J. Lipid Research, October, 1961
Volume 2, Number 4

Review

The metabolism of adipose tissue *in vitro*

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[Received for publication July 18, 1961]

Although the vital role of adipose tissue as a reservoir of fat which can be mobilized to provide energy has long been recognized, it was assumed until relatively recently that the tissue is to a large extent metabolically inert. By 1948 there was available a considerable body of data derived from physiological studies which indicated that adipose tissue is not an inactive storehouse. This view was stressed by Wertheimer and Shapiro (1) in their classic review, despite absence of precise information concerning the enzymes and metabolic pathways in the tissue.

An important barrier to the understanding of adipose tissue metabolism and its relationship to the physiology of the intact organism was the lack of knowledge of the mechanism by which fat is mobilized from adipose tissue and transported to other tissues. Favarger (2) had theorized in 1949 that free fatty acids (FFA) might be the form in which fat is released from adipose tissue and transported in the blood. The first clear-cut evidence in support of this hypothesis came from the studies of Gordon (3, 4), who correlated the concentration of free fatty acids in serum with changes in nutritional state and interpreted these data as evidence that fat is released from adipose tissue and transported in the blood as FFA. Similar observations were also reported by Dole (5). These and numerous subsequent studies were made possible by the development of reliable, relatively simple methods for quantification of free fatty acids in serum and other biological media by Gordon (4) and by Dole (5). The demonstration that fatty acid release and other metabolic processes are influenced by hormones during incubation *in vitro* has led to widespread interest in the activities of adipose tissue.

There are obvious differences in the metabolism of adipose tissue at different locations in the body. Certain depots are depleted more rapidly during starvation and, conversely, certain areas participate little if at all in the development of generalized obesity. To what extent these differences are due to nervous regulation or to variations in vascularity or control of blood supply is not known. The former is undoubtedly of basic importance in the regulation of adipose tissue metabolism in the intact animal. When tissues are studied *in vitro*, these factors are obviously inoperative except in so far as they have affected the composition (chemical and/or enzymatic) of the particular sample of tissue prior to its excision for study.

Several investigators have compared specific aspects of the metabolism of rat adipose tissue derived from different anatomical sites.[1] In studies of fatty acid synthesis Shapiro and Wertheimer (6) found equivalent activity in mesentery and in groin fat. Minced preparations of epididymal, perirenal, and subcutaneous adipose tissue incorporated roughly equal amounts of glucose carbon into fatty acids (7). Glucose uptake and the effects of insulin on this process were of the same magnitude in tissues from subcutaneous, genital, mesenteric, and perinephric regions (8). Using a manometric method, Ball and Cooper (9) found that the responses of epididymal, mesenteric, and perinephric tissues to insulin were quantitatively alike. Adipose tissues from these same sites obtained from female rats (substituting inguinal fat for epididymal) had equivalent activities and were not grossly different from the tissues of male rats in their response to insulin. It has

[1] In all of these statements and in the discussion that follows, "adipose tissue" refers to white (or yellow) adipose tissue. Brown adipose tissue is distinctly different in location, morphology, composition, etc. A few metabolic differences are reported in the studies comparing adipose tissue from various locations (6, 7, 9). In all studies, with exceptions noted in the text, rat tissue was used. Studies of the metabolism *in vitro* of adipose tissue from animals other than the rat are so few that there is no basis for discussion of species differences.

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been found, however, that fatty acid synthesis from glucose and the oxidation of carbon-1 of glucose is greater in adipose tissue from female than from male rats (10).

The information to be summarized here pertains chiefly to the metabolism of the epididymal fat pad which is a particularly convenient source of adipose tissue for study *in vitro*. There is no reason to believe that its metabolism *in vitro* differs significantly from other adipose tissue of the male rat. On the other hand, it is reasonable to assume that the metabolism of adipose tissue from female rats will differ in some particulars.

In this survey, which is based largely on *in vitro* observations, no explicit attempt has been made to assess the physiological significance of the findings. As the available information is summarized and interpreted, however, certain correlations and questions will emerge concerning adipose tissue metabolism and its regulation in the intact animal.

Fatty Acid Metabolism

Reactions leading to the accumulation or removal of FFA in the adipose tissue cell are shown schematically in Figure 1. Release of FFA *in vitro* is readily demonstrable. Although transfer of FFA into the cell can be observed in *in vitro* studies, there is no evidence that

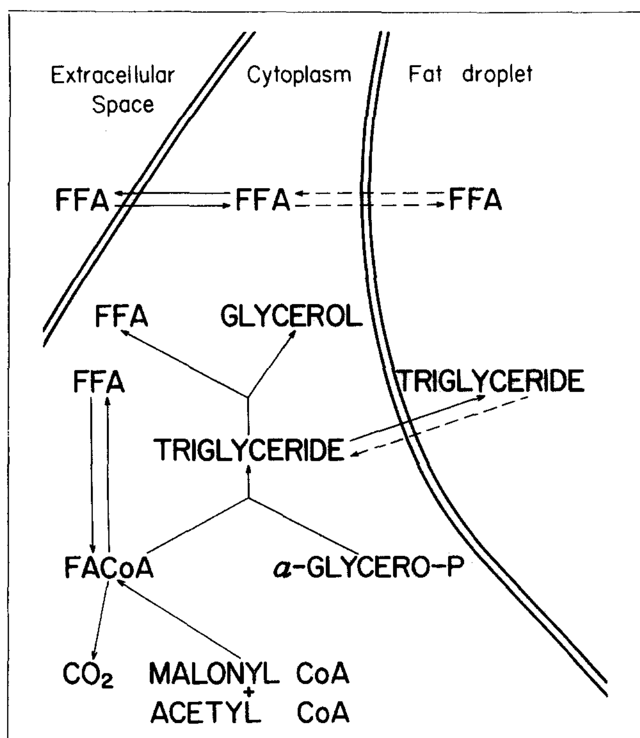

Fig. 1. Production and removal of free fatty acids in adipose tissue.

net uptake of serum FFA by adipose tissue occurs *in vivo*. It is probable that under most conditions *in vivo*, when there is net movement it is in the outward direction.

Fatty acid synthesis is an important function of adipose tissue *in vivo*. The rates of fatty acid synthesis and degradation observed during incubation *in vitro* suggest, however, that these reactions are not involved in the regulation of FFA accumulation and release. It is pertinent in this regard to note that when fatty acid synthesis is most active, fatty acid esterification is also active, and fatty acid release is minimal. Conversely, fatty acid synthesis (and the rate of esterification) is minimal under conditions where FFA release is highest.

Adipose tissue has a large store of triglyceride which, when acted upon by one or another of the lipases found in this tissue, provides the major source of FFA for release. There is considerable evidence to support the view that triglyceride breakdown and resynthesis go on constantly. It is obvious, then, that regulation of the rate of FFA accumulation or release could be effected by altering the rate of lipolysis and/or the rate of esterification. Both of these mechanisms are apparently utilized. Increased FFA release caused by certain hormones is probably associated with increased lipolysis. The suppression of FFA accumulation resulting from the availability of glucose for metabolism likely results from an increase in the rate of esterification of FFA. In this regard it is important to remember that the products of lipolysis, FFA and glycerol, are not the immediate substrates for the esterification reactions. The FFA must first be activated to form fatty acyl coenzyme A derivatives, which in turn react with α-glycerophosphate, not glycerol, in the first steps of glyceride synthesis. Glycerol cannot be utilized to any significant extent by adipose tissue. Thus, the glycerol produced by lipolysis accumulates, providing an index of the amount of triglyceride breakdown; and re-esterification of the fatty acids constantly produced by lipolysis depends on a continuing supply of glucose (or other equivalent substrate) to provide α-glycerophosphate.

The dotted arrows in Figure 1 indicate questions concerning FFA pools and reactions within the adipose tissue cell. The FFA involved in different reactions are indicated separately to suggest that although the tissue fatty acids are frequently referred to as an entity, there is good evidence for the presence of more than one kinetically distinguishable pool. It should not be inferred, however, that each of the "FFA's" on the diagram represents a specific discrete pool. Furthermore, although it is almost certain that at least

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a portion of the tissue FFA is dissolved in the lipid phase of the fat droplet, it is not known how this is related functionally to one or another of the metabolic pools of FFA.

It is generally accepted that triglyceride is synthesized in the cytoplasm of the adipose cell and then transferred into the fat droplet where it is stored. It is not known whether triglyceride is transported back to the cytoplasm for hydrolysis or whether there is lipolytic activity at the surface of the fat droplet, so that intact triglycerides do not leave the fat droplet. Movement of triglycerides across the cell membrane is not included in the diagram but some data relating to this question are discussed below.

Figure 1 offers a highly schematic picture of the adipose tissue cell. It, however, serves to emphasize the reactions which are the basis of the most obvious metabolic activities of this tissue.

FATTY ACID METABOLISM IN INTACT TISSUE

Fatty Acid Release. Data from a number of studies of the release of fatty acids by adipose tissue have been reviewed by Engel and White (11) and by Wertheimer and colleagues (12, 13). Some general observations about FFA release are summarized here as a background for discussion of specific information in subsequent sections. The mechanisms through which the effects of hormones on FFA release and on other aspects of metabolism are produced and interrelated are considered below.

In blood, FFA are almost completely bound to albumin (14). In order to demonstrate FFA release from adipose tissue *in vitro*, albumin must be present in the medium (15). The interaction of FFA with albumin has been studied in detail by Goodman (14), who interpreted his data in terms of three classes of binding sites for FFA on the albumin molecule; one group consisting of two sites with a very high association constant, another group of five sites with a somewhat lower constant, and a third group consisting of a much larger number of sites (of the order of twenty) with a much lower association constant. The concentration of unbound FFA, i.e., the chemical potential of FFA, in solutions containing albumin is a function of the mole ratio of fatty acids to albumin. From the mole ratio using the apparent association constants for the binding reactions it is possible to estimate the concentration of unbound FFA.

The chemical potential of FFA in the tissue cannot be approximated in any similar way. Concentrations of FFA in the adipose tissue range from 1 to 2 μmoles per gram in the fed state and 2 to 3 μmoles per gram in fasting, up to 8 to 10 or more μmoles per gram in tissues that have been incubated with epinephrine (16, 17, 18). A fraction of the total FFA is probably bound to protein in the cytoplasm. Although the quantity and properties of the binding protein are unknown, the amount of FFA in this location must be presumed to be small since the total protein of adipose tissue is low. A large portion of the tissue FFA must be contained in the fat droplet which, as an organic phase, extracts FFA from the cytoplasm (and perhaps also directly from the extracellular space).[2] After homogenization of the tissue, a large fraction of the FFA is found in the fat layer separated by centrifugation. The extent to which this is a reflection of the distribution of FFA in the intact cell is not known and is difficult to determine experimentally. Knowledge of the distribution and state of the FFA within the adipose tissue will be required, however, in order to understand their effects on cellular metabolism.

The amount of FFA in the tissue at any time is the dynamic resultant of metabolic factors that influence the quantity of FFA added to and removed from the cell. If movement of FFA in and out of the cell is not an active process, then in an *in vitro* system FFA should be distributed between medium and tissue in such a way that the chemical potential is the same in both compartments. Thus, the net movement of fatty acids will be determined by the chemical potential of fatty acids in the tissue relative to that in the medium. The effect of the concentration of FFA in the medium on net movement is easily demonstrated (15, 19). At low concentrations of FFA in the medium there is net release which ceases as the concentration is increased. With further increases in medium FFA concentration there is net uptake of FFA by the tissue. By keeping the amount of FFA in the medium constant and altering the amount of albumin, it would also be possible to influence the movement of FFA.

The quantitative relationship between concentration of FFA in the medium and net movement is a function of tissue metabolism. There is net release of FFA from adipose tissue of fasted rats into medium containing added FFA (20). Tissues from fed rats, however, release little FFA even into medium containing none (20). In the presence of epinephrine, output of fatty acids continues despite medium concentrations much higher than those which reverse net movement in the absence of the hormone. These differences reflect differences in effective FFA concentration (chemical potential of

[2] The cytoplasm is accumulated in one area of the adipose tissue cell. Over the surface of most of the fat droplet there is no detectable layer of cytoplasm.

