids represent the major component of fat in adipose tissue. Fat of adipose tissue is stored in pure form yielding about nine calories per gram in contrast to the energy potentials of carbohydrate or protein which, because they are held in aqueous deposits, yield only about one calory per gram of tissue.[61] Butter and the fat mechanically inseparable from the protein of meat are composed chiefly of triglycerides. Uncooked beef, veal, and lamb roasts, in addition to about 60 per cent of water, contain approximately equal per cents by weight of protein and fat. Thus for uncooked roast beef only 25 per cent of its caloric value is supplied by protein while 75 per cent would be derived from fat. This elementary fact is

often forgotten when a diet high in animal protein is needed to alleviate protein deficiency of a patient.

The vital rôle of triglycerides in meeting energy requirements of carnivorous animals has been emphasized because the size of this lipid fraction during transport in plasma would not indicate the magnitude of the rôle which the triglycerides exercise in total metabolism. An extremely rapid turnover of triglyceride fatty acids compared to the slower exchange of phospholipid and cholesterol has been demonstrated by isotope studies.[13, 34, 49, 66] The present concept is that triglyceride fatty acids are the form in which fat is available for fuel.

In Figure 1 the minimum, mean, and maximum fatty acids of triglycerides, phospholipids, and cholesterol esters in the sera of 100 normal men and women in the postabsorptive state are presented.[68] In some normal sera the triglyceride fatty acids were too low to be measured and accounted for only one-third, 3.1 to 6.1 mEq./L, of the total fatty acids for the sera of mean or maximum normal lipid content. Conversion of the maximum concentration to mM. gives the surprisingly small quantity of 2 mM./per L; if all of the fatty acid happened to be palmitic, the weight of palmitate would be only 161 mg. per 100 ml. of serum. As is obvious from the figure, the major compounds of fatty acids in sera are those combined in phospholipids and with cholesterol. In a given individual slight changes throughout the day or from day to day in the cholesterol and phospholipid fractions were exceeded by the variability in triglycerides. This variability in serum triglycerides in a given individual and the excessive elevation of triglycerides in the carbohydrate starvation of diabetic acidosis fit into the pattern of interrelationships of carbohydrate and lipid metabolism.

INTERMEDIARY METABOLISM OF LIPIDS

The biochemical reactions by which fat is metabolized have been reported with such extensive bibliographies that the following references are included only as a key to enter the literature on these subjects—12, 17, 25, 41, 51, 52, 53, 57, 58, 72, 74, 75, 78, 80, 86, 92. Acetyl Co-A is a necessary intermediate in the synthesis and degradation of fatty acids: any substance capable of forming acetyl Co-A can therefore serve as a substrate for fatty acid synthesis. Ability to synthesize fatty acids has now been demonstrated in many tissues including liver, mammary gland, intestinal mucosa, lung, heart, spleen, testis, and adipose tissues.[58] Synthesis and degradation of fatty acids from and to the two carbon acetate fragments occur through the activities of coenzyme A, and the energy-rich reactions of phosphorylation

