PHARMACOLOGICAL REVIEWS
Vol. 18, No. 1, Part I

Printed in U.S.A.

J. CATECHOLAMINE STIMULATION OF FAT MOBILIZATION AND ITS METABOLIC CONSEQUENCES

DANIEL STEINBERG, M.D.

Laboratory of Metabolism, National Heart Institute, Bethesda, Maryland

The most striking and the best studied effect of catecholamines on lipid metabolism is their ability to stimulate the mobilization of free fatty acids (FFA) from adipose tissue (21, 66). Given the fact, one must ask what is its significance for the body economy as a whole? What is the fate of these FFA, once released, and what influence do they have on other metabolic processes? In other words, one must establish a context based on an understanding of basic patterns of FFA mobilization, transport and utilization. The catecholamines must presumably act within this context, modulating quantitatively rather than qualitatively, as in the case of hormone actions generally. My main purpose today is to sketch for you our current concepts in this connection and to indicate the anticipated consequences of excessively rapid FFA mobilization induced by catecholamines.

A second effect of catecholamines on lipid metabolsm has only recently begun to receive attention, and this is an apparent stimulation of lipid catabolism in diaphragm (38) and in heart muscle (9). Whether this effect is a direct and immediate consequence of catecholamine activity or whether it is a secondary result is not yet certain. On the surface, at least, it appears to be quite analogous to the effects of catecholamines in adipose tissue and deserves further study. Space will not allow discussion of this potentially important area in the present review.

There may well be other effects of catecholamines on lipid metabolism, but these are yet to be thoroughly documented. A basic theme of what follows is that a number of metabolic consequences of catecholamine administration, with respect to lipid metabolism, can very likely be traced back to a *primary* effect on FFA mobilization from adipose tissue or breakdown of lipids in muscle and other peripheral tissues.

THE PHYSIOLOGIC FATTY ACID TRANSPORT CYCLE

The schematic diagram shown in figure 1 is convenient for visualizing the overall body economy of FFA. The organism is viewed as a four-compartment system made up of: a) adipose tissue stores of fatty acids, almost exclusively in the form of triglycerides (TG); b) the plasma compartment, in which the extremely rapid FFA turnover is quantitatively most important but which includes as well the several lipoprotein fractions (LP) which also transport fatty acids (in ester forms); c) the liver, probably the most important site of FFA uptake and the site of lipoprotein and ketone body production; and d) the remainder of the utilizing tissues grouped together, it being recognized that this grouping together of heterogeneous tissues represents an extreme oversimplification.

Just as the liver glycogen represents the only very large store of carbohydrate available to provide energy in the fasting state, the adipose tissue triglycerides

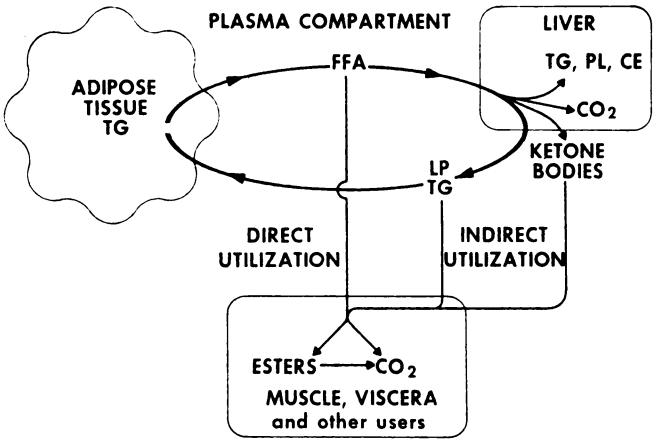

FIG. 1. A schematic representation of fatty acid transport and utilization, indicating the probable functioning of a *physiologic fatty acid transport cycle.* (From Metabolism **13**: 1265, 1964.)

represent by all odds the major store of fat available in the fasting state. Enough calories are stored in the adipose tissue of the average man to provide caloric substrate for more than 30 days in the absence of food intake. There is good reason to believe that fatty acid esters in muscle and other tissues also represent stored substrate that can be called upon as metabolic fuels for immediate local consumption (13, 35, 38), but these stores are relatively small and limited by comparison with those in adipose tissue. The fatty acids of the adipose tissue are mobilized into the plasma compartment as FFA and, as far as we now know, only in this form. This mobilization of FFA increases during fasting and increases enormously under the influence of catecholamines (and a large number of other hormones), and this leads to increased plasma FFA concentration and turnover.

Plasma FFA are taken up and oxidized or stored in virtually every tissue (*direct utilization*), with the possible exception of the central nervous system, and the rate of uptake is a strong function of the plasma concentration. Uptake in the liver, however, is of particular importance, approximately 30% of the FFA presented to the liver being taken up in a single passage through it. Part of what is taken up is esterified and appears in liver triglycerides, phospholipids (PL) and cholesterol esters (CE). Another (smaller) fraction is oxidized completely to carbon dioxide or converted to ketone bodies. The latter cannot be utilized in the liver but are transported through the plasma compartment and further oxidized to CO_2 in the peripheral tissues (*indirect utilization*).

It is now well established that when the rate of delivery of FFA to the liver is elevated, there is a tendency for an increased rate of output of lipoproteins from it into the plasma, but the mechanism underlying this stimulation remains unknown. These newly synthesized lipoproteins include, of course, triglycerides as indicated in figure 1, but they also contain phospholipids, cholesterol, cho-

lesterol esters and protein. In other words, the lipoproteins secreted by the liver at an accelerated rate in the face of a high rate of FFA input are not, as far as we know, qualitatively different from the lipoproteins normally secreted by the liver. At the moment, we are primarily concerned with the triglycerides in these lipoproteins as they fit into our fatty acid transport cycle, and hence the shorthand designation "LP TG" in figure 1. The extent to which lipoprotein triglycerides are utilized as an energy source by the peripheral tissues (a second mode of *indirect utilization*) has not been firmly established. Data on the overall turnover rate of fatty acids in lipoprotein triglycerides suggest that this turnover may be only about 10 to 20 % of that of the plasma FFA, but it is not certain how much of this turnover represents direct combustion and how much deposition in the tissue esters (25).

Rodbell (42) and Bezman, Felts and Havel (4) have shown that isolated adipose tissue can take up significant amounts of chylomicron triglycerides and of very low density lipoprotein triglycerides, respectively. The recent studies by Markscheid and Shafrir (33) on uptake by isolated rat adipose tissue of triglyceride from various density classes of lipoprotein are of interest from a quantitative point of view. They observed uptake of about 1 μmole of triglyceride per gram of adipose tissue in 3 hr of incubation. If uptake in human adipose tissue were of a comparable magnitude, this would correspond to a daily uptake of over 50 g, a figure comparable in magnitude to estimates of *total* plasma glyceride turnover in man. Much more work will be needed before the quantitative importance of lipoprotein triglyceride uptake into adipose tissue can be evaluated with confidence. Nevertheless, it is fair to conclude that at least some of the lipoprotein triglyceride is indeed redeposited in the adipose tissue, and this process closes what we have called the *physiologic fatty acid transport cycle.* Since uptake of FFA by the adipose tissue does not appear to be quantitatively important, the capability of returning fatty acids to the adipose tissue *via* lipoprotein triglycerides may represent an important homeostatic mechanism. When FFA are mobilized in excess of immediate energy requirements or oxidative

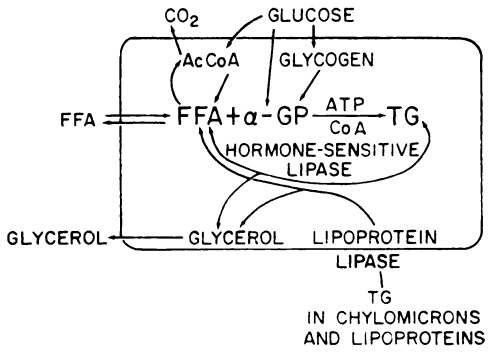

FIG. 2. A schematic representation of the metabolic processes in the adipose tissue cell relevant to mobilization and storage of fatty acids. (From The Control of Lipid Metabolism, Biochemical Society Symposium no. 24, p. 112, 1963.)

