Studies on the Metabolism of Adipose Tissue. XII. The Effects of Insulin and Epinephrine on Free Fatty Acid and Glycerol Production in the Presence and Absence of Glucose*

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Rat epididymal adipose tissue was incubated for 2 hours in a Krebs-Ringer phosphate medium containing glucose, 3 mg per ml, but no albumin. Measurements were made of the oxygen consumption, of the tissue content of free (unesterified) fatty acids, and of the amount of glycerol released into the medium. The addition of epinephrine, 0.1 μg per ml, to the medium resulted in an increased accumulation of tissue free fatty acids and medium glycerol. The rate of oxygen consumption was first increased but then gradually declined to a value below that of untreated tissue. When both epinephrine and insulin (1000 μunits per ml) were added glycerol release was nearly tripled while tissue free fatty acid levels were reduced to very low values. Concomitantly oxygen consumption was stimulated several fold. It is concluded that under these conditions the addition of insulin promotes a rapid reesterification of the free fatty acids formed by lipolysis of tissue fat. When glucose is omitted from the medium the tissue response the epinephrine is not markedly affected. However if insulin is now present in addition the accumulation of both tissue free fatty acids and medium glycerol produced by epinephrine is markedly diminished. This effect may be seen with as little as 10 μunits per ml of insulin. The decline in oxygen consumption otherwise seen in the presence of epinephrine is also prevented. A similar action of insulin is seen when epinephrine is replaced by the lipolytic agents, glucagon and thyrotropic hormone. It is suggested that under these circumstances insulin may exert an inhibitory influence upon the lipolytic processes of the tissue.

The addition of epinephrine to rat epididymal adipose tissue incubated *in vitro* causes a large increase in the rate at which triglycerides are hydrolyzed to free (unesterified) fatty acids and glycerol. When albumin is present in the incubation medium a large fraction of the free fatty acid produced is released from the tissue and appears in the medium as albumin-bound free fatty acid (Gordon and Cherkes, 1958; White and Engel, 1958). It has also been shown that homogenates prepared from adipose tissue which has been exposed to epinephrine contain a higher lipase activity than do homogenates of untreated tissue (Rizack, 1961; Hollenberg *et al.*, 1961). The release of free fatty acids seen in the presence of epinephrine is greatly reduced when insulin and glucose are also present (Gordon and Cherkes, 1958). It has been suggested that this decrease in release of free fatty acids is due to an increase in the rate at which the free fatty acids are reesterified by the tissue (Bally *et al.*, 1960; Steinberg *et al.*, 1960; Raben and Hollenberg, 1960; Wertheimer and Shafrir, 1960), presumably as a consequence of an increase in the rate of formation of glycerophosphate from glucose under these conditions.

We present here data which demonstrate that the accumulation of free fatty acids within the tissue produced by epinephrine is reduced by insulin even when no glucose is added to the incubation medium. This action of insulin differs from that seen in the presence of glucose and epinephrine in that concomitantly glycerol production is diminished rather than increased. Also no marked increase in oxygen consumption is seen. The results suggest that under these conditions insulin in some unknown manner inhibits the conversion of triglycerides to glycerol and free fatty acids.

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METHODS

Epididymal adipose tissue was obtained from Holtzman rats weighing 150 to 200 g and maintained as described previously (Ball and Merrill, 1961). Tissue from two rats was used in each experiment. The fat bodies were cut transversely three times, thus yielding pieces distal, medial, or proximal with respect to the epididymis and weighing from 40 to 100 mg. The twelve pieces of tissue so obtained were distributed into six Warburg vessels in such a manner that each vessel contained one distal and one proximal or two medial pieces. When one piece was taken from the left fat body of one rat, the other piece was taken from the right fat body of the second rat or *vice versa*. The Warburg vessels contained in the main compartment 2.80 ml of Krebs-Ringer phosphate medium modified to contain one half the recommended amount of calcium (Umbreit *et al.*, 1957), 0.20 ml of 0.15 M NaCl or of a hormone solution in the side-arm, and 0.20 ml of 10% KOH in the center well. The vessels were placed in a water bath at 37.3° for 5 minutes, after which the side-arm contents were dumped. The incubation was continued for an additional 2 hours at a shaking rate of 120 cycles per minute. Air served as the gas phase.

Stock solutions of crystalline zinc insulin (Lot #466368, Eli Lilly & Co.), and L(−)epinephrine (free base, Lot #38623, Burroughs-Welcome & Co.) were prepared as previously described (Ball and Merrill, 1961; Hagen and Ball, 1960) and diluted appropriately in 0.15 M NaCl on the day of their use. Where indicated, an amorphous insulin preparation (Lot #192-235B-188, Eli Lilly & Co.) which had been treated to remove zinc and glucagon was substituted for the crystalline preparation. We are indebted to Dr. Otto Behrens of Eli Lilly & Co. for the insulin samples.