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fatty acids) in the tissue, maintained by cell metabolism.

It is obvious that the chemical potential of FFA in neither compartment is constant during incubation of tissues *in vitro*. It has been found that under certain conditions the accumulation of FFA in the medium tends to stop after about 1 hour of incubation (21, 22). When incubation is continued for a total of 3 hours, some of the FFA initially released may be taken up again by the tissue. If, on the other hand, the tissue is transferred at the end of 1 hour to fresh medium (containing no FFA), release of FFA is continued at the initial rate for another hour. In addition to indicating again the role of the medium in the release process, these findings suggest that metabolic adjustments occur in association with changes in FFA movement. This is borne out by data from other studies. It has been observed that during the first 30 minutes of incubation of epididymal fat pads from fasted rats, the amount of FFA that appears in the medium is roughly equivalent to the decrease in tissue FFA content that occurs at the same time, i.e., there is no net production of FFA in this time. At the end of 60 minutes of incubation, more FFA is accumulated in the medium and the concentration of FFA in the tissue is restored to the zero time level, i.e., there is net production of FFA during the second 30 minutes of incubation.[3] Metabolic changes associated with fatty acid movement can be demonstrated also when tissues incubated with and without albumin in the medium are compared. The concentration of FFA is higher in tissues incubated in the absence of albumin. The amount of extra FFA in those tissues is, however, less than the amount of FFA found in the medium containing albumin. This suggests that the elevation of FFA concentration in the tissue without albumin retards further accumulation.[3]

Relatively minor differences in experimental conditions, e.g., in the amount of or the FFA content of the albumin used, or the time of incubation, can produce effects on cell metabolism. These findings lend support to the view that certain of the effects of epinephrine and other hormones with similar action are the result of the elevation of intracellular FFA concentration which they produce (23, 24). The results of studies carried out in adipose tissue homogenates or cell fractions also may be influenced by the concentration of FFA in the tissues from which they were prepared. This can be an important variable whether or not incubation of tissues has been carried out prior to homogenization.

As used in this discussion, FFA refers collectively to the several long-chain fatty acids found in adipose tissue. The fatty acids comprising this mixture differ in solubility and other physicochemical properties. Goodman (14) has observed that there is considerable difference in the association constants for the interaction of albumin with different fatty acids. It is to be expected that there are differences in the physiological behavior of the specific fatty acids. Preliminary observations of Watson and Margolis[4] indicate that the fatty acid composition of FFA released from adipose tissue during incubation differs significantly from that of the glyceride fatty acids of the tissue.

Uptake and Utilization of Fatty Acids. Stern and Shapiro (25) first demonstrated the uptake and esterification of stearic acid-1-C^{14} by adipose tissue incubated *in vitro*. Shapiro and co-workers (26, 27, 28) extended these observations and reported that the nutritional state of animals from which tissues were obtained markedly affected the quantity of C^{14}-FFA taken up and esterified *in vitro*. In general, more than 90% of the C^{14}-FFA taken up by tissues from fed rats is found in the ester (triglyceride) fraction at the end of the incubation. Tissues from fasted rats take up less C^{14}-FFA and of the amount contained in the tissue, less than 70% is in the glyceride fraction[5] (27). The uptake and esterification of C^{14}-FFA by tissues from fasted rats takes place simultaneously with a net release of FFA, whereas the tissues from fed rats release little or no FFA.

Incubation in nitrogen inhibits incorporation of palmitic acid-1-C^{14} into glycerides by only 60%. The addition of 0.01 M sodium fluoride to tissues incubated in nitrogen causes little more inhibition, although in the presence of both 0.1 M sodium fluoride and nitrogen, incorporation of C^{14}-palmitate was almost completely abolished. It is interesting that there is little or no increase in FFA release associated with inhibition of C^{14}-palmitate incorporation by nitrogen and/or fluoride. This means either that fatty acid production (lipolysis) is inhibited along with esterification or that the rate of breakdown and resynthesis of triglycerides is so slow that what appears to be almost complete inhibition of esterification does not lead to a sizable accumulation of FFA during the incubation period. Similarly, apparently large effects of glucose on FFA esterification are associated with relatively small net changes in FFA release (29).

The incorporation of palmitic acid-1-C^{14} into tissue glycerides is increased by small amounts of glucose (0.625 μmole/ml) (19). When glucose (or glucose plus insulin) is added to the medium, the rate of FFA release is decreased and there may be, in fact, a net disap-

[3] M. Vaughan and D. Steinberg, unpublished observations.

[4] W. C. Watson and S. Margolis, unpublished observations.

[5] D. Steinberg, M. Vaughan, and S. Margolis, unpublished observations.

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pearance of FFA (20, 30, 31). The finding that glycerol release continues when FFA release is decreased by glucose (32) suggests that lipolysis continues unchanged, leading to the formation of FFA and glycerol, but that in the presence of glucose the rate of triglyceride synthesis is enhanced so that little or no FFA accumulates.

When the concentration of fatty acids in the medium is increased, an increase in net uptake of fatty acids can be observed (see above). The extra FFA removed from the medium is not accumulated in the tissue and this, together with data on the incorporation of palmitic acid-1-C^{14} into triglyceride in similar experiments (19), suggests that the rate of fatty acid esterification is increased.

It has been found that about 3% of the C^{14}-palmitate present in the tissue at the end of a 1-hour incubation period is in the phospholipid fraction and is recovered in one component after chromatography of this fraction on silicic-acid paper (33). The percentage of tissue radioactivity in this fraction was the same in tissues from fed as from fasted rats, i.e., there was more radioactive palmitate in the phospholipid fraction in the tissues from fed rats. The addition of glucose to the medium had considerably less effect on incorporation of palmitate into phospholipid than it did on its incorporation into triglyceride determined in the same tissue.[6] If glucose stimulates triglyceride synthesis by acting as a precursor for α-glycerophosphate and/or a source of energy for fatty acid activation, then it must be concluded that neither the availability of α-glycerophosphate nor the rate of fatty acid activation is the factor limiting incorporation of palmitate into phospholipid in these experiments.

In the case of the glucose effect, all available evidence supports the interpretation that the increased incorporation of C^{14}-palmitate into neutral lipid results from an increase in the rate of triglyceride synthesis. It is not possible, however, to deduce the true rate of fatty acid esterification in experiments such as those described above since the specific activity of the fatty acids of the precursor pool is not known. From the previous discussion of the state of FFA in adipose tissue, it seems clear that the total FFA of tissue is not the precursor pool. Circumstantial evidence in support of this conclusion is obtained from attempts to calculate a minimum value for the amount of fatty acid esterified, using the specific activity of the tissue FFA at the end of the experiment. (The specific activity of the tissue FFA is zero at the beginning of the experiment, rises relatively slowly, and even at the end of a 3-hour incubation period is only a very small fraction of the specific activity of the medium fatty acid [29, 34]). In many instances the rates of esterification calculated in this way are implausibly high, suggesting that the specific activity of the true precursor pool is much higher than that of the total tissue fatty acids.

Kerpel *et al.* (34) have attempted to assess the extent of participation of the tissue FFA in the incorporation of C^{14}-palmitate from the medium into tissue glycerides and in the formation of medium fatty acids from tissue glycerides. They have concluded that "the tissue free fatty acids do not seem to take a prominent part in assimilation and esterification." In addition, they have presented evidence for the presence of a triglyceride compartment associated with a particulate fraction which had a much higher specific activity than the triglyceride of the floating fat, and have suggested that this "active" glyceride compartment interacts directly with the medium fatty acids without the participation of the tissue FFA. There seems to be agreement on the fact that the total adipose tissue FFA is not the precursor pool for glyceride synthesis, but it is quite possible that the tissue contains a pool that is the precursor.

In an attempt to demonstrate the existence within the adipose tissue cell of fractions containing fatty acids of different specific activity, homogenates of tissues incubated for short periods with C^{14}-palmitate were separated into three fractions by centrifugation.[6] Table 1 contains representative data from a few such studies, showing distinct differences in the specific activity of fatty acids of cell fractions separated in this crude fashion. It is not presumed that any of these fractions represents a single metabolic pool or a specific combination of metabolic pools. No one of them has a

TABLE 1. SPECIFIC ACTIVITY OF FATTY ACIDS IN CELL FRACTIONS AFTER INCUBATION OF ADIPOSE TISSUE WITH PALMITIC ACID-1-C^{14}

	FFA Specific Activity (cpm/μEq FFA)			
Exp. No.	Whole Homogenate	Sediment	Solution	Fat
1	1980	3050	390	921
2	1530	4740	730	1060
3	1260	2690	692	869
4*	800	3420		590
5*	1110	3860	700	739

Both fat pads from each rat (fasted) incubated together for 20 minutes in medium containing 0.25 or 0.5 μc of palmitic acid-1-C^{14}. Fractions separated by centrifugation of homogenized tissue for 30 minutes at 15,000 $\times$ g, at 4°.

* Medium in which tissues were incubated contained epinephrine 0.1μg/ml.

[6] M. Vaughan, unpublished observations.

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specific activity which would be expected for the pool that is the precursor of triglyceride. The composition of these fractions is not known; certainly all are heterogeneous. Even if there are functionally and kinetically distinguishable pools, the possibility of isolating one or another is questionable, since the latter achievement depends on the association (maintained throughout the isolation procedure) of a specific pool with a morphologically distinguishable and isolatable cell fraction. Although the fatty acids in the sediment have the highest specific activity, it is possible that one or another of the fractions includes a very small pool of very high specific activity.

Table 2 contains data of another type, indicating a practical problem and a potential artifact in many kinds of experiments with intact tissues.[6] Intact epididymal fat pads were incubated with palmitic acid-1-C^{14}, then divided into two pieces, a thick (proximal) portion and a thin (distal) portion. Each piece was homogenized separately and the glyceride and fatty acid fractions isolated. The explanation for the differences in fatty acid specific activity in different portions of the same tissue is not readily apparent, but the generally greater accumulation of C^{14} in glycerides of the thin portion, when expressed per gram of tissue, strongly suggests that there may be areas of the thick portion that participate to a variable extent in the esterification of the labeled fatty acid from the medium. In the presence of gradients within the tissue, e.g., only a portion of the cells participating in fatty acid uptake and release, the specific activity of the total tissue fatty acid is not a relevant datum.

If the interpretation of Kerpel *et al.* (34) were correct, i.e., if there were "direct interchange between medium fatty acids and tissue fatty acid esters," it would be possible to approximate (estimate) rates of fatty acid esterification, although the calculation would be complicated, however, by the fact that the specific activity of the medium FFA decreases throughout the incubation. In experiments where there is uptake of labeled fatty acid (tracer amount) associated with net release of FFA, the specific activity of the medium FFA may decline to 25% of its initial value in 1 hour.[3] Changes in medium specific activity have been amply documented by Kerpel *et al.* (34), who observed that even when there was no net release of fatty acids (tissues from fed rats incubated in medium containing glucose), the specific activity of the medium fatty acids fell to less than 40% of the zero time value after 3 hours. Assuming that the medium FFA is very nearly in equilibrium with the precursor pool, it is apparent that calculations of esterification based on the initial medium specific activity would be of questionable usefulness, especially when attempting to compare rates of synthesis in two tissues where there have been gross differences in the change of specific activity of medium fatty acids with time.

At present it seems probable that there is in adipose tissue a pool of fatty acids that is the precursor for triglyceride. It is most likely small and must be of much higher specific activity than the total tissue FFA. Perhaps it is very nearly in equilibrium with the medium fatty acids. The presence of another pool of tissue FFA comprising FFA from triglyceride breakdown in the process of release may also be postulated. The relationship of FFA in the fat droplet to these or other possible metabolic pools remains unknown.