TABLE 1

Activating effect of hormones on lipase activity in preincubated adipose tissue[a]

Hormone	Lipolytic Activity (μmoles glycerol/g/30 min)		
	Control	Hormone-treated	Δ
E	1.0	2.6	+ 1.6 ± 0.2
ACTH	1.6	3.8	+ 2.2 ± 0.2
Glucagon	1.8	4.8	+ 3.0 ± 0.7
TSH	1.6	2.9	+ 1.3 ± 0.5

Data from Vaughan, Berger and Steinberg, 1964.

[a] Paired fat pads incubated for 90 min. Hormones added to one of each pair during final 10 min of incubation.

capacity, they can be deposited as esters in the liver, muscle, and other peripheral tissues, and finally, as just discussed, returned *via* lipoproteins to the adipose tissue triglyceride pool. With this physiologic pattern of fatty acid transport as a background, let us now examine in more detail some of its individual elements.

MOBILIZATION OF FFA FROM ADIPOSE TISSUE

The metabolic reactions immediately relevant to deposition and mobilization of fat from adipose tissue are schematically represented in figure 2. The triglyceride pool (or pools) is in a dynamic equilibrium at all times, as evidenced by the fact that glycerol is continuously released even in the face of net fat deposition (30), and by the fact that incorporation of labeled fatty acids occurs under all circumstances, even when there is net mobilization (64) The synthesis of new triglycerides is effected *via* the following reaction sequence (54):

3 FFA + 3 ATP + 3 CoA → 3 FA CoA + 3 AMP + 3 PP
2 FA CoA + α-glycerophosphate → phosphatidic acid + 2 CoA
Phosphatidic acid → diglyceride + Pi
Diglyceride + FA CoA → triglyceride + CoA

Sum: 3 FFA + 3 ATP + α-GP → Triglyceride + Pi + 3 AMP + 3 PP

Because adipose tissue lacks phosphoglycerokinase activity, there must be a constant supply of the obligatory α-glycerophosphate acceptor in order to maintain steady state. α-Glycerophosphate can be generated either by breakdown of the small stores of adipose tissue glycogen or by glycolysis of exogenously supplied glucose. Here, then, is one point at which catecholamines may directly or indirectly influence FFA mobilization—by influencing the availability of glucose for re-esterification. Indeed, the catecholamines do stimulate the uptake of glucose by isolated adipose tissue (58), and this in itself might be expected to favor glyceride synthesis and thus preserve triglyceride stores. However, the effect on lipolytic rates far outweighs any such effects, and the net effect is one of increased FFA release. The inability of epinephrine (E) to maintain high FFA levels *in vivo* during constant infusions, whereas norepinephrine (NE) can do

TABLE 2

Failure of NE to stimulate "monoglyceridase" activity[a]

Substrate	Lipolytic Activity (μmoles glycerol/g/30 min)		
	Control	Treated	Δ
Endogenous	0.9	2.7	+ 1.8 ± 0.26
Monostearin	50	55	+ 5.0 ± 3.5

Data from Vaughan, Berger and Steinberg, 1964.

[a] Five pairs of tissues incubated for 90 min. NE added to one of each pair during last 10 min of incubation.

just that, has been attributed to the counteracting effect of the higher glucose levels accompanying E administration (17, 26).

As described above, triglycerides supplied in the form of chylomicrons or plasma lipoproteins can also contribute fatty acids to the depot glyceride stores. The uptake of lipoprotein or chylomicron glycerides is correlated with tissue levels of lipoprotein lipase (4), and it is now generally accepted that the role of this enzyme is limited to facilitation of the incorporation of plasma glycerides into depot fat (41). It does not appear to play a role in the breakdown of endogenous adipose tissue glyceride, *i.e.*, does not play a role in mobilization of FFA.

Adipose tissue lipases in relation to FFA mobilization. Studies with intact rat adipose tissue have shown that its lipolytic activity rises very rapidly in response to stimulation with catecholamines and a wide variety of other hormones (5, 28, 34, 40, 61). Only a few minutes of exposure to hormone (NE, ACTH, TSH or glucagon) is sufficient to effect a 2- to 3-fold increase in lipase activity of homogenates prepared from the hormone-treated fat pads (table 1) (61). Some activation could be demonstrated even in fat pads incubated in the absence of hormones but simply homogenized in a medium containing a high concentration of hormones; this indicates that activation begins practically instantaneously.

The enzyme activity responding to hormone stimulation has yet to be purified to any extent. We have designated it noncommittally as "hormone-sensitive lipase" and defined it operationally in terms of the assay conditions used. The tissue also contains, however, an enzyme (or enzymes) much more active in the splitting of lower glycerides. This activity in whole homogenates is at least an order of magnitude greater than that against endogenous substrate, which is essentially all triglyceride, but the activity is not increased by NE (table 2). The monoglyceridase activity has been differentiated from the hormone-sensitive lipase by its pH optimum and temperature-activity curve, and by partial fractionation, in addition to the obvious difference in hormone response (57, 61). Activity against diglycerides is also much greater than that against triglycerides, but it is not yet certain whether this enzyme is distinct from the monoglyceridase, since the activities fractionate together (57, 61). Until the enzymes are more satisfactorily purified, conclusions must remain tentative. However, the results obtained to date suggest a scheme such as is shown in figure 3. The hormone-sensitive lipase is converted from an inactive to an active form under the influence of catecholamines, ACTH, glucagon and a number of other hormonal

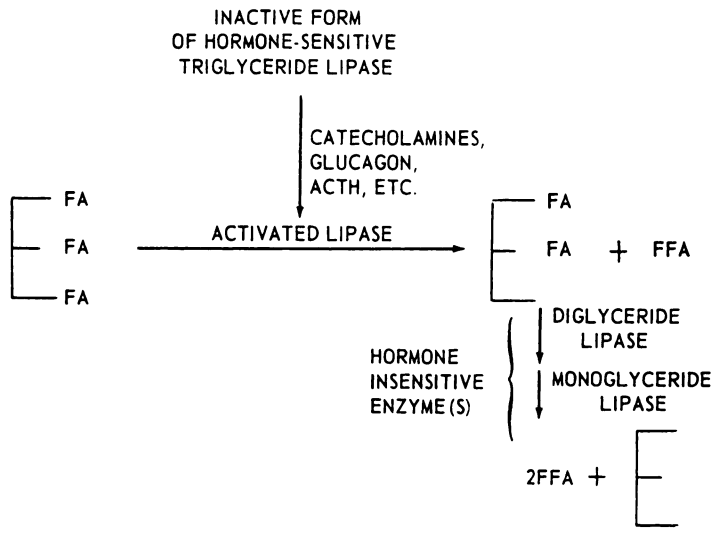

FIG. 3. A schematic representation of the system of lipase activities in adipose tissue as they are probably functioning in breakdown of triglycerides and mobilization of FFA. As discussed in the text but not shown, there is good evidence that the active form of the hormone-sensitive lipase can be quite rapidly converted to its inactive form (see figures 4 and 5).

factors. In its activated form, this lipase splits an ester bond in triglyceride. The diglycerides thus generated are very rapidly hydrolyzed to FFA plus glycerol. It is not yet certain whether the lower glyceridase activity represents a single enzyme or whether there are separate diglyceridase and monoglyceridase activities. It is of interest to note that 2-monoglycerides are more readily attacked than 1-monoglycerides, a specificity the reverse of that of pancreatic lipase.