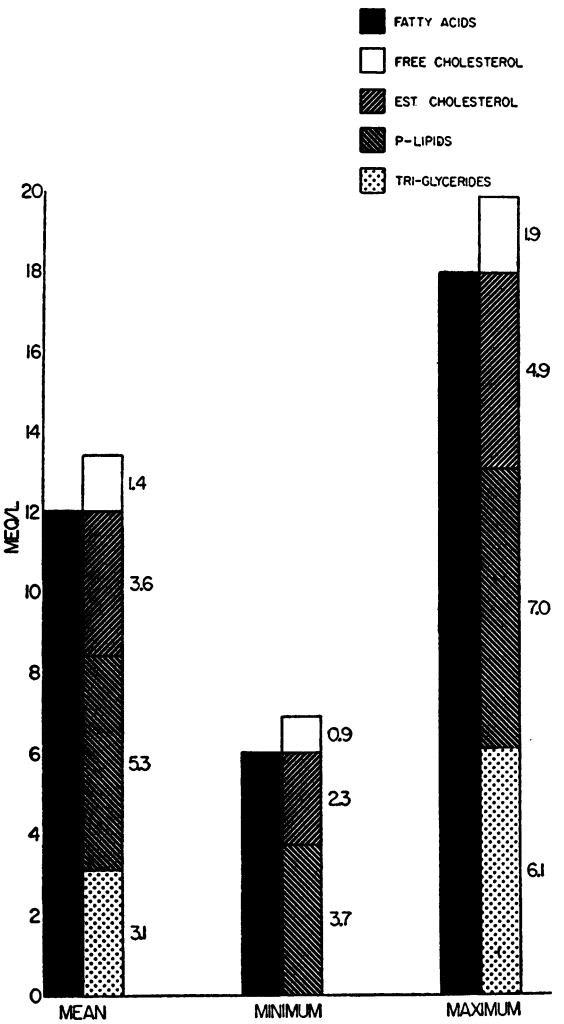

FIG. 1. Distribution of total fatty acids in serum lipid fractions. Cholesterol and lipid phosphorus are represented by the number of mEq./L of fatty acids with which they combine. Minimum and maximum total cholesterol (molecular wt. is 386) would be equivalent to the sums of the free and esterified cholesterol (2.3 + 0.9 mEq.) (386) (100 ml./1000 ml.) and (4.9 + 1.9 mEq.) (386) (100 ml./1000 ml.) or 123 to 262 mg. per cent of serum cholesterol.

Serum phospholipid composition is still being investigated but here 20 per cent of phospholipids is assumed to occur in sphingomyelin carrying 1 mEq. of fatty acids and 80 per cent in phospholipids carrying 2 mEq. of fatty acids per mM. of phosphorus. Phospholipid fatty acids would be calculated

$$\frac{(0.80 \times 2 + 0.20) \text{ lipid P in mg. per liter}}{31 \text{ (wt. of millimole of P)}}$$

Minimum and maximum mEq. of phospholipid fatty acids correspond with 6.4 to 12.0 mg. per cent of lipid phosphorus.

which interplay in energy exchange with the Krebs' cycle, Figure 2.* In the oxidation of fatty acids two mechanisms seem to predominate: either fatty acids are transported to the periphery for complete liberation of energy by degradation into two carbon fragments which are burned via the citric acid cycle, or in the liver fatty acids are broken down by activated coenzyme A into coenzyme A for re-utilization and into products yielding ketones.

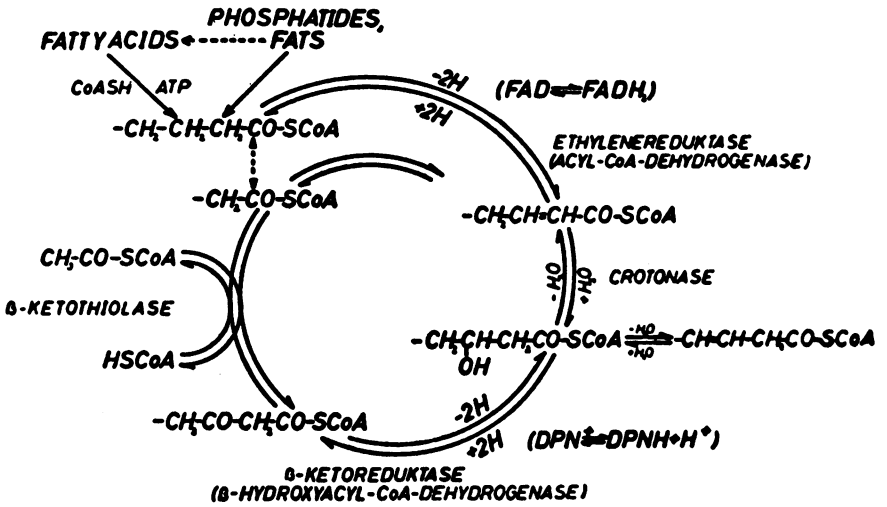

FIG. 2. Fatty acid cycle. Lynen has drawn a simplified diagram for the rôle of CoA in the metabolism of fat. This depicts the "(a) condensation of two molecules of acetyl-CoA to form acetoacetyl CoA and CoASH; (b) reduction of acetoacetyl CoA to B hydroxybutyryl CoA; (c) dehydration of B hydroxybutyryl CoA to crotonyl CoA; and (d) reduction of the crotonyl CoA to butyryl CoA." In this figure DPN (diphosphopyridine nucleotide) and FAD (flavin adenine dinucleotide) are included at steps of hydrogen exchange.[52, 58]