TABLE 2. DISTRIBUTION OF RADIOACTIVITY FROM C^{14}-PALMITATE WITHIN THE EPIDIDYMAL FAT PAD

Tissue No.		Weight	Tissue FFA		Tissue Glycerides
		mg.	*μeq/g*	*cpm/μeq*	*cpm/g*
1	Thick	305	1.8	2,060	3,930
	Thin	370	2.0	1,260	6,510
2	Thick	290	2.2		2,110
	Thin	408	1.9	1,575	7,750
3	Thick	272	1.8	2,120	3,220
	Thin	445	1.7	1,450	3,820
4	Thick	318	1.9	1,950	3,640
	Thin	383	1.6	1,400	3,510
5*	Thick	250	1.4	7,410	38,800
	Thin	256	1.8	1,250	66,900
6*	Thick	359	1.3	2,280	31,100
	Thin	195	1.9	2,790	42,600
7*	Thick	200	1.5	3,950	39,200
	Thin	574	1.1	3,010	57,900
8*	Thick	297	1.2	7,690	
	Thin	475	1.3	1,150	

Each fat pad (intact) was incubated for about 30 minutes at 37° in 3 ml medium containing 0.25μc palmitic acid-1-C^{14}. It was then rinsed, blotted, and divided into a thick (proximal) and thin (distal) portion, each of which was then weighed, homogenized, and the lipids extracted and fractionated.

* In these experiments the albumin in the medium had been extracted to free it of FFA, i.e., the specific activity of medium FFA was much higher than it was in Nos. 1–4.

Fatty Acid Synthesis and Oxidation. The synthesis of fatty acids from various isotopically labeled precursors in intact adipose tissue has been extensively studied. Winegrad *et al.* (35) considered in detail much of the available data on lipogenesis in adipose tissue. Only a brief summary is presented here. Some of the pertinent findings will be mentioned later in considering the effects of hormones, several of which

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have been shown to influence fatty acid synthesis. Shapiro and Wertheimer (6) first demonstrated the synthesis of fatty acids in adipose tissue by measuring the incorporation of deuterium from deuterium oxide. Fatty acid synthesis from C^{14}-labeled acetate, pyruvate, malonate, lactate, acetaldehyde, propionate, and isoleucine has been studied in a number of laboratories (21, 36 to 45). Utilization of glucose for fatty acid synthesis is discussed along with other aspects of glucose metabolism (see below).

Lipogenesis is markedly depressed in adipose tissue from starved rats and also in tissue from rats that have been maintained on a high fat, low carbohydrate diet (7, 39). Fatty acid synthesis from acetate is essentially zero in tissues from starved rats and is only partially restored by the addition of glucose to the medium (39). Furthermore, glucose uptake is low and fatty acid synthesis from glucose itself is diminished in tissues from fasted rats (39, 46). Presumably the effect of fasting on lipogenesis is a manifestation of the close relationship between the rate of fatty acid synthesis and the amount of glucose metabolized through the oxidative pathway. The correlation between lipogenesis and activity of the phosphogluconate shunt has been observed in other tissues under a variety of conditions. As noted earlier, adipose tissue from female rats, which oxidizes carbon-1 of glucose more rapidly than does tissue from male rats, also exhibits a greater rate of lipogenesis (10). When the amount of glucose metabolized through the oxidative pathway is increased by insulin or by increasing medium glucose concentration, fatty acid synthesis is stimulated (43, 47, 48). On the other hand, under conditions in which glucose uptake is low, or when glucose utilization, though high, proceeds to only a limited extent through the phosphogluconate shunt (e.g., in the presence of epinephrine), fatty acid synthesis is depressed (23, 49). It is not known through what mechanism this relationship is maintained. A number of workers have considered that the rate of fatty acid synthesis is in some way dependent upon, or regulated by, the availability of TPNH generated in the oxidation of glucose-6-phosphate. Leboeuf and Cahill (50) have suggested, alternatively, that the availability of acetyl-CoA for fatty acid synthesis influences the pathway taken by glucose.

It may be noted that although liver and adipose tissue respond to fasting similarly with a marked depression of fatty acid synthesis, the two tissues differ in their response to other variables. Thus, exposure of rats to cold, which abolishes hepatic lipogenesis (51), has very little effect on lipogenesis in adipose tissue (52). The latter is also unaffected by the addition of ethanol, which stimulates fatty acid synthesis from acetate in liver slices. (The absence of an effect of ethanol in adipose tissue may be related to the fact that ethanol-1,2-C^{14} is apparently not oxidized in this tissue [53].) The addition of insulin *in vitro* does not restore fatty acid synthesis in slices of liver from diabetic rats (54), whereas the depression of fatty acid synthesis in the adipose tissue can be relieved by addition of insulin to the incubation medium (35, 43).

Oxidation of C^{14}-labeled fatty acid by adipose tissue *in vitro* has been observed in several laboratories (19, 21, 26, 27, 55). The amount of fatty acid oxidized is not large and the formation of $C^{14}O_2$ from labeled fatty acid is depressed by the addition of small amounts of glucose to the medium (19). Shapiro and co-workers (26, 27) found that the fraction of labeled fatty acid taken up by the tissue that was converted to carbon dioxide was larger in tissues from fasted rats than in tissues from fed rats. The actual amount oxidized was, however, approximately equal in both types of tissues. Production of $C^{14}O_2$ from palmitic acid-1-C^{14} was roughly proportional to the total concentration of fatty acids in the medium (19). For reasons discussed above, however, it is difficult to calculate in such experiments the actual rates of fatty acid oxidation.

Uptake and Release of Triglycerides. It is generally agreed that adipose tissue *in vivo* derives its supply of preformed fatty acids from the triglycerides of low density lipoproteins. A role for lipoprotein lipase in this process has been postulated. It has been suggested that lipoprotein lipase situated on the endothelium of capillaries in adipose tissue (or in some similar position near the circulating blood) hydrolyzes triglycerides of the lipoprotein, yielding FFA and perhaps partial glycerides, which then readily pass from the vascular space into the adipose tissue cells (56).

Such a mechanism would likely not be observable in *in vitro* studies, since under these conditions the substrate is not delivered to the adipose cells through the capillary circulation. Thus, Rodbell (57) concluded on the basis of experiments in which epididymal fat pads were incubated in medium containing chylomicrons or a synthetic triglyceride emulsion that triglyceride uptake might be occurring by pinocytosis. Addition of diisopropyl fluorophosphate, an inhibitor of lipoprotein lipase, to the medium did not interfere with uptake. Furthermore, the triglycerides were apparently taken up intact without prior hydrolysis. Following uptake by the tissue into a compartment no longer exchangeable with triglycerides in the medium, the triglycerides were apparently hydrolyzed and fatty acids re-esterified prior to storage in the fat droplet. In tissues from fasted rats the process of hydrolysis and re-esterification was

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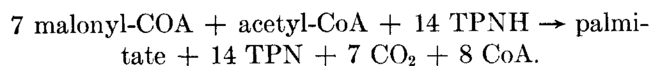
less active than it was in tissues from fed rats, although the uptake of triglycerides was the same in the two groups.

It has been generally assumed that triglycerides are not released from the adipose tissue cell. Just as the release of fatty acids requires the presence of albumin, triglyceride release into an aqueous medium is not likely to occur in the absence of a suitable acceptor. The nature of the "suitable acceptor," if one exists, is unknown, but lipoprotein comes to mind as a reasonable possibility and it has been reported by Shapiro (28) that release of triglyceride can be demonstrated when β-lipoprotein is included in the incubation medium. This process has not been further characterized and, like the problem of triglyceride uptake, offers many questions for solution.

ENZYMATIC PATHWAYS OF FATTY ACID METABOLISM

Fatty Acid Synthesis. Wakil (58) has recently summarized in detail the information concerning the mechanisms of fatty acid synthesis in living organisms. The mitochondrial system which seems to be concerned chiefly with the addition of two carbon units to existing fatty acids has not been studied in adipose tissue. Ganguly (59) studied the distribution of the nonmitochondrial or malonyl-CoA system in various animal tissues. In a comparison of beef tissues, when activity was expressed per milligram of tissue protein, the tissues with highest activity were suprarenal fat, mammary gland, and adipose tissue. Brain was the only other tissue with activity in a similar range. In the past year Martin *et al.* (60) have described the partial purification of this enzyme system from the supernatant solution obtained by centrifugation of homogenates of rat epididymal fat pad for 60 minutes at $105{,}000 \times g$. They have determined that when malonyl-CoA, acetyl-CoA, and TPNH are incubated with the enzyme, palmitic acid is the principal product. The ratio of malonyl-CoA to acetyl-CoA utilized for palmitic acid synthesis is 7:1, indicating that the over-all reaction is:



$$\text{7 malonyl-COA} + \text{acetyl-CoA} + \text{14 TPNH} \rightarrow \text{palmitate} + \text{14 TPN} + 7\ CO_2 + \text{8 CoA}.$$

It has been suggested that the acetyl moiety from acetyl-CoA forms only the methyl terminus of the palmitate molecule. The rest of the molecule is formed stepwise with two carbons derived from each malonyl-CoA. Consonant with this interpretation, it has also been shown that when a branched-chain or an odd-numbered fatty acyl-CoA derivative is substituted for acetyl-CoA, the corresponding long-chain fatty acid is formed with the acetyl-CoA substitute in the methyl terminal position (61). It is of interest that in all instances, whatever the nature of the acyl-CoA acceptor, the fatty acid produced contained 15, 16, or 17 carbon atoms. It differs in this regard from the yeast enzyme system described by Lynen (62), which yields chiefly fatty acids of 18 carbons in length. Presumably, the palmitic acid and any other fatty acids that are synthesized by this system can be elongated by the mitochondrial system.

There is no evidence available concerning the presence or absence of a soluble system for synthesis of short-chain fatty acids analogous to that described in mammary gland by Hele *et al.* (63). The older literature contains a few reports of enzymes that desaturate fatty acids (64 to 67), but the mechanism by which double bonds are introduced into fatty acids in animal tissues is still under investigation and the system in adipose tissue has not been characterized.

Fatty Acid Esterification: Glyceride Synthesis. The synthesis of glycerides by homogenized adipose tissue has been investigated in detail by Steinberg and co-workers (68, 69). Their observations are compatible with a pathway similar in all respects to that previously described in liver homogenates (70 to 73).

(1) Palmitate + ATP → palmityl-CoA + AMP + PP
(2) 2 Palmityl-CoA + α-glycerophosphate → phosphatidic acid
(3) Phosphatidic acid → diglyceride + phosphate
(4) Diglyceride + palmityl-CoA → triglyceride

Table 3 indicates the composition of the system used in studies of triglyceride synthesis in adipose tissue homogenates and the effects of omission of one or another of the components. All of the enzymes required for the synthesis of triglyceride are found in a particulate fraction of the adipose tissue homogenate prepared by centrifugation at $105{,}000 \times g$ for 1 hour. Active particles can be obtained after suspension and resedimentation of this fraction, although such preparations rapidly lose activity if stored in dilute suspension for a few hours in the cold.

In studying some of these reactions in adipose tissue microsomes, it has been possible to substitute palmityl-CoA for ATP and CoA[7] (74). In this and other homogenate fractions, however, the utilization of acyl-CoA is complicated by the presence of acyl-CoA deacylase activity.[8] Shapiro and co-workers (26), using the hydroxamate method, demonstrated the presence in extracts of adipose tissue of a long-chain fatty acid activating system dependent on the addition of ATP

[7] S. Margolis and M. Vaughan, unpublished observations.
[8] S. Margolis, unpublished observations.

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and CoA. This is perhaps the same enzyme that catalyzes reaction 1 in the system described in Table 3 and has been referred to as fatty acyl-CoA synthetase (75) or acyl-CoA thiokinase (76).

In the absence of added α-glycerophosphate there is always some palmitate esterified, even in dialyzed homogenates. This may be due to the presence of small amounts of α-glycerophosphate in the enzyme preparation. It has been found that dialysis under conditions used in preparation for the incorporation studies removes only two-thirds of a tracer amount of α-glycerophosphate-1-C^{14} added to the homogenate. Adipose tissue contains 0.1 to 0.2 μmole α-glycerophosphate per gram tissue (wet weight).[8] Diglycerides, present or formed in the homogenate, also may serve as acceptors for the small amount of fatty acid esterified in the absence of added α-glycerophosphate.