Studies in our laboratory of the rates of lipolysis in hormone-treated tissues have shown that in some instances this stimulation is transient, lipolytic rates having returned to control values by 30 min even though highly significant increases were apparent during the first 30 min of incubation (62). This indirect evidence for the presence of both lipase-activating and lipase-inactivating systems has been extended in recent studies with homogenates (63). As shown in figure 4, the lipolytic activity of homogenates falls progressively when the pH is held at 6. By contrast, there is an initial increase in lipolytic activity in homogenates incubated at pH 7.4, with a drop in activity toward the end of a 1-hr incubation. Partial reactivation of lipolytic activity could be restored if after 45 min of incubation at low pH an aliquot of the opalescent fluid fraction (15,000 $\times$ g for 30 min) was added (fig. 5). If adipose tissue is preincubated for 3 hr and an homogenate prepared with water or 10^{-3} M EDTA is centrifuged, the lipase activity of the fat layer floating to the top is low, as is also the intrinsic lipase activity of the immediate infranatant fluid (which, however, still contains cell particles). Incubation of these two fractions together, each with low intrinsic

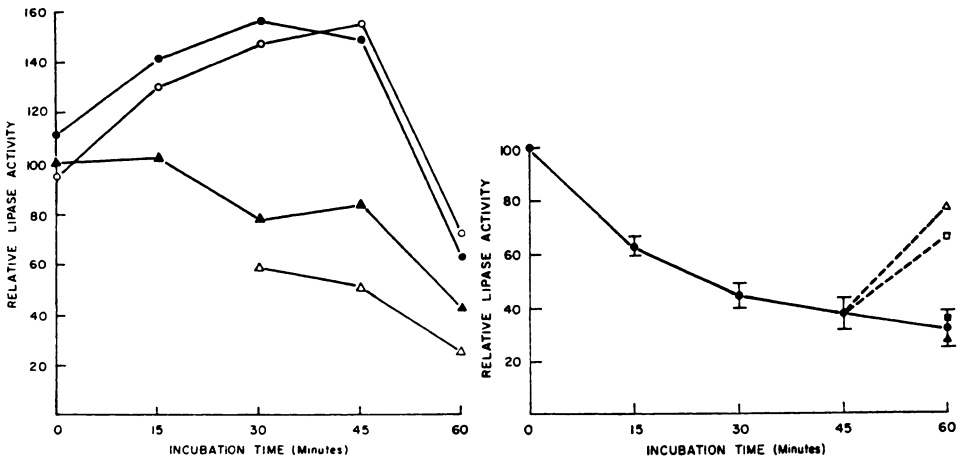

FIG. 4 (left). Time course of changes in lipase activity during incubation of homogenate at 37°C. Fat pads were homogenized in 0.154 M KCl (20 mg tissue/ml homogenate). During the incubation period approximately 20 mg homogenized tissue, or lower fat fraction recovered from 40 mg tissue, was present in 0.5 ml medium as indicated. Whole homogenate (●); lower fat fraction (○); fractions incubated in 0.03 M KCl, 0.02 M sodium phosphate buffer, final pH 7.5. Whole homogenate (▲); lower fat fraction (△); fractions incubated in 0.03 M KCl, 0.002 M sodium acetate buffer, final pH approximately 6.

FIG. 5 (right). Changes in lipase activity during incubation of homogenate at pH 5.9 and stimulation after addition of fluid fraction at 45 min. ●——●, composite curve based on data from 5 experiments with different homogenates incubated in 0.02 M sodium acetate, 0.03 M KCl, final pH 5.9 (mean ± standard error). In one experiment, the monoglyceride lipase activity (3) measured at 45 min was 95% of that at zero time. In two other experiments, lipase activity was assayed at zero time and samples of homogenate were incubated as described above except that the volume was 0.4 ml. After 45 min, 0.1 ml fluid fraction derived from another homogenate prepared in 0.154 M KCl was added to some samples (□,△) and 0.1 ml 0.154 M KCl (■,▲) to others. Incubation was continued for 15 minutes more and lipase activity was assayed.

lipase activity, leads to a highly significant increment in lipase activity beyond the sum of the individual activities (table 3). No cofactor additions were necessary, and it was shown that the activity of the fluid fraction was partially retained after dialysis and lyophilization. Thus we are dealing with a labile system in which the lipase is probably constantly subject both to activation and to inactivation. The net lipolytic activity at any time would reflect the balance between these processes. Note that there was essentially no activation of monoglyceridase (table 3). The rapidity of the hormone-sensitive lipase activation and the fact that it occurs in an unfortified system virtually eliminates the possibility that the catecholamine effect represents enzyme induction. Furthermore, Fain has recently shown directly that puromycin does not interfere with E-stimulated lipase activation (16). Thus, we are dealing with conversion of inactive to active enzyme, but the nature of the enzyme modification remains unknown.

TABLE 3

Activation of adipose tissue lipase in a cell-free system

Tissue Fraction	Hormone-sensitive Lipase Activity	Monoglyceride Lipase Activity
	μEq FFA/20 min	
1. Fluid alone	0.04	0.66
2. Lower fat layer alone	0.04	0.28
3. Fluid plus fat, after 15-min preincubation	0.24	0.96
Increment	0.16 (200%)	0.02 (2%)

Data from Vaughan, Steinberg, Lieberman and Stanley, 1964.

A great deal of evidence points to a mechanism for lipase activation analogous to that for phosphorylase activation, including a role for cyclic 3′,5′-AMP. This has been discussed by Dr. Vaughan in her introductory remarks (Section II I) (60) and is presented in detail in Dr. Butcher's paper in Section II K (6).