The latter after transfer to the periphery are burned by steps of the citric acid cycle. In tissues but not in liver "transferase" enzymes permit the utilization for energy of compounds, such as acetoacetate, which have been elaborated by the liver.[78]

Much published evidence, for which only a few articles are cited here, has confirmed not only the direct utilization for fuel of ketones but of fatty acids by tissues including heart and skeletal muscle, although the exact mechanism of transfer of fatty acids into muscle cells is not defined too pre-

* Since submission of this paper nomenclature for enzymes of fatty-acid metabolism has been suggested in the following reference: Beinert, H., Green, D. E., Hele, Priscilla, Hoffman-Ostenhof, O., Lynen, F., Ochoa, S., Popják, G., and Ruyssen: Nomenclature of enzymes of fatty-acid metabolism. Science, 1956, *124*, 614-616.

cisely.[28, 33, 76, 84, 87, 88] The major rôle which fat can assume in energy-producing reactions of the whole organism is indicated by the small percentage of oxygen utilized for combustion of glucose by fasting human subjects. Andres, Cader, and Zierler[7] studied the utilization of glucose by forearm muscle of postabsorptive human subjects and found that combustion of glucose accounted for only 7 per cent of the oxygen uptake, the remainder presumably being used for combustion of lipid and protein.

METABOLISM OF FAT IN RELATION TO THAT OF CARBOHYDRATE

The preponderance of metabolism of carbohydrate or fat is governed by the availability of these substances for fuel. Thus on a mixed diet, carbohydrate is liberally ingested during the day and burned as fuel. Excess carbohydrate is converted to glycogen and to fat for storage in the depots. During the overnight fast, fat is mobilized from the depots and by morning constitutes 60 per cent or more of the metabolic mixture.[61] From 8:00 a.m. to 10:00 p.m. in nonfasting individuals 60 grams of glucose are used by body muscle, compared to 30 grams used from 10:00 p.m. to 8:00 a.m.[26] As carbohydrate is further depleted with continued fasting, oxidation of both fatty acids and ketones increases to compensate for the energy requirements inadequately satisfied by carbohydrates. Separate reactions do not occur in a series. A continuously changing power supply involves carbohydrate ingestion, preferential combustion of carbohydrate and synthesis of fat during carbohydrate surplus; but when excess carbohydrate is spent, the newly formed fatty acids are immediately recalled for oxidation. Since the resultant RQ of these simultaneous reactions might be one, the RQ alone does not reveal the metabolic activity of fat in energy metabolism.[62] In a sense fat is the chief form in which fuel is burned by the body.

The velocity of carbohydrate combustion governs the *net* exchange of fat toward synthesis or combustion although certain adaptive phenomena and species differences alter the metabolic pathways for fat. Fat synthesis cannot generally occur without exogenous carbohydrate, but fat combustion in the absence of carbohydrate proceeds freely albeit with ketosis. This phenomenon was recognized by observation of severely diabetic, emaciated patients before the insulin era. That the nondiabetic animal could liberally synthesize fatty acids, with the exception of the essential, unsaturated compounds, was established by numerous investigations on the amount and composition of body fat of animals fed on "fat-free" diets or food of minimal fat content.[41, 64, 65] The extent of this phenomenon was revealed from experiments in which rats were fed on a carbohydrate diet containing glucose labelled with deuterium. Schoenheimer[70] demonstrated the dynamic, simul-

taneous breakdown and synthesis of fatty acids from the speed with which the label from glucose appeared in the fatty acids.