TABLE 3. Incorporation of Palmitic Acid-1-C^{14} into Neutral Lipid by Adipose Tissue Homogenate

Additions	Relative Count Incorporation	
	Whole Homogenate	Defatted Homogenate
Complete system*	100	100
Complete system minus ATP	0	1
Complete system minus CoA	4	7
Complete system minus αGP	27	17
Complete system minus Mg^{++}	35	57
Complete system minus cysteine	51	
Complete system minus NaF	32	56
Complete system minus buffer	0	
Complete system with Tris buffer in place of phosphate		93
Complete system minus creatine phosphate	58	
Complete system minus ATP plus 2 μmoles of ADP and 7.5 μmoles of creatine phosphate		45
None	0	
Buffer only	2	
Complete system, held at 100° for 5 minutes	0	

* Each flask contained 2 ml of a 1:5 homogenate of epididymal fat pad in 0.15 M KCl (representing about 400 mg wet weight of adipose tissue) with 10 μmoles of αGP, tracer amount (less than 0.01 μmole) palmitic acid-1-C^{14}, potassium salt, and the following cofactors: 2 μmoles of ATP; 3 μmoles of $MgCl_2$; 0.1 μmole of CoA; 25 μmoles of cysteine; 125 μmoles of NaF; 125 μmoles of potassium phosphate buffer, pH 7.0; water to make a final volume of 3 ml. In addition, incubation done with the whole homogenate contained 7.5 μmoles of creatine phosphate; incubations with the defatted homogenate contained no creatine phosphate except where indicated. Incubation carried out in 25-ml Erlenmeyer flasks for 20 minutes at 37° with shaking.

Reproduced by permission from (69), Steinberg, D., M. Vaughan and S. Margolis. *J. Biol. Chem.* **236**: 1631, 1961.

Labeled phosphatidic acid has been isolated after addition to the system of either palmitic acid-1-C^{14}, α-glycerophosphate-1-C^{14}, or P^{32}-α-glycerophosphate.[7] Phosphatidic acid is the only labeled phospholipid that is synthesized in detectable amounts from any of these precursors in the system described.

Essentially, all of the phosphatidic acid phosphatase activity (reaction 3) is present in the microsomes. Activity varies little with pH between 6.5 and 8.0, where it is maximal. In contrast to the phosphatidic acid phosphatase of liver (71), the adipose tissue enzyme is not inhibited by $MgCl_2$ at concentrations equimolar with substrate.[7]

It might be convenient to call the enzyme that catalyzes reaction 4 diglyceride transacylase. To do so assumes that this enzyme differs from glycerophosphate acylase (76) (or phosphoglycerol transacylase [75]), the enzyme that catalyzes reaction 2. Although not absolutely proved, it seems likely that this assumption is correct. Goldman and Vagelos (74) have studied this reaction in microsomes using C^{14}-labeled palmityl-CoA and oleyl-CoA and several diglycerides. In general, palmityl-CoA was more reactive than oleyl-CoA, and diglycerides containing myristate were more reactive than those containing palmitate, which were more reactive than those containing stearate. The only α,α′-diglyceride tested was unreactive whereas all α,β-diglycerides tested were utilized. The α,β-diglycerides containing an unsaturated fatty acid in either position were apparently more reactive than those containing two saturated fatty acids.

As might have been expected from the results of studies with C^{14}-labeled glycerol in intact adipose tissue[5] (21, 27, 46), α-glycerophosphate cannot be replaced by equimolar amounts of glycerol (69). When added in very high concentration, glycerol can be esterified in a system containing the CoA ester of palmitic acid-1-C^{14} and either microsomes or a soluble supernatant fraction. Certain other alcohols can also serve as fatty acid acceptors in this system.[8] From data obtained in the studies with glycerol, it was deduced that monoglycerides also can be esterified, although it has not been possible to demonstrate directly the esterification of monoolein. When monoolein (10 μmoles) was substituted for α-glycerophosphate in the system described in Table 1, 40% of it was hydrolyzed during the 30-minute incubation period (69).

No evidence for the formation of α-glycerophosphate from glycerol and ATP (glycerokinase activity) in adipose tissue has been found[8] (77) and it seems clear that glycerol is not an effective precursor for glyceride synthesis at concentrations which for α-glycerophosphate are optimal. In suitably fortified homogenates, any

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of the metabolic intermediates between glucose-6-phosphate and α-glycerophosphate can be substituted for the latter in supporting glyceride synthesis (78). (See below.)

In homogenates of adipose tissue in which glyceride synthesis is studied, lipolysis goes on simultaneously with esterification (69). When unfractionated homogenate is incubated at 37° without additions, there is a net increase in FFA in the system. When the homogenate is supplemented for glyceride synthesis, as in Table 3, FFA accumulation is diminished and in some instances there is a net disappearance of FFA. It has been shown that there is essentially no synthesis or oxidation of FFA under these conditions, so that it may be presumed that this difference represents a net synthesis of glycerides. The specific activity of the FFA decreases markedly over the course of such experiments, indicating that lipolysis is continuing even when there is a net decrease in FFA. Table 4 contains data from an experiment with homogenate from which most of the neutral lipid (and some of the FFA) had been removed. In the flasks incubated without added FFA there was no net change in fatty acid but a decrease in the specific activity of the FFA to about 12% of the initial level in 20 minutes. In flasks to which 1.2 μmoles of FFA had been added there was a net disappearance of 0.8 μeq of FFA and no change in specific activity.

TABLE 4. Effect of Increasing Initial FFA Concentration on Net Changes in FFA and Palmitic Acid-1-C^{14} Incorporation*

	FFA Added†	Zero Time	20 Minutes
Total FFA	0	0.4	0.4
(μeq per flask)	+	1.5	0.7
FFA specific radioactivity	0	97,000	12,000
(cpm per μeq)	+	25,000	28,000
Neutral lipid radioactivity	0	200	20,400
(cpm)	+	200	12,900

* Defatted homogenate incubated as described in Table 3, including creatine phosphate.

† 1.2 μeq of FFA prepared by saponification of epididymal fat pad lipids.

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When there are large changes in the specific activity of the FFA during an incubation, it is difficult, if not impossible, to draw conclusions about rates of esterifications. Alterations in the incorporation of radioactivity in neutral lipid could be the result of effects on the precursor specific activity (i.e., changes in rates of lipolysis) rather than effects on esterification. Although this presents a complicating factor in studying glyceride synthesis in certain types of homogenate preparations, it offers a system in which regulation of fatty acid metabolism might be investigated, since esterification and lipolysis seem to be the processes chiefly involved in removal and production of FFA. It is of interest that there is no transesterification, i.e., no incorporation of palmitic acid-1-C^{14} into glycerides in the absence of ATP and/or CoA, associated with lipolysis in this system. Similarly, the data of Shapiro *et al.* (79) suggest that there is no incorporation of palmitic acid-1-C^{14} into glycerides by transesterification in intact mesentery.

Lipases of Adipose Tissue. There are apparently several lipases in adipose tissue. Some of the earliest studies of enzymes in this tissue were those of Quagliariello and Scoz (80), who observed hydrolysis of tributyrin and triolein by extracts of adipose tissue. Lipase (or perhaps better, esterase) activity has also been investigated using various Tweens as substrates (81) Mashburn and co-workers have recently described the presence of three presumably different esterases in extracts of adipose tissue (82). One hydrolyzes naphthol acetate, one naphthol caprylate, and the third catalyzes the hydrolysis of tributyrin. The activity of the last of these enzymes was increased in tissues that were incubated with epinephrine or with ACTH prior to homogenization for assay of lipase activity. The partial purification and some of the properties of a lipase that hydrolyzes a wide range of triglycerides but does not attack triacetin or triolein has also been described by Lynn and Perryman (83). No one of these enzymes has been extensively purified and characterized, nor is it possible to assign a physiological role to any one of them.

Lipoprotein lipase is probably the most studied and best characterized of the adipose tissue lipases, although attempts to purify it have been for the most part unsuccessful (84 to 89). The enzyme catalyzes the hydrolysis of glycerides of long-chain fatty acids when they are in the form of lipoproteins, either chylomicrons, low density serum lipoproteins, or artificial substrates consisting of a triglyceride emulsion mixed with serum lipoproteins. Products of reaction are fatty acids, glycerol, and an uncharacterized lipoprotein moiety. The reaction requires any one of several cations for activation and albumin or Ca^{++} as a fatty acid acceptor (88).

Lipoprotein lipase hydrolyzes all three ester bonds of the triglyceride molecule and apparently has no positional specificity (89). It differs therein from pancreatic lipase, which hydrolyzes preferentially those ester bonds involving the α positions of glycerol (90, 91).

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This enzyme is characterized by its sensitivity to a number of inhibitors, e.g., protamine, ethylenediaminetetraacetate, phosphate, and sodium chloride (86, 87). Lipoprotein lipase is inactivated by extracts of bacteria adapted to grow on heparin but not by extracts of unadapted bacteria (86). Extracts of both types of this organism contain a mucopolysaccharase that degrades chondroitin sulfates A and C and hyaluronic acid but only the extracts of the adapted bacteria can degrade heparin. On the basis of these observations Korn has suggested that the enzyme may be a mucoprotein with a heparin-like prosthetic group (86).

The precise location of lipoprotein lipase in adipose tissue is not known. Robinson and French (92) have shown that when heparin is injected into the femoral artery of a rabbit, lipoprotein lipase and heparin appear simultaneously in the blood collected from the cannulated femoral vein. They also have found that lipoprotein lipase appears in the blood after injection of dextran sulfate with a molecular weight of 2 million. On the basis of these observations they have suggested that the enzyme must be located very near the circulating blood, perhaps in the capillary endothelium.

When rat epididymal fat pads are incubated in medium containing heparin, lipoprotein lipase is released into the medium (88, 93). Tissues obtained from fasted animals release much less enzyme than do tissues from fed rats (88, 93). The concentration of lipoprotein lipase is also lower in the tissues from fasted rats (93, 94) and it has been demonstrated that this is not due to the presence of an inhibitor in these tissues (93). Incubation of tissues from fasted rats in medium containing glucose and insulin causes an increase in the activity of the enzyme (93).

The available information suggests that lipoprotein lipase functions in the transport of fat from the blood into the adipose tissue and is not involved in the liberation of free fatty acids from adipose tissue glycerides. The fact that triglyceride in the form of lipoprotein is the preferred substrate, the evidence suggesting a location in the capillary wall, and the data correlating enzyme activity with fat uptake by adipose tissue but not with the rate of fatty acid release, provide support for this view. It has been suggested that FFA formed from triglyceride by the action of lipoprotein lipase move into the extracellular space and then into the adipose tissue cell where they are esterified and stored as triglycerides (56). It is of significance in this regard that fatty acid esterification is most active in adipose tissue from fed rats where lipoprotein lipase activity is also at a maximum.

Rizack (95) has described an "epinephrine-sensitive lipolytic system" in adipose tissue. This lipase is contained in particles that do not sediment with centrifugation for 30 minutes at $105{,}000 \times g$ but do sediment when centrifugation is continued for 12 hours. It is assayed using diluted Ediol® (a commercial coconut oil emulsion), albumin, and phosphate buffer, pH 6.8. Increasing the concentration of phosphate from 2.4×10^{-3} M to 6×10^{-3} M increases enzyme activity about 2.5-fold. There is no change in activity with higher concentrations. It is not inhibited by phosphate, protamine, sodium chloride, or ethylenediamine-tetraacetate as is liproprotein lipase. Sodium fluoride at concentrations that do not affect lipoprotein lipase activity causes marked inhibition.

Hollenberg and co-workers (96) have studied an enzyme that is very similar to the one investigated by Rizack. The two enzymes are assayed in similar systems, have similar pH optima, decline in activity in tissues incubated *in vitro*, and, most striking, are increased in activity in tissues that have been incubated with epinephrine (95, 96) or ACTH (96) but not with growth hormone (96). The evidence strongly suggests that it is the same enzyme that has been studied by both workers. Robinson (97) also may have been observing this enzyme when, in the course of studies on lipoprotein lipase, he noted that extracts from tissues of starved rats hydrolyzed the triglycerides of adipose tissue but not those of added chyle.