METABOLIC CONSEQUENCES OF ENHANCED FFA MOBILIZATION

1. FFA uptake, a function of FFA concentration. The rate of uptake and utilization of FFA is, in every tissue that has been examined, a function of the concentration of FFA to which it is exposed (51). Most of the studies on the effects of FFA concentration on uptake have employed a constant concentration of albumin so that, as the FFA concentration was raised, there was simultaneously an increase in the molar ratio of FFA to albumin $\bar{\nu}$. Shtacher and Shafrir (48) showed that in muscle slices, however, the uptake and utilization was also increased by increasing $\bar{\nu}$ at *constant* FFA concentration. Recent studies by Dr. Arthur A. Spector in our laboratory have shed some light on the quantitative importance of the parameters governing both the transfer of FFA from albumin to cell surface and the subsequent utilization of FFA (49, 50). We have used suspensions of Ehrlich ascites tumor cells as a model system and studied first the rapid initial uptake of FFA. As shown in figure 6, the cells rapidly take up FFA, reaching within 1 min a high value which does not then much change in the course of the succeeding hour of incubation. The amounts of radioactivity appearing in CO_2 and in lipid esters, however, increase progressively over the hour of incubation. We then studied the characteristics of the rapid initial uptake at 1 min and found that if the total palmitate concentration in the medium was held constant but the molar ratio of fatty acid to albumin was varied, this initial uptake increased markedly as a function of $\bar{\nu}$. If on the other hand, the $\bar{\nu}$ was held constant while the total fatty acid concentration was varied over a wide range, there was little or no effect of total fatty acid concentration. Now, the amount of unbound FFA (anions in aqueous solution) in equilibrium with FFA-albumin complexes is determined by $\bar{\nu}$. If one estimates the concentration of *unbound* FFA and replots the data on uptake *versus* estimated *unbound* FFA concentration, one obtains a roughly linear relationship, at least at low values of $\bar{\nu}$.

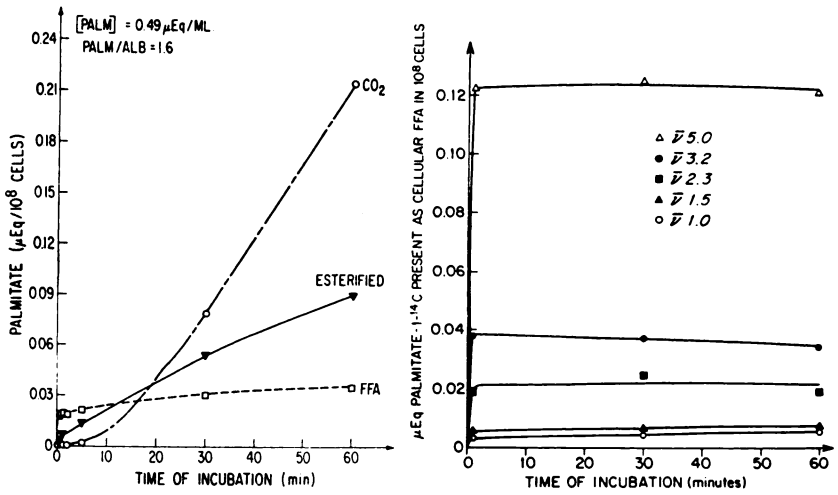

FIG. 6 (left). Uptake and metabolism of palmitate-1-C^{14} by Ehrlich ascites tumor cells during a 60-min incubation. (From J. biol. Chem. **240:** 1032, 1965.)

FIG. 7 (right). Equilibrium FFA uptake in relation to the palmitate-albumin $\bar{\nu}$ of the incubation medium during a 60-min incubation. (From J. biol. Chem. **240:** 3747, 1965.)

In figure 7, we see that at various values of $\bar{\nu}$, the time course of FFA uptake is similar in each case, an equilibrium concentration of cell FFA being established within the first minute or so of incubation with no further increase during a subsequent hour of incubation. The amount of fatty acid oxidized in 30 and in 60 min increases with increasing values of $\bar{\nu}$ for the FFA-albumin complex in the medium, as shown in figure 8. In contrast, when $\bar{\nu}$ is held constant but the total FFA concentration is varied from 0.2 up to 1.2 μEq/ml, the rate of $C^{14}O_2$ production remains constant (fig. 9). These results are compatible with the scheme shown in figure 10. It seems likely that the FFA must first dissociate from albumin and that the molecular species taken up is the unbound form. Thus, it is understandable that the uptake is a function of molar ratio of FFA to albumin, since the latter determines the concentration of unbound FFA. This in turn determines an equilibrium cell content of FFA, probably adsorbed to the surface of the cells, although the latter is not proved. The fact that isolated red cell ghosts strongly adsorb FFA (20) is compatible with this interpretation, as is also the fact that none of the usual metabolic inhibitors interferes with the rapid 1-min uptake by Ehrlich ascites tumor cells (50). The rate of subsequent utilization of bound FFA for oxidation to CO_2 (as shown in fig. 8) and for conversion to ester forms (not shown) then appears to be a function of the steady state FFA concentration on (or in) the cells. Ordinarily, the concentration of plasma albumin in the intact animal remains rather constant and so an increase in total FFA concentration in short-term studies is equivalent to an increase in $\bar{\nu}$. These studies show, however, that the relationship between FFA concentration and utilization is more specifically related to $\bar{\nu}$ and the quantitative relationship appears to be a complex one. One would anticipate that the re-

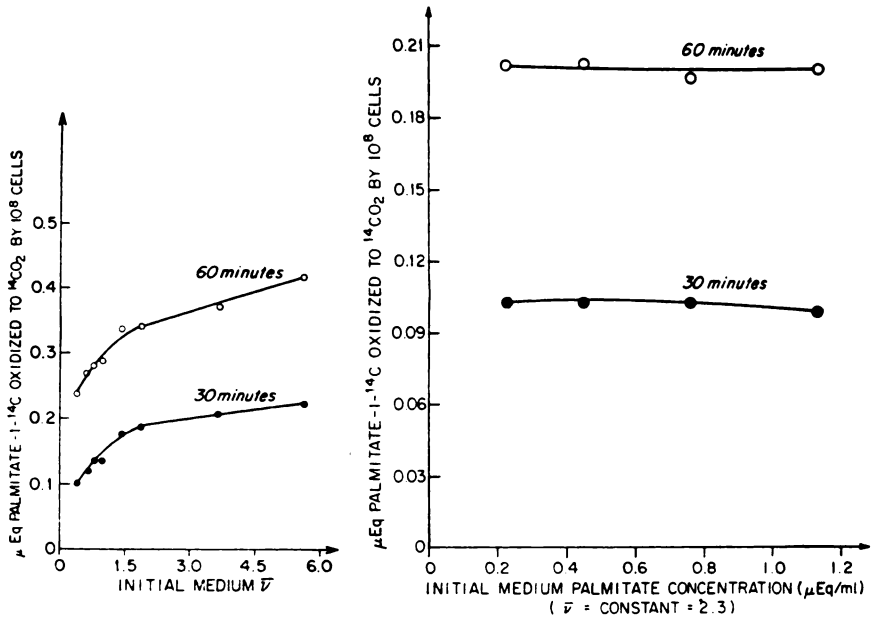

FIG. 8 (left). The effect of extracellular FFA-albumin $\bar{\nu}$ on the oxidation of palmitate-1-C^{14} to $C^{14}O_2$ during a 30- or a 60-min incubation. (From J. biol. Chem. **240:** 3747, 1965.)

FIG. 9 (right). Effect of concentration of palmitate in the medium at a constant palmitate albumin molar ratio (2·3) on the cellular oxidation of palmitate-1-C^{14} to $C^{14}O^4$. (From J. biol. Chem. **240:** 3747, 1965.)

lationships might be altered in patients with the nephrotic syndrome or with hypoalbuminemia of other origins. Epinephrine, by elevating ambient FFA concentrations and thus $\bar{\nu}$, would be expected to, and does, increase the rate of uptake and utilization in the peripheral tissues.