Following these early experiments, abundant evidence has accumulated that the synthesis of fat is dependent on the combustion of carbohydrate.[15] Haugaard and Stadie[37] found that the ability of liver to synthesize fatty acids from acetate was directly related to its glycogen content, and suggested that glucose not only provided the 2-carbon fragments, but also the energy for fatty acid synthesis. Since the rate of fatty acid synthesis varies with the supply of glycogen, and since liver tissue has a relatively high content of glycogen, investigations on liver tissue of the utilization of substrates between glycolysis and fat synthesis are complicated by the amounts of glycogen in the tissue. On the other hand, mammary tissue of lactating animals, which stores little glycogen, affords an excellent tissue to study the utilization for fat synthesis of such intermediates as glucose, fructose, pyruvate, or acetate.[39, 52, 67, 72, 86] Ruminants are less dependent than nonruminants on glucose for fat synthesis by mammary gland.[9] In this tissue the primary mechanism for fat synthesis depends on condensation of two carbon units rather than on elongation of the fatty acid chain. Glucose favors formation of triglycerides from the sodium salts of specific acids such as octanoic.[40]

While fatty acid synthesis requires the presence of glucose or products of the glycolytic cycle, these are not essential for fatty acid oxidation. The net exchange of fat in the direction of combustion is not facilitated but rather decreases when glucose or glycolytic products are available.[27, 88] Lossow and Chaikoff[50] followed the fate of labeled fatty acids injected as emulsions of triglycerides or as free fatty acids in rats with and without prefeeding with carbohydrate. The oxidation of all fatty acids of the even series from octanoic to palmitic was spared by carbohydrate, and the longer the chain, the more pronounced was the sparing. The intact labeled fatty acids were identified both in liver and in the whole carcass. Previous high carbohydrate diets, used in these experiments, may have intensified this effect.

The foregoing experiments demonstrate clearly that the active metabolism of glucose stimulates fatty acid synthesis and displaces fatty acid oxidation to a subsidiary position, while in the absence of carbohydrate fatty acid oxidation proceeds at an increased efficiency to meet the caloric demands and fatty acid synthesis fades into inactivity. It follows that all factors influencing carbohydrate metabolism might be expected to have an effect on fat metabolism opposite to their effect on carbohydrates. The effect of hormones in altering fat metabolism appears to be inseparable from their action on carbohydrate. It has been repeatedly demonstrated that insulin stimulates the formation of fat from glucose. The initial belief that insulin was necessary for a specific stage of fatty acid synthesis is not entirely dis-

proved[14] but has been replaced by the hypothesis that the action of insulin is secondary to its stimulating effect on carbohydrate metabolism.[75]

The inhibition of carbohydrate metabolism by the hormones of the anterior pituitary and the adrenal cortex is associated with decreased fatty acid synthesis and increased fat combustion.[14] In rat mammary slices the addition of cortisone *in vitro* inhibited lipogenesis.[8] Growth hormone has been reported to spare carbohydrate and protein *in vivo* by accelerating catabolism of fat provided dietary or carcass fat is available (Gurin in Najjar[58]). Allen, Medes, and Weinhouse[6] could not demonstrate an *in vitro* effect of growth hormone on fatty acid oxidation and enumerated the difficulties in using isolated tissues for such studies.

EFFECT OF GLUCOSE ON LIPIDS IN TRANSPORT

As a corollary to the fat-sparing, fat-synthesizing activities of glucose one might expect a surfeit of glucose to facilitate the deposition of fat in the depots, and the absence of glucose to favor fat mobilization. Since the plasma is the major vehicle for the transport of lipids, it might be anticipated that the relative predominance of mobilization or deposition of fat would be detected by changes in serum lipids; and indeed an increase in at least one and often all components of the serum lipids occurs when carbohydrate combustion is severely restricted. An example is the change occurring during fasting. Probably the earliest detectable change is a rise in the nonesterified fatty acids[19, 29] which occurs after an overnight fast, and which can be reversed by feeding 100 gms. of glucose. Gordon[29] gave evidence that the rise of nonesterified fatty acids is associated with mobilization of fat from the depots. There is considerable species variation in starvation. Man responds with a slight rise in serum cholesterol after five days of starvation, monkeys respond with a marked rise, and dogs display no consistent change in serum lipids and little ketosis.[43] The changes in man are prevented by the ingestion of as little as 100 gms. of carbohydrate a day.[43] In semi-starvation on a diet which is predominantly carbohydrate such changes as ketosis and elevation of lipids do not occur.[45] It was stressed by Peters that the ketosis and elevated serum cholesterol in starvation result from a specific lack of carbohydrate for some operative function rather than a caloric deficit.[61]

Factors which depress carbohydrate metabolism other than starvation may also cause elevations of serum lipids. An increase of triglycerides and other lipid fractions occurs in uncontrolled human diabetes, in glycogen storage disease, and in alloxan or pancreatectomy diabetes in experimental animals. Cortisone, growth hormone, and epinephrine favor fat mobilization and have been reported to cause lipemia in experimental animals.[1, 30]

Whether the effects of those substances on fat metabolism are primary or merely secondary to their inhibition of carbohydrate metabolism is a problem of no minor significance.