It is of considerable interest that Rizack (95) has observed activation of the lipase when extracts of tissues that had lost activity during incubation were incubated with epinephrine and ATP. Information presently available is insufficient, but it seems likely that further investigation will establish this lipase as the one responsible for the breakdown of adipose tissue triglycerides and perhaps for the effects of certain hormones on the release of FFA.

Carbohydrate Metabolism

METABOLISM OF HEXOSES IN INTACT TISSUE

Metabolism of Glucose in Intact Tissue: Role in Fatty Acid Synthesis and Esterification. Data concerning the fate of C^{14}-labeled glucose in adipose tissue and the pathways of glucose metabolism in relation to fatty acid metabolism have been summarized in detail by Cahill and co-workers (46, 49, 98) and by Winegrad *et al.* (35).

By comparing the incorporation of C^{14} from glucose-1-C^{14} and from glucose-6-C^{14} into CO_2 and lipids, Winegrad and Renold (47) have obtained evidence for the presence in adipose tissue of an active phosphogluconate pathway in addition to the glycolytic pathway for glu-

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cose metabolism. It was estimated in their experiments that about 50% of the metabolized glucose traversed the glycolytic pathway and 50% the oxidative or another alternate pathway. Other workers using somewhat different assumptions have arrived at roughly the same estimate (98). Winegrad *et al.* (99) have suggested the presence of an active glucuronic acid pathway in adipose tissue on the basis of data obtained in studies of the effects of growth hormone on the metabolism of specifically labeled glucose. In this regard, it has been shown that C^{14} from glucuronic acid-6-C^{14} is incorporated into CO_2 but not into fatty acids, which is the result to be expected on the basis of the known reactions of the uronic acid pathway (35).

More than 70% of the labeled glucose taken up by adipose tissue is recovered in CO_2, glyceride glycerol, fatty acids, and glycogen, the last representing the quantitatively least significant of these components (43, 46, 48, 98). In the presence of low concentrations of glucose in the medium, a large fraction of the total uptake (30% to 50%) is recovered in glyceride glycerol. As the concentration of glucose is increased, glucose uptake rises and the fraction converted to glycerol decreases (46, 48, 98, 100). When tissues are incubated in an atmosphere of nitrogen instead of oxygen there is a marked decrease in glucose uptake, but the incorporation into glyceride glycerol is diminished to a lesser extent than is incorporation into CO_2, glycogen, or fatty acids (46). Tissues from fasted rats exhibit a depressed rate of glucose uptake. Conversion to fatty acids and glycogen is almost abolished and oxidation is markedly diminished. Incorporation of C^{14} from glucose into glyceride glycerol is also affected but to a much lesser extent (46, 100). In all of these circumstances, then, it appears that fatty acid synthesis is most responsive to the availability of glucose within the tissue, whereas production of α-glycerophosphate, the precursor of glyceride glycerol, is maintained with relatively small alterations in the face of large changes in total glucose utilization. The relationship between glucose utilization and lipogenesis has been observed under numerous conditions in adipose tissue and in other tissues. More specifically, it appears that the rate of fatty acid synthesis is related to the amount of glucose oxidized via the phosphogluconate shunt (see above).

These generalizations fit readily into the picture of an adipose tissue cell in which triglyceride breakdown and synthesis go on constantly with a continuing supply of α-glycerophosphate required for the latter process. When α-glycerophosphate production becomes sufficiently limited, esterification no longer keeps pace with lipolysis and net accumulation of FFA results. Conversely, when glucose utilization reaches a certain level, the rate of esterification exceeds that of lipolysis and there is a net decrease in FFA in the system. There is very little fatty acid synthesis until glucose utilization has reached a level at which production of glycerophosphate is approaching a maximum. In a descriptive sense, this may mean that in the intact organism glucose is converted to fat for storage only after the requirements of the tissue for retention of the fatty acids already present are met. This description obviously does not take into account changes in the rate of lipolysis. It is not known whether glucose metabolism itself can influence the rate of triglyceride breakdown.

Metabolism of Other Sugars. Using a manometric measure of over-all changes in gas pressure, Ball and Cooper (9) were able to demonstrate an effect of insulin on the utilization of mannose and fructose as well as of glucose by adipose tissue. The pattern of metabolism of uniformly labeled C^{14}-mannose was observed by Wood *et al.* (101) to be similar to that of glucose. In general, the amount of mannose used for synthesis of CO_2, fatty acids, glyceride glycerol, and glycogen was found to be 60% to 90% of that of glucose. It might be concluded that this difference is conditioned either by a difference in the rate of entry into the cell, or by the rate of phosphorylation prior to further metabolism. In the view of Wood and co-workers (101), the available evidence favors the latter possibility.

Glucose oxidation in adipose tissue is almost completely blocked by 2-deoxy-glucose. A glucose analogue, 6-deoxy-6-fluoroglucose, that presumably cannot be phosphorylated by hexokinase, also inhibits the oxidation of glucose but the inhibition is not complete. In adipose tissue it reaches a maximum at about 70% inhibition, when further increases in inhibitor concentration have little effect. These findings, which are qualitatively similar to those obtained in other tissues with 6-deoxy-6-fluoroglucose, have been interpreted to mean that there are two pathways for glucose uptake by cells, only one of which is inhibited by this analogue (102, 103).

ENZYMATIC PATHWAYS OF CARBOHYDRATE METABOLISM

Glycogen Synthesis and Degradation, Including the Phosphorylase System. A summary of the enzymatic reactions involved in glycogen synthesis and degradation is found in Figure 2. Though the phosphorylation of glucose has not been studied in homogenates of adipose tissue, hexokinase (*a*) is undoubtedly present. As indicated by the blocked arrow, there is apparently no glucose-6-phosphatase (*b*) activity in adipose tissue (18, 104). Phosphoglucomutase (*c*) has been assayed (104, 105) and, as in most other tissues, it appears to

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exceed in activity the other enzymes involved in glycogen synthesis and degradation (105). In all tissues where it has been investigated there appear to be separate pathways for the formation and the breakdown of glycogen. Leloir and Cardini (106) have described an enzyme, UDPG glycogen transglucosylase (*e*), that catalyzes the synthesis of glycogen from uridine diphosphate glucose (UDPG). This enzyme, although it is very likely present in adipose tissue, has not been directly demonstrated. UDPG pyrophosphorylase (*d*) has been assayed in adipose tissue (105).

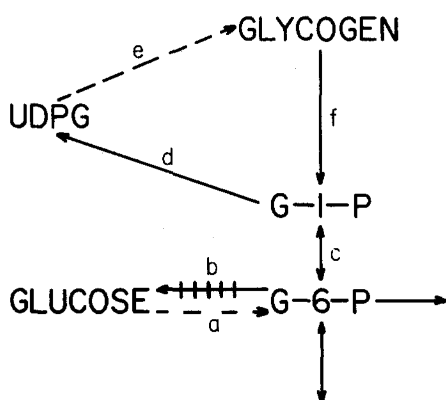

Fig. 2. Pathways of glycogen metabolism.

The reaction catalyzed by phosphorylase (*f*), an enzyme that has been extensively studied in several tissues, was long considered to be involved in both the synthesis and degradation of glycogen, although several indisputable physiological facts were difficult to integrate into such a picture. Most of these latter observations can be readily explained within the framework of the present view that glycogen synthesis proceeds via reactions (*d*) and (*e*), whereas phosphorylase (*f*) is involved only with the degradation of glycogen. The importance of the role of phosphorylase in adipose tissue is difficult to assess. Tuerkischer and Wertheimer (107) found no detectable glycogen in tissues from normally fed rats. Transient accumulation of glycogen in the adipose tissue was demonstrable shortly after consumption of a carbohydrate-rich feeding, particularly if this was preceded by a period of fasting. In a recent study the glycogen content of fat was reported as 0.22 mg/g in tissue from fed rats and 0.11 mg/g in tissues from fasted rats (105). In the absence of substrate it is difficult to understand what effect alterations in phosphorylase activity per se may have on tissue metabolism.

Figure 3 is a schematic representation of the reactions involved in the regulation of phosphorylase activity. Adipose tissue phosphorylase has not been isolated and characterized. Thus, the intimate mechanism of its inactivation and reactivation is not known. The enzyme is rapidly inactivated when intact or homogenized adipose tissue is incubated *in vitro*. This inactivation is inhibited by fluoride (18). In this and in other of its characteristics the adipose tissue system is similar to that in liver described by Sutherland and co-workers (108, 109) and differs from the muscle system.

The enzyme that inactivates phosphorylase in liver is a phosphatase and inactive phosphorylase is dephospho-phosphorylase (108). The nature of the inactivating enzyme in adipose tissue has not been demonstrated. Likewise, the mechanism of reactivation of phosphorylase in adipose tissue is not known. In the liver system reactivation consists in phosphorylation of the inactive enzyme by ATP catalyzed by a phosphokinase (109). The reaction is accelerated by cyclic 3′,5′-adenosine monophosphate (3′,5′-AMP) (110). The formation of 3′,5′-AMP in a particulate fraction of adipose tissue homogenate has been demonstrated (18, 111). Accumulation of 3′,5′-AMP in this system is enhanced by epinephrine as it is in liver, skeletal muscle, cardiac muscle, and brain (111). The mechanism of the effect of epinephrine (and of other active catecholamines) is not known. Sutherland and co-workers (111) have also demonstrated that the diesterase that inactivates 3′,5′-AMP is present in adipose tissue.

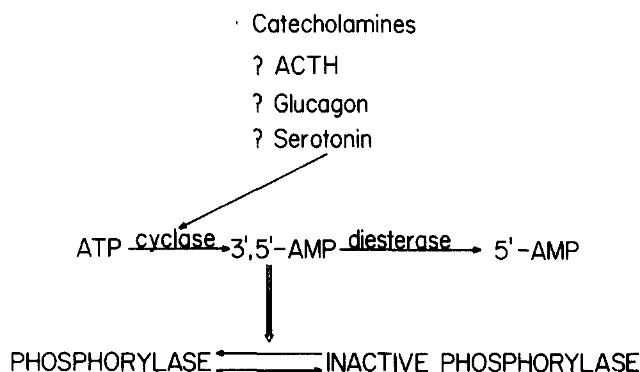

Fig. 3. Reactions involving phosphorylase and cyclic 3′,5′-AMP.

It is presumed that the stimulation of phosphorylase activity in adipose tissue induced with epinephrine or one of several other hormones is mediated by 3′,5′-AMP. Addition of 3′,5′-AMP to the medium in which tissues are incubated causes, however, none of the changes produced by epinephrine under similar conditions, including the stimulation of phosphorylase activity (18, 31). This does not, of course, exclude the possibility that the nucleotide formed *within* the tissue

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plays a role in the effects of epinephrine and certain other hormones.

It should not be concluded that the action of 3′,5′-AMP and/or its formation or degradation is related necessarily to phosphorylase only. The information is summarized here since this is the context in which the system was discovered and its workings elucidated. It is quite probable that 3′,5′-AMP and/or the processes related to its formation and removal will be found to have effects on cellular function in addition to its well-documented effects in the phosphorylase system. It has been reported, for example, that this nucleotide enhances the activity of phosphofructokinase in extracts of the liver fluke, Fasciola hepatica (112).

Glucose Dissimilation and Oxidation, Including α-Glycerophosphate Synthesis and Breakdown. In Figure 4 are summarized some of the enzymatic reactions discussed below.

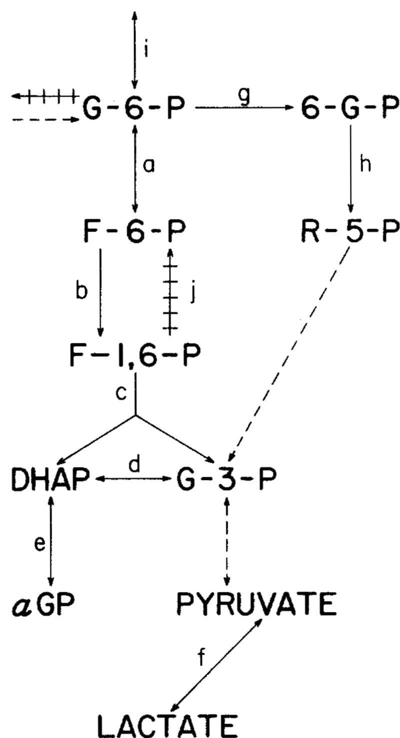

FIG. 4. Some pathways of glucose metabolism in adipose tissue.