2. Development of fatty liver. As early as 1926, Wertheimer (65) showed that fatty liver induced by phloridzin treatment could be prevented by sectioning the spinal cord in the upper thoracic region. Many other early studies also suggested strongly that the development of fatty liver was in some instances dependent upon the ability of the animal to mobilize depot fat (reviewed in (45)). When it was recognized that depot fat was mobilized in the form of FFA, it became possible to study this problem more directly. Feigelson *et al.* (17) showed that a fatty liver could be produced acutely in normal dogs by infusing NE at a constant rate over an 8-hr period. Liver glyceride content increased 5- to 7-fold. That this was not due to an effect of NE directly on liver metabolism was shown by giving identical infusions *via* the portal vein. Under these circumstances, the NE was largely inactivated before it reached the systemic circulation (31); there was little or no rise in systemic plasma FFA levels, and there was little or no increase in liver triglyceride concentration. Further evidence that the deposition of fat is directly attributable to the elevated FFA levels produced comes from studies of the isolated perfused liver. When the perfusate contains high

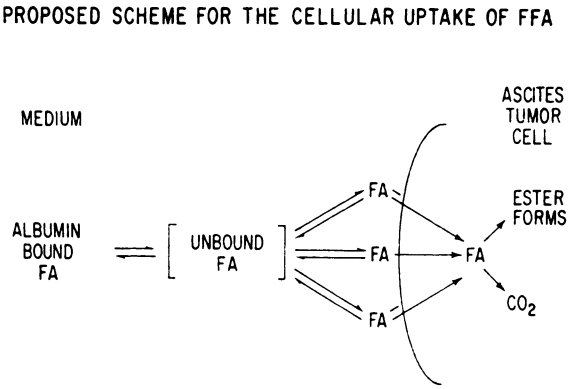

FIG. 10. A schematic representation of the transfer of fatty acids from albumin via unbound fatty acid anions in free solution to the cell surface, followed by transfer into metabolic pathways. As described in the text, the data suggest that the concentration of FFA at or near the cell surface governs the subsequent rate of utilization for esterification or oxidation.

concentrations of FFA, there is a progressive and marked deposition primarily of triglycerides and smaller amounts of phospholipid (18, 36). It was possible to produce as much as a 50% increase in liver glyceride concentration in 90 min of perfusion. Further evidence that it is simply the elevated rate of delivery of FFA to the liver that induces fat deposition has been obtained by Carlson and Liljedahl (8). They showed that the fatty liver produced by NE infusion could be prevented by simultaneously administering nicotinic acid, which blocked the FFA response to NE. Rudman and co-workers (43) have shown that excessively rapid mobilization of FFA induced by Factor H, a pituitary peptide fat-mobilizing hormone, can also lead to deposition of fat in the liver. These studies make it clear that one important element favoring deposition of fat in the liver is rapid mobilization from the periphery. The studies cited show that if hepatic uptake is excessive, a fatty liver can develop in the absence of any specific damage to the liver. On the other hand, if the capacity of the liver to oxidize fatty acids or to remove them by secretion as esters in lipoproteins is impaired, then net deposition occurs even with a normal rate of delivery of FFA. The rate of fatty liver formation under these circumstances, however, would still be influenced by factors regulating mobilization from the periphery, and here autonomic nervous system activity might be highly relevant.

3. Increased output of lipoproteins from the liver. Shortly after the striking effects of E on blood glucose levels were discovered, a number of attempts were made to demonstrate analogous effects on blood lipid levels. The results were conflicting but for the most part negative. In retrospect, this was probably because the studies were directed at uncovering acute responses, like those of E on glucose metabolism, but the important FFA fraction, which *does* respond acutely, was not yet discovered. Actually, there are significant changes in plasma lipoprotein concentrations in response to catecholamine injection, but they

occur late. After subcutaneous injection of E in oil into dogs, there is an immediate rise in plasma FFA levels, reaching a peak at about 2 hr, but the plasma cholesterol and phospholipid concentrations do not change perceptibly during the first 6 hr (45, 46). Between 12 and 24 hr, the plasma lipoprotein levels rise, and by 24 hr there is a very highly significant increase in plasma lipoprotein levels. A similar delayed response was seen by Rudman and co-workers following injection of their porcine Fraction H into rabbits, although the time scale was condensed. Plasma FFA levels had reached a maximum value by 2 hr, but plasma triglyceride levels did not increase significantly until 8 hr after injection (43).

Once again, it appears that at least a partial explanation for these responses lies in the increased rate of FFA delivery to the liver. Perfusion of the isolated rat liver with a medium containing high concentrations of FFA will stimulate the secretion of lipoproteins into the perfusing medium (18, 27, 36). The pattern of the new lipoprotein production stimulated by FFA, which determines whether cholesterol, phospholipids, triglycerides or all three will be elevated, seems to vary with the species and possibly with the nutritional state of the animal. In just what way increased FFA uptake leads to increased lipoprotein output is not known.

4. Increased production of ketone bodies. The literature concerning the factors regulating ketone body production is extensive and suggests that many factors are involved directly or indirectly (44). Here we only wish to call attention to the possible direct effect of FFA load to the liver. In collaboration with Dr. S. S

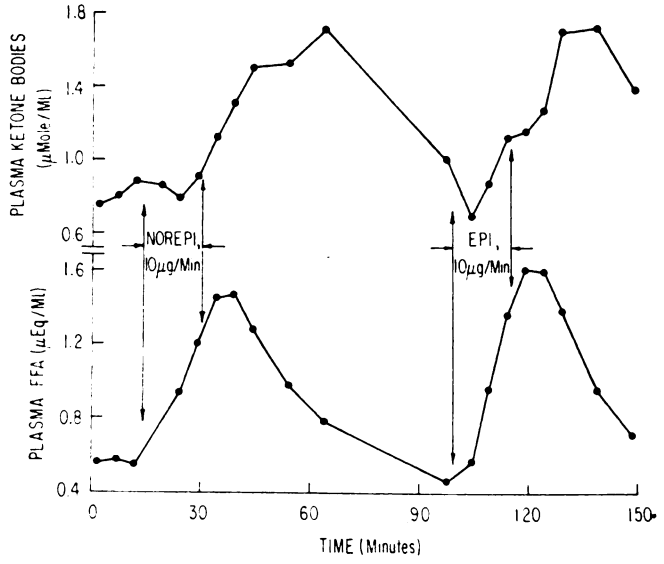

FIG. 11. Plasma FFA and ketone body response of a normal subject to intravenous infusion of NE and E, each at 10 μg per min. (Data from Steinberg, Chernick and Eaton, unpublished experiments.)

Chernick and Dr. R. P. Eaton, we have determined changes in blood ketone body levels in normal human volunteers during infusion of E or NE. As shown in figure 11, the infusions were accompanied by the expected rise in FFA levels; there was also a very significant rise in blood ketone body levels, but this was delayed in time relative to that of the FFA. Like the rise in FFA levels, it was transient. The time relationship is certainly suggestive of a direct relationship. It should also be pointed out that Gordon and Fredrickson (22) have carried out preliminary isotopic studies showing that the specific radioactivity of urinary ketone bodies and that of plasma FFA in subjects given injections of C^{14}-palmitate were comparable, a relationship that suggests a rather direct interconversion. In other words, it appears that the plasma FFA do not undergo any very extensive dilution prior to conversion to ketone bodies. While there certainly must be a variety of other metabolic factors involved, the possibility that the excessive ketone body production in starvation and in diabetes, for example, is directly attributable to excessive loading of the liver with FFA beyond its capacity to esterify them or oxidize them completely, deserves consideration.