The hyperlipemia associated with impaired glucose metabolism is frequently attributed to intense mobilization of fat into the blood stream. That there might also be a delayed removal of fat from the blood stream is self-evident. Only sporadic experiments on this subject have appeared in the past. As early as 1918 Bang[10] showed in dogs that the feeding of oil alone produced a larger alimentary rise of plasma total fatty acids than when carbohydrate in the form of bread was fed with the same amount of oil. Rony and Ching[69] found that the rise in total fatty acids of plasma after a fat meal fed to dogs who had been fasted for 7 to 14 days could be abolished or decreased by the simultaneous administration of 1 gm. of glucose per pound of body weight, regardless of whether the glucose was administered orally, intravenously, or subcutaneously. Insulin had a similar effect, and epinephrine no effect. None of these substances had a demonstrable influence on the serum fatty acids of fasting dogs not given oil.

A more precise study of this phenomenon in human subjects was recently carried out in this laboratory,[8] using more detailed lipid analyses than were available to the above authors. The changes in serum lipids following a standard fat meal ingested after an overnight fast were compared with the changes occurring when extra glucose was administered orally with the fat meal. The fat meal consisted of 60 gms. of fat, 23 gms. of protein, and 42 gms. of carbohydrate. The glucose was given as lemonade in equal doses, one hour and again one-half hour before the breakfast. Occasionally, a third dose was given after breakfast. When the fat meal alone was given as a control to 12 normal individuals on 20 occasions, the only consistent change in the serum lipids was a rise in the emulsified triglyceride fraction from a mean fasting level of 3.1 mEq./L to a mean level of 6.6 mEq./L three hours after the fat meal.[4] The average increase was 3.5 mEq./L, with the minimum and maximum 1.5 and 5.5 mEq./L.* Lactescence occurred at triglyceride values between 3 and 11 mEq./L. In pathological lactescence of plasma in certain diseases the lipids appear in particulate form only when triglyceride concentrations exceed 15 to 20 mEq./L.[5] In such particles a large proportion of the plasma cholesterol and phospholipid is also found. If any cholesterol and phospholipid are present with the triglyceride particles of alimentary lipemia, their concentration is too small to be detected by the methods used. In striking contrast to the control series seven of eight sub-

* In previous experiments using a meal containing much more fat,[54] in addition to the rise in triglyceride fatty acids, lipid phosphorus increased slightly.

jects, each serving as his own control, responded to the administration of 120 gms. or less of extra glucose with a diminished or absent rise in triglycerides after the fat meal. Simultaneously, lactescence was less apparent in the serum. From these findings,[4] it was concluded that active carbohydrate metabolism facilitated the escape of particulate lipids from plasma following a fat meal but that interpretation of individual differences might disentangle the metabolic processes involved in this phenomenon. Any substance capable of stimulating carbohydrate metabolism might have the same effect without the necessity of giving carbohydrate itself, and substances inhibiting carbohydrate combustion might have an opposite influence. This prediction has been confirmed thus far with two substances—glucagon, which diminishes, and epinephrine, which if anything increases alimentary lipemia.[4] Other investigators found more unabsorbed fat in intestinal chyme obtained near Treitz' ligament during the two hours after ingestion of 45 gms. of vegetable fat alone than when bread, jelly, sugar, and coffee were consumed with the same amount of fat,[86] verifying our opinion that the effect of glucose is not a local action on the gastrointestinal tract.