Studies of the metabolism of differentially labeled glucose in intact adipose tissues have indicated that the oxidative pathway plays an important part in glucose metabolism. Glucose-6-phosphate dehydrogenase (*g*) activity expressed per milligram tissue nitrogen is several times greater in adipose tissue than it is in liver (104). The ratios of its activity to that of 6-phosphogluconate dehydrogenase (*h*), phosphohexoseisomerase (*b*), or phosphoglucomutase (*i*) are also many times the ratios observed for the same activities in liver. Thus, in absolute amount and, perhaps more important, relative to the activity of the other enzymes associated with glucose-6-phosphate utilization, the enzyme introducing glucose-6-phosphate to the oxidative pathway is very active.

In the intact tissue the rate of triglyceride synthesis might be regulated by changes in the activity of one of the enzymes involved, by the availability of ATP or one of the other required cofactors, or by the availability of substrates. Considering the last possibility, there is probably a constant supply of fatty acids derived from lipolysis. The availability of α-glycerophosphate may, however, be more variable and, as has been suggested by several workers, may in certain circumstances be an important factor in determining the rate of glyceride synthesis. In adipose tissue, glycerophosphate cannot be formed by the phosphorylation of glycerol and thus must be formed from dihydroxyacetone phosphate, probably derived chiefly from glycolysis. It has been shown that α-glycerophosphate can be synthesized from glucose-6-phosphate, fructose-6-phosphate, fructose-1,6-diphosphate, glyceraldehyde-3-phosphate, or dihydroxyacetone phosphate by adipose tissue homogenates suitably fortified with ATP, $MgCl_2$, and DPNH. Thus, it is likely that phosphohexoseisomerase (*a*), phosphofructokinase (*b*), aldolase (*c*), triosephosphate isomerase (*d*), and α-glycerophosphate dehydrogenase (*e*) are all present in adipose tissue (78). These reactions can be observed in the supernatant solution prepared by centrifugation of homogenates at 105,000 × *g* for 60 minutes.

Lactic dehydrogenase and α-glycerophosphate dehydrogenase activity are present in roughly equivalent amounts in rat adipose tissue (113). This is true also in liver, whereas in muscle lactic dehydrogenase activity is considerably greater than that of α-glycerophosphate dehydrogenase (113). It is not known whether the amounts or relative amounts of these two enzymes play any part in determining the quantity of lactate versus α-glycerophosphate formed in tissues.

Adipose tissue homogenates hydrolyze α-glycerophosphate. There is a large fraction of activity with a pH optimum of about 5.5 and a lesser amount of activity in the alkaline range with an optimum pH about 9.5. Acid phosphatase activity is present chiefly in microsomes and in the soluble fraction. There are differences in the behavior of the acid phosphatase in these two fractions, e.g., the microsomal enzyme, in contrast to the soluble enzymes, hydrolyzes α-glycerophosphate more rapidly than it does β-glycerophosphate and is inhibited 40% by an amount of potassium

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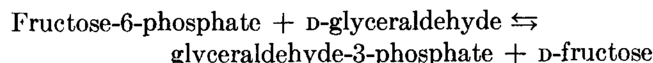
linoleate that has no effect on the soluble enzyme (78). It is not known whether in the intact cell any glycerol is formed directly from α-glycerophosphate by the action of a phosphatase or whether it is all derived from glycerides via hydrolysis.

There is good evidence that the other reactions of the oxidative pathway and the reactions between glyceraldehyde-3-phosphate and pyruvate are operative in adipose tissue but they have not been demonstrated or assayed directly. Eichel (114) has reported on the activity of several respiratory enzymes assayed in unfractionated homogenates of adipose tissue, among them cytochrome *c* oxidase, succinic oxidase, succinic dehydrogenase, lactic oxidase, lactic dehydrogenase, and DPNH cytochrome *c* reductase. He has observed also malic and isocitric dehydrogenase activities but did not find evidence of choline, glucose, glutamic or β-hydroxybutyric dehydrogenases of D-amino acid oxidase.

The possibility of an active transaldolase in adipose tissue has been suggested by Cahill *et al.* (98) as an explanation for the finding that more carbon-1 of glucose is recovered in glycerol than in fatty acids. The presence of this reaction would tend to favor the utilization of carbons 4, 5, and 6 of glucose in fatty acid synthesis. Glyceraldehyde has been shown to serve as an acceptor in transaldolase reactions (115):


Fructose-6-phosphate + D-glyceraldehyde ⇆ glyceraldehyde-3-phosphate + D-fructose

Evidence for the presence of transaldolase activity in adipose tissue has been obtained in experiments demonstrating the formation of α-glycerophosphate from glucose-6-phosphate and glyceraldehyde in the presence of DPNH but with no ATP added (78).

The apparent absence of fructose-1,6-diphosphatase has been reported (104) and would indicate that glycolysis is irreversible. This might explain the observations of Rose and Shapiro (39), who found no glycogen synthesis from acetate-1-C^{14} or from pyruvate-2-C^{14} under conditions in which uniformly labeled C^{14}-glucose was actively incorporated into glycogen.

EFFECTS OF HORMONES ADDED IN VITRO ON ADIPOSE TISSUE METABOLISM

The sensitivity of adipose tissue to a wide variety of hormones has been demonstrated in *in vitro* studies, many of which have been summarized recently by Engel and White (116). Some of these hormones, e.g., insulin and the catecholamines, undoubtedly are of basic importance in the regulation of the metabolism of this tissue in the intact animal. The physiological role of certain others is more difficult to evaluate at this time. There is no evidence, for example, that makes it possible to relate the effects of serotonin or of growth hormone as they are observed *in vitro* to the regulation of adipose tissue metabolism in the intact animal.

INSULIN

It was first demonstrated by Krahl (8) that the uptake of glucose by adipose tissue incubated *in vitro* is stimulated by the addition of insulin *in vitro*. In studies designed to provide information concerning the effects of insulin on the pathways of glucose metabolism in adipose tissue, Winegrad and Renold (47) compared the metabolic fate of C^{14} from glucose specifically labeled in carbon-1 or carbon-6. They observed, as reported previously by Milstein (117), that insulin stimulates the oxidation of carbon-1 much more than it does the oxidation of carbon-6. Fatty acid synthesis from both carbon-1 and carbon-6 is markedly increased by insulin. These workers drew attention to the striking association of large effects on fatty acid synthesis with large increases in the oxidation of carbon-1 of glucose. The correlation between the rate of glucose oxidation via the phosphogluconate pathway and the rate of lipogenesis has been frequently noted. In a discussion of lipogenesis in adipose tissue, Winegrad *et al.* (35) considered in detail the effects of insulin on fatty acid synthesis. In summary, the stimulation by insulin of fatty acid synthesis from numerous C^{14}-labeled substrates in the presence of glucose has been amply confirmed in a number of laboratories (21, 23, 48, 118). This effect, as do most other effects of insulin on adipose tissue metabolism (with exceptions as noted below), requires the presence of glucose (31, 43) and can be explained in terms of the demonstrated action of insulin on glucose uptake and metabolism.

The effect of insulin on over-all changes in gas pressure (the resultant of oxygen uptake and carbon dioxide production) during incubation of adipose tissue *in vitro* has been investigated by Ball and co-workers (9, 119 to 122). With or without glucose in the medium there is a slow decline in pressure, indicating that oxygen uptake is greater than carbon dioxide production. Within 10 minutes of the addition of insulin there is an increase in gas pressure, which continues to rise linearly with time for periods up to 3 hours. Since oxygen uptake is little changed by insulin in these experiments, it is presumed that the positive pressure response is due to the evolution of carbon dioxide, a reflection of increased lipogenesis. This effect of insulin also is demonstrable only when glucose, fructose, or mannose is present in the medium.

Insulin increases the incorporation of C^{14} from glucose

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into glyceride glycerol. This effect is relatively less than that on fatty acid synthesis from glucose and is participated in to an equal extent by carbons 1 and 6 (21, 23, 48, 99). It has been presumed that the increased incorporation of C^{14} from glucose into glyceride glycerol reflects an increased rate of triglyceride synthesis. In this regard it has been shown that insulin (in the presence of glucose) decreases the rate of release of FFA (11, 18, 30, 31, 116) and stimulates the incorporation of C^{14}-labeled fatty acids into tissue glycerides (19), changes which are most simply explained as the results of an increased rate of triglyceride synthesis. It has been suggested that glucose, in its role as the major precursor of α-glycerophosphate (and perhaps by providing a supply of energy for the esterification processes), is of basic importance in the regulation of triglyceride synthesis (see above).

In general, the pattern of glucose metabolism is essentially the same whether glucose uptake is stimulated by insulin or by increasing the concentration of glucose in the medium (48). Fatty acid synthesis from both carbon-1 and carbon-6 is greatly increased. The incorporation of both of these carbons into glycerol is enhanced but to a lesser degree. Oxidation of carbon-1 to CO_2 is markedly stimulated and oxidation of carbon-6 only slightly stimulated. These findings are compatible with the view that the action of insulin is exerted before the divergence of the metabolic pathways for glucose, i.e., at the site of entry of glucose into the cell or of glucose phosphorylation. Data from studies of the effect of insulin on incorporation of C^{14} from mannose into glycogen, carbon dioxide, fatty acids, and glyceride glycerol is also consistent with this hypothesis (101). The effects of insulin on glycogen synthesis, however, seem to be more specific and may require another explanation. When equivalent increases in glucose uptake are produced by increasing glucose concentration, or by prolactin or insulin, only in the last instance is there a significant increase in the incorporation of C^{14}-glucose into glycogen. Stimulation of glucose uptake and glycogen synthesis (nonradioactive) by tolbutamide *in vitro* has been reported (123).

Stimulation by insulin of lactic acid formation from glucose can be observed under certain conditions (122). In the presence of glucose, incorporation of C^{14}-glycine into adipose tissue protein is also enhanced by insulin (124).

Adipose tissue *in vitro* is remarkably sensitive to insulin. As little as 10 micro units/ml stimulates the oxidation of glucose-1-C^{14} (125). With an assay method based on this effect, it has been found that adipose tissue is influenced by insulinlike activity present in serum to which the rat diaphragm *in vitro* is insensitive (126). After incubating serum with extracts of adipose tissue, insulinlike activity in the diaphragm assay can be demonstrated. A fraction of adipose tissue homogenate containing the "insulin-activating" component has been prepared using ethanol fractionation and paper electrophoresis. Neither the nature of the "inactive" insulin nor the mechanism of action of the adipose tissue extracts is known (127).

In a few instances effects of insulin have been demonstrated in the absence of glucose. These obviously cannot be explained as the result of enhancement of glucose uptake. Haugaard and Marsh (128) observed a relatively small but a significant stimulation of oxygen uptake by insulin when the medium contained either glucose, succinate, lactate, acetate, or pyruvate. There was no stimulation of oxygen uptake in the absence of substrate. Increased incorporation of C^{14} from pyruvate-3-C^{14} or from acetate-1-C^{14} into adipose tissue protein by insulin in the absence of glucose has been reported. Incorporation of C^{14} into lipids determined in the same experiments was not affected (129).

Barrnett and Ball (130 to 133) have explored the effects of insulin on the morphology of adipose tissue cells as revealed by electron microscopy. Tissues incubated for as little as 10 minutes in medium containing insulin exhibit invaginations of the plasma membrane, collections of numerous minute vesicles in the region of the plasma membrane, and occasionally what appeared to be long channels lined with membrane and connecting with the surface membrane. This picture is not seen in tissues incubated without insulin. The authors have suggested that the morphologic changes represent pinocytosis, the result of insulin action. These changes are produced by insulin whether or not glucose is present in the medium.