5. The calorigenic effect of catecholamines in relation to fat metabolism. Despite extensive study, there is still no consensus regarding the mechanisms by which catecholamines increase oxygen consumption and there are almost certainly several. No attempt can be made here to review the many aspects of this complex problem.

Here we wish to consider only one question: Does the catecholamine-induced FFA mobilization play a significant role in calorigenesis? This question is really 2-fold: a) Is FFA mobilization essential to support the calorigenic effect? and b) Does the FFA mobilization play a *causative* role in calorigenesis? We have found that pronethalol, shown by Pilkington *et al.* (37) to inhibit markedly the NE-induced mobilization of FFA, reduces or even abolishes completely the calorigenic action of NE in man without altering markedly the pulse or blood pressure responses (52). Because this inhibitor of β-adrenergic responses may have simultaneously altered other metabolic effects of the catecholamine, the results by no means establish a cause-and-effect relationship between the FFA-mobilizing action *per se* and the calorigenic action. Havel *et al.* (24) have shown, however, that nicotinic acid, like pronethalol, also suppresses both FFA mobilization and increase in O_2 consumption after NE administration in man. The inhibition of the increase in O_2 consumption was only partial. These results have been confirmed (13). The fact that two different blockers of FFA mobilization, probably operating by different mechanisms, both influence calorigenesis somewhat strengthens the possibility that the FFA mobilization is essential for some part of the calorigenic effect. On the other hand, the less-than-complete effectiveness of nicotinic acid in this regard, even though it was highly effective in preventing the increase in FFA levels, implies that the calorigenic action is at most only partially dependent on the simultaneous FFA mobilization.

If FFA play a role in calorigenesis, do they do so simply by providing the substrate to support the effect, mediated *via* some direct catecholamine effect on the tissues, or do they themselves elicit an increase in O_2 consumption?

Studies by Dr. Paul Nestel in our laboratory have shown that the O_2 consumption of the isolated perfused rat liver can be increased by using very high concentrations of FFA in the perfusing fluid (51), but the effect is not obtained consistently. Isolated slices of rat liver or skeletal muscle show no increase in Q_{O_2} in the presence of high medium FFA, even though the rate of FFA utilization is increased (Eaton and Steinberg, unpublished results). Isolated Ehrlich ascites tumor cells again show no FFA-induced increase in Q_{O_2} (Spector and Steinberg, unpublished results). On the other hand, Evans and Mueller (15) have reported that guinea pig leukocytes do show an elevated Q_{O_2} in the presence of high medium concentrations of FFA. Recently Dr. Challoner in our laboratory has found a consistent FFA stimulation of Q_{O_2} in the perfused rat heart (10) accompanied by an increase in flow rate. The effect was also seen in the arrested heart perfused at a constant flow rate. The mechanism underlying the effect is not known. The findings in the several tissues studied are conflicting, and it is too early to draw conclusions. Further work with various perfused tissues will be needed to permit assessment of the possible quantitative importance of direct FFA stimulation of Q_{O_2} consumption. From what has been said above, however, particularly with regard to the incomplete effectiveness of nicotinic acid, it seems unlikely that any such effect will afford more than a partial explanation of catecholamine calorigenesis *in vivo*.

It has been repeatedly postulated that many of the manifestations of hyperthyroidism might be attributable to an increased sensitivity to catecholamines. We have examined the possibility that the excessively rapid mobilization of FFA in the hyperthyroid state might be essential in maintaining the hypermetabolic state (13). Normal volunteer subjects were treated with triiodothyronine and then studied metabolically in the hyperthyroid state. Their plasma FFA turnover was found to be elevated as was their $\dot{V}O_2$. When nicotinic acid was administered intravenously, the plasma FFA levels and turnover fell markedly, in some cases to levels as low as those found in normal subjects by Carlson *et al.* (7), but the $\dot{V}O_2$ was not altered even though plasma FFA turnover was reduced by 50% or more; the results indicate that the hypermetabolic state could be maintained independently of high plasma FFA levels and turnover. It was calculated that during nicotinic acid treatment oxidation of the fatty acids turning over in the plasma compartment could not account for the total oxygen consumption of the patient. Since the respiratory quotient remained below 0.8, this leads us to conclude that there must be sizeable stores of endogenous lipid in the tissues that can be called upon as substrate when delivery of FFA from the periphery is interrupted. It should be stressed that these were short-term studies and do not rule out some relationship between FFA mobilization and rates of total substrate utilization. It could be postulated that some degree of adaptation takes place during treatment with triiodothyronine and thus permits a continuation of the hypermetabolic state for some time after the stimulus of high FFA levels has been removed.

Another aspect of these studies involved treatment of the patients with the β-adrenergic blocking agent, pronethalol. This did not prevent the development

of the hypermetabolic state (13). Yet pronethalol can reduce the hypermetabolic effects of intravenously administered catecholamines (52). The results do not support the proposition that the hypermetabolism in the hyperthyroid state is mediated by catecholamines.

FACTORS MODIFYING THE FAT-MOBILIZING POTENTIAL OF CATECHOLAMINES

1. Glucose and insulin. The basal release of FFA from adipose tissue *in vitro* is inhibited by glucose and insulin. The stimulation of FFA release by catecholamines has been reported to be diminished in the presence of glucose and insulin, although results have not been entirely consistent. In the intact animal there does appear to be a modifying effect. For example, long-term infusions of NE will maintain FFA levels at a high plateau value, but similar infusions of E, sufficient to cause an equal initial rise in plasma FFA levels, fail to sustain the initially high levels (17, 26). This may be attributed to the greater hyperglycemia accompanying E administration. The FFA-mobilizing effect of E can be suppressed in experimental animals by simultaneous administration of glucose and insulin, but very large doses are required (46).

2. The permissive role of corticosteroids. Shafrir, Sussman and Steinberg first showed both in dogs and in rats that the capacity of adipose tissue to respond *in vitro* or *in vivo* to stimulation by catecholamines is reduced when the animal has been adrenalectomized (45, 47). This finding has been confirmed by Reshef and Shapiro (39) and by Maickel and Brodie (32). Addition of corticosteroids to the system *in vitro* does not restore this responsiveness, but prior treatment of the animal does. The doses of the corticosteroids used do not by themselves cause acute elevation of FFA levels. The results suggest that the catecholamine-sensitive system in adipose tissue depends in some way on corticosteroid activity for its maintenance in a fully responsive state. This type of interaction is reminiscent of others described for the corticosteroid hormones and designated "permissive" by Ingle (29).

It should be pointed out that corticosteroids do have the ability to directly stimulate fatty acid release from adipose tissue *in vitro*, but the time scale of these responses is longer than that for responses to E.

3. The permissive role of thyroid hormone. Adipose tissue taken from hypothyroid animals is also less responsive than normal to the stimulating action of catecholamines, while adipose tissue from hyperthyroid animals is hyper-responsive (11, 12). Again, the addition of triiodothyronine itself *in vitro* has not been demonstrated to influence either FFA release or responsiveness to catecholamines. The steady-state FFA levels of hypothyroid patients are decidedly low, and those of hyperthyroid patients decidedly high (23). In triiodothyronine-treated volunteer subjects, FFA levels are elevated and FFA turnover is elevated (13). These subjects also show an exaggerated response to intravenously administered catecholamines. Whether or not the high rate of FFA mobilization in the hyperthyroid state is due exclusively to a hypersensitivity of adipose tissue to stimulation by catecholamines cannot be stated with certainly. The fact that intravenous nicotinic acid, an inhibitor of catecholamine stimulation of adipose

tissue, restores the FFA turnover to virtually normal levels, is compatible with such a possibility (13).