The mechanism by which glucose reduced the rise in triglyceride fatty acids after the standard breakfast is unknown. Most workers in this field have directed their attention to alimentary lipemia after dietary fat without due consideration of the status of carbohydrate metabolism. The two most common metabolic processes for reduction of any fuel such as plasma triglycerides are oxidative combustion or deposition and storage. That fat is preferentially spared in carbohydrate surplus has been emphasized, leaving the alternate explanation that glucose facilitates removal of triglycerides for deposition and storage. Spitzer[73] in agreement with others has called attention to the capacity of the tissues of the dog to remove fat from the blood when he observed less lactescence in venous than in arterial blood of the leg of the hepatectomized dog previously given olive oil. Bergström and associates,[18] from quantitative comparisons of specific activities in fatty acids of lymph and plasma triglycerides, assumed that special properties of emulsified fat from thoracic duct lymph promoted its absorption from systemic blood. Although direct entry of emulsified lipids into adipose tissue and utilization by muscle cells of triglyceride fatty acids are two possible metabolic pathways for reduction of plasma lipids (Chaikoff and Brown in Greenberg[80]), the liver may play a major preparatory rôle after fat in the chyle enters the blood stream. To the liver is generally attributed the rôle of lipoprotein formation from circulating triglyceride particles removed for incorporation into lipoproteins; the latter are then released to the plasma for transport to the periphery where the fatty acids from triglycerides are used for fuel. Waddell *et al.*[85] demonstrated in rats that the removal of intra-

venously injected fat emulsions was considerably retarded by hepatectomy. Friedman *et al.*[34] attributed to the Kupffer cells of the liver removal of cholesterol-containing chylomicrons. Spitzer[78] reported that the turbidity of serum in dogs fed cod liver oil actually increased following functional hepatectomy. The liver has an important rôle in the metabolism of mobilized as well as administered fat. A transfer of depot fat to the liver occurs under the stimulus of growth hormone[79] and in female rats, following starvation.[91] If removal of fat from the liver is blocked by administering ethionine, a substance which is thought to interfere with protein formation, striking hypolipemia and fatty liver develop.[22] In all the studies quoted above, carbohydrate metabolism may have been impaired by starvation, by growth hormone, by hepatectomy, or by the administration of fat with little or no carbohydrate. If the absence of exogenous glucose favors mobilization and combustion of fat, the foregoing studies elucidate the fate only of fat destined to be burned, and leave unknown its disposition when an ample supply of carbohydrate favors its storage. It seems entirely possible that carbohydrate plenty activates enzyme systems of the depots to remove circulating fat directly, and in the absence of sufficient carbohydrate enzymatic functions of the depots to remove circulating fat are temporarily inactive. Circulating fat would then persist in the plasma until removed by the liver and other organs.

ADIPOSE TISSUE

Shapiro and Wertheimer[71] cite recent vivid reports of the rapid biochemical activity of adipose tissue. The reactions, which include synthesis of glycogen and fat, vary not only from animal to animal but with the anatomical locations and mechanical function of the fat. Oxygen consumption does not differ, but respiratory quotients are higher if tissues from animals fasted for 24 hours are compared with fat depleted tissues. Fat synthesis persists into the fasting state in adipose tissue at a time when it is greatly curtailed in liver.[11] Feller,[28] using male mice fed ad libitum, found greater synthesis of fatty acids in adipose tissue than in liver, as did Hausberger and Milstein,[36] while Itzhake and Wertheimer (cited by Shapiro and Wertheimer[71]), who worked with normally fed rats, reported that respiratory activity of adipose tissue was half that of liver. In each instance the large amounts of fat and water in adipose tissue and liver necessitate the conversion of data to nitrogen content of fat free dry weight. Feller[28] found different activities dependent on the position of the labelled carbon of acetate. Stern and Shapiro[77] incubated for four hours adipose tissue in serum or serum enriched with fat and noted transfer from serum to adipose

tissue of 1.3 to 4.2 mEq. of fatty acids. *In vivo* preferential selection by adipose tissue of specific fatty acids occurs, but after feeding fat of atypical nature the character of the fat in depots approaches the composition of dietary fat (Chaikoff and Brown in Greenberg[30]).[65, 72] The presence in adipose tissue of a lipoprotein lipase having clearing activities[47] further implicates this tissue in the clearing of alimentary lipemia. Adipose tissue may also play a vital rôle such as desaturation[71] in preparing ingested fat for subsequent use as fuel, in addition to providing storage space for fat. From this mass of diverse experimental results it might be conceded that adipose tissue derives triglycerides not only by direct deposition from the blood stream, but also by synthesis from carbohydrate.