The available evidence suggests, as has been stated by Winegrad and Renold, that "adipose tissue is a major anatomical site of insulin action" (36). The mechanism of action of insulin in adipose tissue is likely very similar to that in other tissues and remains to be elucidated. As in other tissues where insulin effects have been studied, an action of insulin facilitating the entry of glucose (or of certain other hexoses) into the metabolic machinery of the cell would account for many of its effects. But in adipose tissue, as in other tissues, there are certain observations that appear to be unexplained by such an hypothesis.

EPINEPHRINE AND NOREPINEPHRINE, INCLUDING THE EFFECTS OF 3′,5′-AMP AND 5′-AMP

Epinephrine has been used in *in vitro* studies more extensively than has norepinephrine. They are ap-

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parently alike in actions and in effective concentrations and will be discussed together. The data are presented in some detail, and because they are descriptive also of the effects of ACTH, the section on the latter hormone is brief and refers frequently to this one. The effects of glucagon are similar to those of the catecholamines and ACTH and will be discussed within the same framework.

The effects of epinephrine in adipose tissue metabolism are several. Since the mechanism through which they are produced is unknown and since it is not clear at present in what manner some of the apparently diverse actions are related, they will be considered separately: (*a*) Effects on fatty acid release, lipolysis, and triglyceride synthesis, (*b*) effects on glucose uptake and metabolism, and (*c*) effects on phosphorylase activity and 3′,5′-AMP.

In the early studies of Gordon and Cherkes (20) and of White and Engel (16, 17) it was demonstrated that fatty acid release is stimulated by the addition of epinephrine or norepinephrine to the medium *in vitro*. The concentration of FFA in epinephrine-treated tissues rises before accumulation in the medium is evident, and at the end of the incubation period the tissue FFA concentration is higher in the epinephrine-treated tissues than it is in the controls (16 to 19). The magnitude of the epinephrine effect is not altered by incubation in nitrogen[9] (21) or by the additon of 5×10^{-3} M iodoacetate (21). It has been shown that addition to the medium of phentolamine, dibenamine, dibenzylene, or dihydroergocornine[9] (31, 134) inhibits the effects of the catecholamines on FFA release.

Since triglyceride breakdown and resynthesis goes on constantly in adipose tissue, producing and removing FFA, alteration in the rate of either of the processes should lead to a net change in the amount of FFA in the system. The decreased rate of fatty acid release induced by the presence of glucose probably results in large part from an increase in the rate of triglyceride synthesis. The effects of epinephrine on the rate of triglyceride synthesis have been difficult to assess and will be considered after discussing the evidence for what is probably the chief factor in the increased fatty acid release, an increased rate of lipolysis.

When adipose tissue is incubated *in vitro*, glycerol (21, 32) as well as FFA is liberated into the medium. These are the products of hydrolysis of triglycerides. As discussed above, the fatty acids can be re-esterified, the rate of this process depending probably to a large extent on the supply of α-glycerophosphate. Glycerol is not metabolized to any significant extent[9] (27, 46). The amount of glycerol accumulated during an incubation is therefore an indication of the amount of lipolysis, whereas the amount of FFA accumulated represents the difference between the amount formed and the amount removed. When release of FFA is increased by the addition of epinephrine, the amount of glycerol released is also increased, strongly suggesting that the rate of triglyceride breakdown is increased (21, 32). The only other apparent source of glycerol in adipose tissue is α-glycerophosphate. The presence of α-glycerophosphatase activity has been demonstrated (78) but it is not known whether this reaction is operative in intact adipose tissue. Furthermore, the concentration of α-glycerophosphate in adipose tissue is low (0.1 to 0.2 μmole/g tissue wet weight)[8] and the effect of epinephrine on glycerol release has been observed in the absence of glucose in the medium, i.e., when it is presumed there is little synthesis of α-glycerophosphate. Lynn *et al.* (21) have found that the glycerol released from tissues incubated with C^{14}-glucose and epinephrine contained no radioactivity, strong evidence that it came from triglyceride, not α-glycerophosphate.

Positive evidence in support of the postulated effect of epinephrine on lipolysis is provided by the work of Rizack (95) and of Hollenberg *et al.* (96), who have observed increased lipase activity on adipose tissues incubated with epinephrine (and with ACTH). In summary, it has been found that when epididymal fat pads are incubated with one of these hormones, then homogenized, and the lipase assayed in a system containing buffer (pH about 7), albumin, and an artificial coconut oil emulsion, the activity is higher than it is in control tissues incubated without hormone. Rizack has reported also that the lipolytic system in a cell-free extract of adipose tissue can be activated by incubating the extract with epinephrine and either ATP or tissue sediment. The activity of this lipase in homogenates is inhibited by sodium fluoride, as is the effect of epinephrine (or ACTH) on FFA release in intact tissues (96). A role for this lipase in the action of epinephrine is strongly suggested. The magnitude of the changes in activity induced by the hormone seems hardly great enough to account for the large increases in the rate of fatty acid release in intact tissues. However, this may mean only that the conditions thus far employed are not optimal for demonstration of changes in the activity of the specific lipase affected by epinephrine.

It has been shown that the incorporation of palmitic acid-1-C^{14} into glycerides in the epididymal fat pad is markedly decreased in the presence of epinephrine (29). The tissues incubated with epinephrine contained more FFA than did the controls in these studies, but they also contained more C^{14}-palmitate and the specific activity of the total tissue FFA was actually higher in the epi-

[9] D. Steinberg and M. Vaughan, unpublished observations.

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nephrine-treated tissues. The specific activity of the medium FFA was, on the other hand, considerably lower in the flasks containing epinephrine than it was in the control flasks. Steinberg *et al.* (29) have discussed the problems in interpretation presented by these experiments. For reasons discussed in detail above, it is impossible to calculate in experiments such as these the rate of fatty acid esterification, nor is it reasonable to assume that when changes in the rate of incorporation of labeled fatty acid are induced, they are necessarily proportional to alterations in the rate of fatty acid esterification. The specific activity of the precursor pool of fatty acids for triglyceride synthesis is not known at any time during the course of the experiment. It has not been isolated and determined nor can it be predicted.

It has been shown that the incorporation of glycerol-1,3-C^{14} and of pyruvate-1-C^{14} into triglycerides by adipose tissue *in vitro* is decreased by epinephrine[9] (29, 46). The interpretation of these data also is complicated by the question of precursor specific activity. Leboeuf *et al.* (32) have postulated that triglyceride synthesis is increased by epinephrine on the basis of the observation that epinephrine increased the incorporation of C^{14} from glucose into glyceride glycerol. The specific activity of α-glycerophosphate from which glyceride glycerol is formed is not known in these studies and the possibility cannot be excluded that changes in the amount of C^{14} found in glyceride glycerol are the result of changes in precursor specific activity.

Prior to considering the effects of the catecholamines on glucose metabolism it may be pertinent to point out that the effects of these hormones on fatty acid release (31), on phosphorylase activity (18), on incorporation of glycine-1-C^{14} into protein (124), on lactic acid production (121), and the characteristic effects on net gas exchange (121) are evident whether or not glucose is present in the medium.

The uptake of glucose by adipose tissue is increased in the presence of epinephrine or norepinephrine (21, 31, 32). It is possible, however, with low concentrations of these hormones (0.1 μg/ml) to produce large effects on fatty acid release with no measurable change in glucose uptake (31).

Amounts of epinephrine that cause little or no increase in glucose uptake do have marked effects on the pattern of glucose metabolism as observed in studies with C^{14}-labeled glucose (31). Carbon dioxide production from glucose-6-C^{14} is stimulated to a greater degree by epinephrine than is oxidation of glucose-1-C^{14} (21, 23). In tissues from fed rats incubated with uniformly labeled C^{14}-glucose, the most striking effect of epinephrine is on the incorporation of C^{14} into glyceride glycerol, which is markedly increased (23). In tissues from fasted rats in which fatty acid synthesis from glucose is already low, the most consistent effect of epinephrine is to decrease the incorporation of glucose carbon into fatty acids, although an increased incorporation into glyceride glycerol is also evident (31). In tissues from fed rats incubated with insulin, it has been shown that the addition of epinephrine decreases synthesis of fatty acid from labeled glucose (23). Incorporation of acetate-1-C^{14} from medium containing glucose into total lipid of adipose tissue has been found to be inhibited by epinephrine (118).

It has been suggested that the effects of epinephrine (and ACTH and growth hormone) on C^{14}-glucose metabolism might be the result of the elevated concentration of FFA in the tissue produced by these hormones (23, 135). By increasing the concentration of fatty acid in the incubation medium it is possible to produce an increase in the incorporation of glucose carbon into glyceride glycerol, i.e., an effect qualitatively like those of the hormones mentioned (23, 135).

It has been found that in the presence of epinephrine, fatty acid release can be significantly inhibited by dihydroergocornine with no effect on the incorporation of glucose carbon into glycerides (31). In these experiments, however, the concentrations of FFA in the ergocornine-treated tissues were still above the usual control levels, perhaps sufficiently elevated to affect glucose metabolism. Certainly the relationship (not necessarily causal) between FFA release and the pattern of glucose metabolism is indicated by the observations that (*a*) glucose does not abolish FFA release in the presence of epinephrine as it does in its absence (31), and (*b*) in C^{14}-glucose experiments, when both epinephrine and insulin are present, the "epinephrine pattern" of metabolism of C^{14}-glucose prevails (23).

Epinephrine and norepinephrine both increase the amount of active phosphorylase in adipose tissue (18). It is not clear what effect this has on adipose tissue metabolism since the amounts of glycogen present in the tissue under most circumstances are minimal and no other substrate for phosphorylase is known. The increase in phosphorylase activity is mediated by the action of 3′,5′-AMP, the production of which is accelerated by epinephrine (see above). It is quite possible that 3′,5′-AMP has effects in addition to its action on phosphorylase. Either these or effects resulting from the formation of 3′,5′-AMP from ATP and its subsequent hydrolysis may account for some of the metabolic changes produced by epinephrine. The production of 3′,5′-AMP and of active phosphorylase is clearly not related directly to stimulation of FFA release and effects on pathways of glucose metabolism, since when

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serotonin produces the former effects in adipose tissue, there is no change in FFA release or in the pattern of C^{14}-glucose metabolism (31). Serotonin does, however, increase glucose uptake (31). Addition of 3′,5′-AMP to the medium does not increase phosphorylase activity in adipose tissue nor does it reproduce any of the other effects of epinephrine (18). Both 3′,5′-AMP and 5′-AMP (at equal concentrations) decrease fatty acid release and stimulate glucose uptake slightly. However, they enhance the incorporation of C^{14} from uniformly labeled C^{14}-glucose into both moieties of the triglyceride, i.e., fatty acids and glycerol, to an equal extent (18, 31).

When 3′,5′-AMP is inactivated in tissues, the product is 5′-AMP. This reaction is inhibited by caffeine. It has not been possible, however, to use caffeine inhibition to determine whether the effects observed with 3′,5′-AMP are all due to its conversion to 5′-AMP by the tissue since caffeine itself affects the adipose tissue. It inhibits glucose uptake and causes a slight stimulation of FFA release (31). Thus, the data available at present are inconclusive with respect to the role played by 3′,5′-AMP (or its synthesis or degradation) in production of most of the effects of epinephrine in adipose tissue.

Epinephrine enhances the accumulation of 3′,5′-AMP, which in turn increases the amount of active phosphorylase and may be involved in the stimulation of glucose uptake. It is not known whether the latter effect could result from an increase in phosphofructokinase activity, which has been produced with 3′,5′-AMP in extracts of Fasciola hepatica (136) but has not been demonstrated in adipose tissue. Rizack (95) has suggested that 3′,5′-AMP is not the mediator of the epinephrine-induced increase in lipase activity which most likely is the cause of increased FFA release. It is possible that the accumulation of FFA within the tissue influences cellular metabolism and that this is reflected in changes in the pattern of C^{14}-glucose metabolism.

ADRENOCORTICOTROPIC HORMONE (ACTH)

The effects of ACTH on the metabolism of adipose tissue *in vitro* are qualitatively and quantitatively like those of the catecholamines. One difference is that the effect of ACTH on the release of FFA *in vitro* cannot be demonstrated in medium containing no calcium, whereas the effect of epinephrine does not require the presence of calcium (137). It is of interest that calcium is also required for the effect of ACTH steroid production by adrenal slices (138).