4. Adrenergic blocking agents. A wide variety of blocking agents has been shown to suppress FFA release both *in vivo* and *in vitro*. Since this problem is discussed in later papers in THIS SYMPOSIUM, no attempts will be made to review this area.

5. Prostaglandin. As first shown in this laboratory, prostaglandin E_1 (PGE_1) is a potent inhibitor of the catecholamine effect on lipolysis in intact fat pads (55, 56). Concentrations as low as 5.6×10^{-8} M were effective; effects could be obtained with concentrations lower on a molar basis than that of NE used to stimulate lipolysis. PGE_1 suppressed not only the lipolytic action of the catecholamines but also that of glucagon, ACTH and TSH. As discussed by Dr. Vaughan in her Introductory Remarks (60), PGE_1 appeared to suppress activation of lipase, assayed in homogenates at the end of incubation, to a greater extent than it inhibited activation of phosphorylase.

PGE_1 was also shown to counteract E-induced fat mobilization in anesthetized dogs (55, 56), and this effect has been confirmed and extended (2). Recently we have tested the effects of single large doses of PGE_1 in unanesthetized dogs (53). In dogs with initially high fasting levels of plasma FFA there was consistently a dramatic sudden drop in plasma FFA level (fig. 12). The very low levels reached

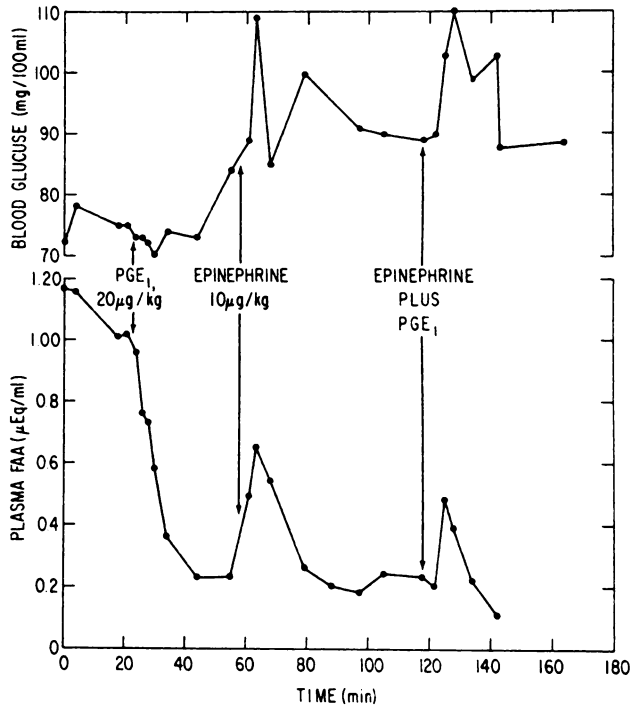

FIG. 12. Plasma FFA and blood glucose responses of two unanesthetized dogs to single intravenous injections of PGE_1, E and a combination of the two. (Data from Steinberg and Pittman, unpublished experiments.)

after PGE_1 injection remained low for 1 to 2 hr. The animal still responded with a rise in FFA when given E, but the response was smaller than that ordinarily seen in control animals, and the response was further reduced when PGE_1 was given together with the E. The blood glucose response did not seem to be affected. These results stand in contrast to those obtained in man by Bergström *et al.* (1). Infusions of PGE_1 at a rate of 0.1 μg per kg per min here caused an elevation of plasma FFA levels. In unanesthetized dogs, we have not been able to elicit a *rise* in FFA levels by infusing PGE_1 at a similar rate. Whether the difference in results reflects a species difference or some difference in experimental conditions is not yet clear.

The hypotensive action of prostaglandin was recognized by Goldblatt and by von Euler independently some 30 years ago (14, 19). Studies with the pure compound isolated by Bergström *et al.* (3) confirmed this effect for the crystalline material. Studies in this laboratory (55, 56) and in Stockholm (2) showed that the hypertensive action of E or NE in dogs can be counteracted by PGE_1. It is tempting to speculate that there may be some relationship at the biochemical level between effects of PGE_1 on E-induced lipolysis and on E-induced hypertension. However, in view of the intrinsic hypotensive action of prostaglandin, it is equally possible that its effects and those of E on blood pressure are oppositely directed but biochemically different.

SUMMARY

The most striking and possibly the most important effect of the catecholamines on lipid metabolism lies in their ability to accelerate acutely the mobilization of FFA from adipose tissue. This effect is attributable to hormone-stimulated conversion of an inactive lipase in adipose tissue to its active form. A number of lines of evidence strongly implicate cyclic 3′,5′-AMP in this activation, as described in more detail in subsequent papers of This Symposium.

It has also been shown that lipase in adipose tissue is subject to inactivation, which can be demonstrated both in the intact tissue and in homogenates. Because the effective lipase activity is thus determined by the balance of a complex dynamic system for activation and inactivation, the mechanisms for effecting net changes in lipase activity can be quite different in different circumstances. Clarification of the mechanisms involved will be difficult until further progress has been made in isolating and characterizing the several enzyme systems involved.

Several metabolic consequences flow from the primary effect of catecholamines on FFA mobilization. The uptake and utilization of FFA is probably increased in all of the peripheral tissues. Recent studies using ascites tumor cells as a model system indicate that the relevant parameter governing FFA uptake is the molar ratio of FFA to albumin. These studies show that the subsequent rate of utilization of FFA for esterification and oxidation to CO_2 is a function of the steady state "load" of FFA on the cell, the latter in turn being a function of the molar ratio of FFA to albumin in the bathing fluid.

The consequences of accelerated uptake in the liver are several: 1) a tendency

to deposition of triglycerides, of relevance in the development of fatty liver; 2) an increase in the rate of ketone body production, of relevance to the ketotic states in fasting and in diabetes; 3) a stimulation of the rate of output of lipoproteins into the plasma, of relevance in determining plasma levels of cholesterol, phospholipids and triglycerides. Since the adipose tissue can take up significant amounts of triglycerides carried in lipoproteins, a mechanism is available by which fatty acids mobilized in excess of current requirements can find their way back to the depot triglyceride stores, *i.e.*, there is probably a ***physiologic fatty acid transport cycle.***

The rate of turnover of this cycle is increased by the catecholamines, and the rates of the several metabolic processes flowing from accelerated FFA mobilization are simultaneously increased. Thus, many changes in lipid metabolism, the end results of catecholamine administration or of excessive autonomic nervous system activity, can be understood as secondary results of accelerated FFA mobilization.