The effect of glucose on alimentary lipemia might be mediated through alterations in cellular permeability to triglycerides of fatty acids. Insulin, which is probably secreted in response to administered glucose, may increase cellular permeability to certain sugars,[74, 75] but it is not known whether this pertains to other substances. Adlersberg *et al.*[1] suggested that corticosteroids have the opposite effect of decreasing tissue permeability, a finding of particular interest in view of the antagonistic action of insulin and cortisone on carbohydrate metabolism.

A comparison of the effect of glucose with that of heparin in reducing alimentary lipemia is inevitable. *In vivo* visual clearing of lipemic serum following heparin injection or *in vitro* clearing after incubation with serum from heparinized animals[82] is accompanied by a rise of albumin-bound nonesterified fatty acids released by hydrolysis of triglycerides.[31, 68] *In vivo* studies indicate not only a physical change but also an escape of fat from the blood stream following heparin.[78] Jeffries[42] claimed no definite relationship between clearing activity caused by heparin and clearing activity which he observed in plasma of rats after feeding fat. Intravascular hydrolysis of triglycerides and subsequent removal of the liberated fatty acids may be a part of the physiological mechanism for clearing alimentary lipemia. By removal of the end product, nonesterified fatty acids, further hydrolysis of triglycerides might be promoted. Diminution of nonesterified fatty acids has been reported after feeding glucose.[19, 29] On the other hand, glucose might, consistent with its sparing action on fatty acids,[88] inhibit the hydrolysis of triglycerides, facilitating rather their removal *in toto* for storage. Bergström and Borgström[19] have just reviewed about 40 investigations on the clearing factor and have discussed the rôle of numerous substances, of lipoproteins, of albumin and calcium as acceptors of nonesterified fatty acids, of glycogen, of alimentary lipemic plasma in activity of the clearing factor. Their conclusions, strangely appropriate to the observations in our alimentary lipemia experiments, are that *in vivo* lipemic blood is cleared by passage through the

capillaries of such tissues as heart and adipose tissues, and that overflow from these sites of clearing factor accounts for its presence in plasma. Much evidence does suggest that such overflow occurs following increased activity of fat storage.

SPECIES DIFFERENCES

Wide species variations in carbohydrate and fat metabolism have been emphasized.[62, 72] These differences are briefly mentioned again because they are important in selection of experimental animals to study alimentary lipemia. Animals such as dogs, which normally eat little carbohydrate, respond to starvation with little ketosis or change in serum lipids.[43] Rony[69] noted that lipemia rarely increased in dogs after a fat meal after a 24-hour fast. He noted that a preliminary fast of 7 to 14 days was necessary to promote a consistent alimentary lipemia. Conversely, the carbohydrate-dependent rabbit, which is remarkably sensitive to carbohydrate withdrawal, develops lipemia with facility not only after fat feeding but also following almost every conceivable stimulus. It appears that after an overnight fast animals which are able to conserve their carbohydrate on fasting demonstrate less alimentary lipemia than those which become rapidly depleted of glycogen.

ADAPTATION

The foregoing data would suggest that in view of the reciprocal relationship of fat to carbohydrate metabolism, metabolic pathways of fat are governed by dietary supply of carbohydrate. Many observations, however, indicate that this relationship persists only in animals receiving ample dietary carbohydrate. Animals maintained for a long period of time on a carbohydrate-limited diet develop adaptive changes which may relieve fat metabolism of its dependency on carbohydrate. This phenomenon has been studied by comparing the response to starvation of animals previously adapted to a high carbohydrate diet with those adapted to a high fat, low carbohydrate diet. As outlined above, the carbohydrate-adapted rat, upon withdrawal of carbohydrate, rapidly becomes ketotic, depleted of liver glycogen, and loses the ability to synthesize fatty acids. Such an animal continues to burn his available stored glucose until severe carbohydrate depletion results. However, these changes are minimized by previous high fat rations for several weeks. Whitney *et al.*[89, 90] summarized a number of metabolic changes which they had reported previously as occurring in fat-adapted animals. On food withdrawal, these animals develop less ketosis and are less sensitive to insulin. These responses correlate with *in vitro*

studies of liver slices, in which glycogen stores were guarded during fasting, and which, on incubation with glucose, utilized glucose sparingly with a corresponding reduction in glycogenesis, carbon dioxide production, and lipogenesis. Rat diaphragm oxidized more added palmitic acid when obtained from a fat-adapted than from a carbohydrate-adapted animal.[88] The fat-adapted animal is thus able to burn fatty acids before carbohydrate depletion occurs. Such an animal guards his carbohydrate supply by curtailing the metabolic activity of glucose.