When ACTH is added to adipose tissue *in vitro*, the release of FFA and the concentration of FFA in the tissue are increased (16, 17, 18, 137). Lipase activity is increased (96) and incorporation of palmitic acid-1-C^{14} into glycerides is decreased (29). Glucose uptake is stimulated (31, 48, 135). The effects of ACTH on the incorporation of glucose carbon into glyceride glycerol and into fatty acids mimic those of epinephrine (31, 48, 135). Incorporation of acetate-1-C^{14} into total lipids is also depressed by ACTH in medium containing glucose (118). Phosphorylase activity is increased presumably through the mediation of 3′,5′-AMP (18).

Phenotolamine *in vitro* blocks the effect of ACTH (just as it does the effects of catecholamines) on the release of FFA (134). When the effect on FFA release has been partially inhibited, there is no change in the ACTH induced pattern of C^{14}-glucose metabolism (31).

The relationship of peptide structure to actions of ACTH in different tissues is of interest. Engel and White (116) have summarized data suggesting that the N-terminal serine of the ACTH molecule may be required for its adrenal effects but not for the effects on adipose tissue. α-Melanophore stimulating hormone, a peptide consisting of 18 amino acids identical in sequence with the N-terminal portion of the ACTH molecule, causes release of FFA from adipose tissue but is not as potent in this regard as is ACTH. The activities of a group of related peptides have also been examined.

It is rather surprising to find that molecules as different in structure as a catecholamine and ACTH, a polypeptide, produce apparently identical effects. It is difficult to visualize a single enzyme or "receptor" that would be capable of interacting with two molecules of such dissimilar configuration. It is almost necessary to conclude that they do not act at the same site. This conclusion is favored by the recent work of Paoletti and Smith,[10] who have found that the effects of ACTH on FFA release are not obtained in adipose tissue that is depleted of catecholamines (chiefly norepinephrine). Their data strongly suggest that norepinephrine stored in the tissue is in some way "released" by the action of ACTH and produces the effects attributed to the latter hormone.

GLUCAGON

The effects of glucagon resemble in many ways those of ACTH and the catecholamines but differ in some particulars. The rate of release and the tissue concentration of FFA are increased by glucagon whether or not glucose is present in the medium (18, 22, 31, 139). The maximal effect of glucagon is, however, much

[10] R. Paoletti and R. L. Smith, personal communication.

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smaller than those obtained with epinephrine or ACTH. Glycerol release is also increased (22), suggesting an increased rate of lipolysis.

Glucagon stimulates glucose uptake (22, 31, 140, 141). Its effect is somewhat greater than those of epinephrine and ACTH. As with the latter hormones, it is possible to demonstrate effects on the release of FFA with concentrations of glucagon that do not cause a measurable increase in glucose uptake (31). It is of interest to know whether any or all of the effect of glucagon on glucose uptake might be due to the presence of insulin in the glucagon preparations. The effect of glucagon on glucose uptake is not altered by treatment of the hormone with cysteine, which inactivates insulin (31, 140), although Lee and co-workers (140) have reported that the insulinlike effect of glucagon on the oxidation carbon-1 of glucose is diminished by cysteine treatment. Hagen (22) has found that the addition of insulin to an amount of glucagon that is maximally effective in stimulating of glucose uptake causes a further increase. She has also observed that the stimulation of oxygen uptake caused by glucagon (in the presence of glucose but not in its absence) is enhanced by the addition of insulin, which alone has little effect. Insulin also enhances the stimulatory effect of epinephrine on oxygen uptake. All of these findings support the view that the effects on glucose metabolism are due to glucagon itself, not to traces of insulin.

Glucagon increases the incorporation of C^{14} from uniformly labeled C^{14}-glucose into glyceride glycerol and causes a more variable decrease in incorporation into fatty acids in tissues from fed rats (141). Inhibition of lipid synthesis from acetate-1-C^{14} has been observed (118). With tissues from fasted rats, glucagon has little effect on fatty acid synthesis from uniformly labeled C^{14}-glucose but greatly enhances glyceride glycerol formation (31). These findings differ at least quantitatively from the effects of epinephrine and of ACTH observed under similar conditions but are clearly not like those of insulin (31). In terms of the hypothesis of Cahill *et al.* (23), concerning the part played by tissue fatty acids in determining the pattern of glucose metabolism, it is possible that differences in the effects of glucagon and of epinephrine, e.g., on the fate of C^{14}-labeled glucose, are due to differences in extent of FFA accumulation induced by the two hormones (18).

Like epinephrine, norepinephrine, ACTH, and serotonin, glucagon increases phosphorylase activity in adipose tissue (18, 22). As mentioned in the discussion of the effects of ACTH, one wonders that so many different molecules produce what appear to be the same effects. It seems probable that this is not due to a lack of specificity in some key reaction. More likely, it will be found, as is suggested by the evidence available concerning ACTH,[10] that the "receptor" or primary site of action is different for each of these hormones.

SEROTONIN

The effects of serotonin on adipose tissue have not been investigated in detail. It increases phosphorylase activity and stimulates glucose uptake when added to the medium in which intact fat pads are incubated (18, 31). Similar effects of serotonin on the liver fluke, F. hepatica, have been reported by Mansour and Lago (142), who have established that serotonin in the fluke enhances the accumulation of 3′,5′-AMP, which in turn mediates the effects on phosphorylase activity (112). It is probable that the effects on adipose tissue phosphorylase are similarly produced. The mechanism of the effect on glucose uptake is less clear. It has been shown that 3,5′-AMP increases phosphofructokinase activity in extracts of F. hepatica (136) but it is not established that such an action would lead to an increase in glucose uptake. Nor is it known whether fructokinase in adipose tissue is similarly affected by 3′,5′-AMP.

Serotonin has proved most interesting because of its lack of effect on release of FFA from adipose tissue (18). Epinephrine, norepinephrine, ACTH, and glucagon, all of which enhance phosphorylase activity in this tissue apparently through the mediation 3′,5′-AMP, also stimulate the release of FFA. The observations with serotonin, therefore, indicate that either the effects of the several hormones on the release of FFA are unrelated to their influence on the 3′,5′-AMP-phosphorylase system or that serotonin, in addition to this action shared with the other hormones, in some manner interferes with fatty acid release. No evidence for the latter alternative has been obtained in an investigation of the effects of serotonin on the release of FFA from adipose tissue in the presence of epinephrine.[6]

SOME PITUITARY HORMONES THAT HAVE EFFECTS ON ADIPOSE TISSUE

Information concerning several pituitary hormones that have effects on adipose tissue metabolism is briefly summarized here. The effects of these hormones *in vitro* have not been extensively studied and in many cases the preparations employed are impure or possibly impure. Thus, it is not possible to draw any conclusions about the mechanism of action or the part played by these hormones in adipose tissue metabolism.

Growth Hormone. The release of FFA from adipose tissue *in vitro* is stimulated by growth hormone (17,

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135). High concentrations of the hormone are required to obtain this effect, although the injection of growth hormone in the intact animal raises serum FFA concentration more dramatically than does ACTH.

It has been found that when glucose is present in the medium, oxygen uptake is increased by the addition of growth hormone (122). Glucose uptake is stimulated but there is no increase in lactic acid production (122). The effects of growth hormone preparations on the pathways of glucose metabolism are complex and difficult to interpret. Leboeuf and Cahill (135) have concluded that the action of growth hormone on glucose metabolism is like that of ACTH and have suggested that it is the result of the accumulation of FFA in tissues incubated with the hormone. Winegrad and coworkers (35, 99) have observed that growth hormone preferentially increases the oxidation of carbon-6 of glucose with little effect on the oxidation of carbon-1. On the basis of their data they have suggested that growth hormone stimulated the activity of the glucuronic acid pathway. It has been found also that fatty acid synthesis from acetate-1-C^{14} or from pyruvate-2-C^{14} is decreased by growth hormone whether or not glucose is present in the medium (35, 118). This and several other effects of growth hormone on adipose tissue metabolism are apparently similar to those of epinephrine and ACTH. Growth hormone does not, however, increase lipase activity as do the latter two hormones (96) and differs in other characteristics as well.

Fat-Mobilizing Factor from Human Urine. Chalmers *et al.* (143, 144, 145) have isolated from the urine of fasting humans a fraction which, when injected into mice, causes an increase in liver fat, blood lipids, and blood ketones. The active principle has been purified by these workers to the extent that as little as 1 μg of the purified material per milliliter of incubation medium stimulates the release of FFA from adipose tissue *in vitro.* This fat-mobilizing factor is apparently a relatively small peptide. Its effects, both *in vivo* and *in vitro*, are in many ways similar to those of ACTH but it differs in chemical characteristics from ACTH. It is much more active than growth hormones in stimulating fatty acid release *in vitro* and its effects *in vivo* are not like those of growth hormone.

Fasting does not induce the urinary excretion of the active factor in patients with pituitary deficiency or in hypophysectomized goats. The administration of human growth hormone to two patients with pituitary deficiency did not lead to the appearance of activity in the urine. Fat-mobilizing factor activity has been found in the urine of fasting humans, goats, sheep, horses, and dogs. Weil and Stetten (146) had reported earlier the presence of what is probably a similar material in the urine of fasting rabbits. It is not present in the urine of normal humans consuming a regular diet but has been found in the urine of persons consuming a diet restricted in calories and very low in carbohydrate. The administration of ACTH to nonfasting subjects does not lead to the appearance of fat-mobilizing activity in the urine.

Much remains to be learned about this factor and its physiological role in the regulation of adipose tissue metabolism. The only information in the literature at present concerns its effects on the release of FFA.

Pituitary Fraction H. Rudman and co-workers have described the presence in extracts of hog pituitary gland of a component, Fraction H, which differs from previously described pituitary hormones (147, 148, 149). When injected into rabbits, this material causes a marked increase of serum FFA within 2 hours and an increase in total blood lipids 4 to 10 hours after injection (149, 150). Less than 1 mg of the purified Fraction H is required to produce these effects. Stimulation *in vitro* of the release of FFA from rabbit adipose tissue has been demonstrated with this material. (The effectiveness of Factor H from hog pituitary varies markedly with the species of the test animal.) There is no further information presently available concerning its action in adipose tissue.

A preparation of Factor H that behaves as a single component on paper electrophoresis has been obtained by Rudman and co-workers (151). The active substance is nondialyzable, insoluble in 5% trichloroacetic acid, and is destroyed by trypsin. Activity survives treatment with pepsin but is then found to be dialyzable and soluble in 5% trichloroacetic acid. Fraction H activity appears to reside in a relatively small molecule that, when isolated, is either an integral part of, or tightly bound to, a larger protein molecule (151).

Prolactin, Thyrotropin, and Follicle-Stimulating Hormone. Prolactin at concentrations up to 100 μg/ml has been found to be without effect on the release of FFA (17). At a concentration of 1 mg/ml prolactin has been shown to affect glucose metabolism in adipose tissue in a manner that much resembles that of insulin (35, 36, 99). When prolactin is present, oxidation of carbon-1 is markedly increased, with a much smaller increase in the rate of CO_2 formation from carbon-6. Fatty acid synthesis from C^{14}-glucose (or from acetate-1-C^{14} or pyruvate-2-C^{14} in the presence of glucose) is greatly enhanced (35, 36). Prolactin does not, however, stimulate glycogen synthesis from glucose to the extent that insulin does (35, 36). In addition, although glucose uptake by adipose tissue from alloxan diabetic rats is increased by prolactin, this hormone does not

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alleviate the defect in fatty acid synthesis as does insulin (35).

White and Engel (17) have reported that the release of FFA from adipose tissue *in vitro* is stimulated by thyrotropin and by follicle-stimulating hormone in high concentrations. As has been pointed out by the authors, the hormone preparations that were used were impure. Thus, the significance of the effects is difficult to evaluate.

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Errata

In the paper by Martha Vaughan (Vol. 2, No. 4, October, 1961), the sentence on page 313, second column, beginning on the fifth line from the bottom, should have read:

> Prolactin does not, however, stimulate fatty acid synthesis from glucose to the extent that insulin does (35).

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