REFERENCES

1. Bergström, S., Carlson L. A., Ekelund, L. G. and Orö, L.: Proc. Soc. exp. Biol. Med. **118**: 110, 1965.
2. Bergström, S., Carlson, L. A. and Orö, L.: Acta Physiol. Scand. **60**: 170, 1964.
3. Bergström, S., Duner, H., Euler, U.S. v., Pernow, B. and Sjövall, J.: Acta Physiol. Scand. **45**: 145, 1959.
4. Bezman, A., Felts, J. M. and Havel, R. J.: J. Lipid Res. **3**: 427, 1962.
5. Björntorp, P. and Furman, R. H.: Am. J. Physiol. **203**: 316, 1962.
6. Butcher, R. W.: This symposium, Section II V.
7. Carlson, L. A., Havel, R. J., Ekelund, L. G. and Holmgren, A.: Metabolism **12**: 837, 1963.
8. Carlson, L. A. and Liljedahl, Sten-Otto: Acta Med. Scand. **173**: 787, 1963.
9. Challoner, D. and Steinberg, D.: Nature **205**: 602, 1965.
10. Challoner, D. and Steinberg, D.: Clin. Res. **12**: 177, 1964; Am. J. Physiol., in press.
11. Debons, A. F. and Schwartz, I. L.: J. Lipid Res. **2**: 86, 1961.
12. Deykin, D. and Vaughan, M.: J. Lipid Res. **4**: 200, 1963.
13. Eaton, R. P., Steinberg, D. and Thompson, R. H.: J. Clin. Invest. **44**: 247, 1965.
14. Euler, U.S. v.: J. Physiol. **88**: 213, 1936.
15. Evans, W. H. and Mueller, P. S.: J. Lipid Res. **4**: 39, 1963.
16. Fain, J. N.: Biochim. Biophys. Acta **84**: 636, 1963.
17. Feigelson, E. W., Pfaff, W., Karmen, A. and Steinberg, D.: J. Clin. Invest. **40**: 2171, 1961.
18. Gidez, L. I., Roheim, P. S. and Eder, H. A.: Fed. Proc. **21**: 289, 1962.
19. Goldblatt, M. W.: J. Physiol. **84**: 280, 1935.
20. Goodman, D. S.: J. Clin. Invest. **37**: 1729, 1958.
21. Gordon, R. S., Jr. and Cherkes, A.: Proc. Soc. exptl. Biol. Med., N. Y. **97**: 150, 1958.
22. Gordon, R. S., Jr. and Fredrickson, D. S.: Cited by Gordon, R. S., Jr. in Diabetes **7**: 190, 1958.
23. Harlan, W. R., Laszlo, J., Bogdonoff, M. D. and Estes E. H.: J. Clin. Endocrinol. Metab. **23**: 33, 1963.
24. Havel, R. J., Carlson, L. A., Ekelund, L. G. and Holmgren, A.: Metabolism **13**: 1402, 1964.
25. Havel, R. J., Felts, J. M. and Van Duyne, C. M.: J. Lipid Res. **3**: 297, 1962.
26. Havel, R. J. and Goldfien, A.: J. Lipid Res. **1**: 102, 1959.
27. Heimberg, M., Weinstein, I., Klausner, H. and Watkins, M. L.: Am. J. Physiol. **202**: 353, 1962.
28. Hollenberg, C. H., Raben, M. S. and Astwood, E. B.: Endocrinology **68**: 589, 1961.
29. Ingle, D. J.: Pediatrics **17**: 407, 1956.
30. Leboeuf, B., Flinn, R. B. and Cahill, G. F., Jr.: Proc. Soc. exptl. Biol. Med., N. Y. **102**: 527, 1959.
31. Lund, A.: Acta Pharmacol. Scand. **7**: 299 1951.
32. Maickel, R. P. and Brodie, B. B.: Ann. N.Y. Acad. of Sci. **104**: 1059, 1963.
33. Markscheid, L. and Shafrir, E.: J. Lipid Res. **6**: 247, 1965.
34. Mosinger, B. and Kujalova, V.: Rev. Czech. Med. **8**: 285, 1962.
35. Neptune, E. M., Jr., Sudduth, H. C., Foreman, D. R. and Fash, F. J.: J. Lipid Res. **1**: 229, 1960.
36. Nestel, P. J. and Steinberg, D.: J. Lipid Res. **4**: 461, 1963.
37. Pilkington, T. R. E., Lowe, R. D., Robinson, B. F. and Titterington, E.: Lancet **2**: 316, 1962.
38. Randle, P. J., Garland, P. B., Hales, C. N. and Newsholme, E. A.: Lancet **1**: 785, 1963.
39. Reshef, L. and Shapiro, B.: Metabolism **9**: 551, 1960.
40. Rizack, M. A.: J. biol. Chem. **236**: 657, 1961.
41. Robinson, D. S.: Adipose Tissue. In: Handbook of Physiology, ed. by A. E. Renold and G. F. Cahill, Jr. Section 5, American Physiological Society, Washington, D.C., 1965.

42. Rodbell, M.: J. biol. Chem. **239**: 375, 1964.
43. Rudman, D., Hirsch, R. L., Kendall, F. E., Seidman, F. and Brown, S. J.: Rec. Prog. Hormone Res. **18**: 89, 1962.
44. Scow, R. O. and Chernick, S. S.: Rec. Prog. Hormone Res. **16**: 497, 1960.
45. Shafrir, E. and Steinberg, D.: J. Clin. Invest. **39**: 310, 1960.
46. Shafrir, E., Sussman, K. E. and Steinberg, D.: J. Lipid Res. **1**: 109, 1959.
47. Shafrir, E., Sussman, K. E. and Steinberg, D.: J. Lipid Res. **1**: 459, 1960.
48. Shtacher, G. and Shafrir, E.: Arch. Biochem. Biophys. **106**: 205, 1963.
49. Spector, A. A. and Steinberg, D.: J. biol. Chem. **240**: 3747, 1965.
50. Spector, A. A., Steinberg, D. and Tanaka, A.: J. biol. Chem. **240**: 1032, 1965.
51. Steinberg, D. In: Control of Lipid Metabolism, ed. by J. K. Grant, Vol. 24, p. 111, Academic, Press, Inc, London, 1963.
52. Steinberg, D., Nestel, P. J., Buskirk, E. R. and Thompson, R. H.: J. Clin. Invest. **43**: 167, 1964.
53. Steinberg, D. and Pittman, R.: Unpublished experiments.
54. Steinberg, D., Vaughan, M. and Margolis, S.: J. biol. Chem. **236**: 1631, 1961.
55. Steinberg, D., Vaughan, M., Nestel, P. and Bergström, S.: Biochem. Pharmacol. **12**: 764, 1963.
56. Steinberg, D., Vaughan, M., Nestel, P., Strand, O. and Bergström, S.: J. Clin. Invest. **43**: 1533, 1964.
57. Strand, O., Vaughan, M. and Steinberg, D.: J. Lipid Res. **5**: 554, 1964.
58. Vaughan, M.: J. biol. Chem. **236**: 2196, 1961.
59. Vaughan, M.: J. biol. Chem. **237**: 3354, 1962.
60. Vaughan, M.: This Symposium, Section II I.
61. Vaughan, M., Berger, J. E. and Steinberg, D.: J. biol. Chem. **239**: 401, 1964.
62. Vaughan, M. and Steinberg, D.. J. Lipid Res. **4**: 193, 1963.
63. Vaughan, M., Steinberg, D., Lieberman, F. and Stanley, S.: Life Sci. **4**: 1077, 1965.
64. Vaughan, M., Steinberg, D. and Pittman, R.: Biochim. Biophys. Acta **84**: 154, 1964.
65. Wertheimer, H. E.: Pflüg. Arch. ges. Physiol. **213**: 262, 287, 1926.
66. White, J. E. and Engel, F. L.: Proc. Soc. exptl Biol. Med., N. Y. **99**: 375, 1958.