In human subjects Sweeney[81] showed that two days of starvation or of a high fat, carbohydrate-free diet had similar effects in impairing carbohydrate tolerance. Ketosis and hypoglycemia diminished when human volunteers have starved for periods of 2½ days[82] at six-week intervals. This adaptive response of long duration has also been reported for obese individuals maintained on diets containing 1 gm. of protein and 0.6 gms. of carbohydrate per kg. ideal weight, but supplying only 400-600 calories per day.[21] During the first few weeks, not only fat but stored carbohydrate and nitrogen were lost. Later nitrogen equilibrium was established, and only body fats were burned. The adaptive changes on a high fat diet may actually be a result of the low carbohydrate in these diets. In the regulation of dietary fat intake the fact should not be neglected that a low fat diet is usually a high carbohydrate diet, and that the interaction of carbohydrate and fat metabolism is subject to adaptive changes. A portion of ingested carbohydrate is normally converted to fat before being burned and this proportion may be increased on a low fat, high carbohydrate diet thus to some extent negating the intent of the low fat diet.

The adaptive response evoked by a low carbohydrate, high fat diet has clinical application to the problem of functional hypoglycemia, and probably accounts for the success of high fat, high protein, low carbohydrate diets used to treat this condition. Such a diet has also been used in the treatment of obesity[59] on the premise that the absence of carbohydrate favors fatty acid oxidation and thus the burning of endogenous fat. With adaptive changes carbohydrate sparing should prevent hypoglycemic hunger attacks and subsequent gorging so common in the obese patient on a restricted diet. The ability of a patient to gain weight and retain nitrogen on a prolonged (5 months) diet consisting solely of fat and protein[46] demonstrates that, with adaptation, fat storage *can* occur in the absence of exogenous carbohydrate. A part of the adaptive phenomenon appears to be the ability to revise certain functions normally dependent on exogenous carbohydrate when the source of the latter is limited.

Adaptive changes occurring during long periods of high-fat feeding or successive starvation are not understood. They may represent changes in

activity of enzyme systems or in hormonal control. There is some support for an "adipokinetic" hormone of the anterior pituitary which mobilizes fat and increases fat combustion. Li[48] discussed the fat-burning, protein-sparing activities of the pituitary as properties of growth hormone. Whatever the mechanisms, the end result favors the conservation of endogenous carbohydrate by the preferential burning of fat for energy. The fat-adapted animal, having already substituted fat for carbohydrate as the major fuel, develops less severe carbohydrate depletion and subsequent ketosis during starvation.

The significance of carbohydrate in human diets seems to have been pushed aside in many discussions of human disease in which abnormalities of lipid metabolism occur. Not only does carbohydrate play a major rôle in governing combustion and synthesis of fat in the framework of metabolism, but the relative quantities of carbohydrate given for energy supply influence interrelated protein and lipid metabolic processes essential to the normal catabolic pathways followed by fat. The differences in specific activities of fat after giving C-14 stearic or palmitic triglycerides[18] afford only one example of the many differences in combustion and storage of diverse forms of fat.[39, 44, 73] The influence of protein and amino acid supply on plasma lipids is mediated through their rôle in phospholipid and lipoprotein formation by the liver. Unsaturated, essential fatty acids play a distinct but not completely defined rôle in formation and transport of phospholipids and esterified cholesterol.[2, 18, 41]

The studies on adaptation demonstrate singularly well the futility of studying a single fuel without due consideration of the status of other foodstuffs and the metabolic and nutritional state of the animal. Whether the effects of many of the factors that alter the metabolism of fat are secondary to their inhibition or facilitation of carbohydrate metabolism is a problem of major significance in the investigation of energy exchange.

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