The procedure for measuring the tissue content of free fatty acids has been described previously (Frerichs and Ball, 1962). Glycerol was determined according to the enzymatic procedure of Wieland (1957) employ-

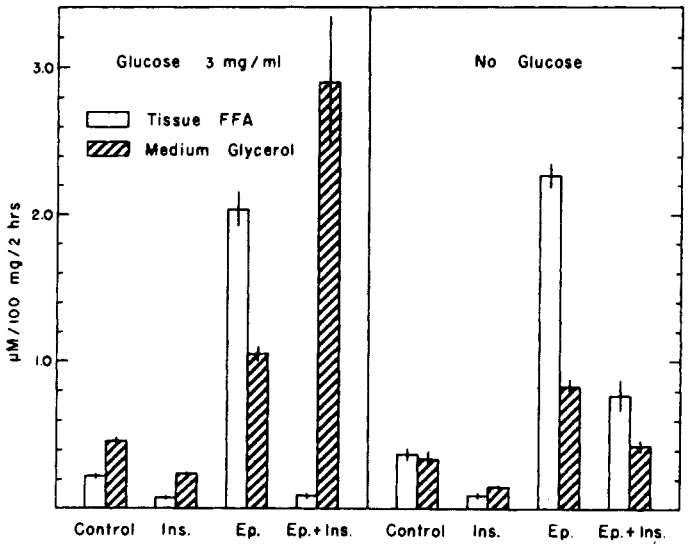


FIG. 1.—The effects of epinephrine and insulin on the free fatty acid and glycerol production of adipose tissue incubated with and without glucose. The data were collected from a total of ten experiments. In five experiments six pairs of tissues were utilized, namely, control ± glucose, epinephrine ± glucose, and epinephrine plus insulin ± glucose. In the other five experiments only four pairs were used: control ± glucose, and insulin ± glucose. The control values from the two sets of experiments were very similar and have been combined in the figure. Tissue pairs were selected as described in the methods section. The vertical lines designate two standard errors of the means.

ing glycerokinase and α-glycerophosphate dehydrogenase. The enzymes employed were purchased from the California Corporation for Biochemical Research. Medium aliquots were deproteinized with one-fifth volume of 30% w/v perchloric acid, neutralized with 40% KOH, and then assayed. Tissue glycerol analyses were performed on homogenates made with 5% w/v perchloric acid. With this procedure the recovery of known amounts of glycerol exceeded 95%.

RESULTS

The effects of epinephrine and insulin on the free fatty acid and glycerol production of adipose tissue are shown in Figure 1. The free fatty acid analyses were made on the tissue, while the glycerol measurements were performed on medium aliquots. It should be noted that no albumin was added to the incubation medium. For this reason the free fatty acids produced within the tissue during the incubation were not released into the medium, but accumulated within the tissue. No free fatty acids could be detected in the medium. On the other hand the glycerol liberated by triglyceride lipolysis within the tissue was released nearly quantitatively into the medium. It should be recalled that adipose tissue has a very poor capacity for metabolizing free glycerol, apparently because of the absence of glycerokinase in this tissue (Wieland and Suyter, 1957; Margolis and Vaughan, 1962). The amount of glycerol found in freshly excised tissue averaged 0.08 μmoles per 100 mg wet weight. After incubation for 2 hours in the absence of hormones this value fell on the average to 0.03 μmoles. Tissue exposed to epinephrine or to epinephrine and insulin for 2 hours in the presence of glucose gave average values of 0.09 and 0.13 μmoles respectively. These small quantities of tissue glycerol were considered negligible in relation to that in the medium, and such measurements were, therefore, not routinely performed.

The experiments shown in the left half of Figure 1 were performed with 3 mg per ml of glucose in the incubation medium. The addition of 0.1 μg per ml of epinephrine caused a large increase over the control values of both tissue free fatty acids and medium glycerol. Similar results have been described by others (White and Engel, 1958; Leboeuf *et al.*, 1959). When insulin was present in addition to epinephrine, there was a further increase in glycerol release while at the same time the tissue free fatty acid level fell to a very low value. The addition of insulin alone depressed both tissue free fatty acid and medium glycerol levels to values below those of the control tissue.

It would appear that very little of the glycerol which is released into the medium under these conditions is derived directly from glucose. This premise is supported by the fact that when uniformly labeled glucose-C^{14} is present in the medium only very small quantities of C^{14} appear in the medium glycerol in the presence of epinephrine or of epinephrine and insulin (Flatt, J. P., and Ball, E. G., unpublished results; Lynn *et al.*, 1960). The total amount of free fatty acids produced by the tissue should therefore equal at least three times the amount of glycerol formed. For example, in the case of the control tissue incubated with glucose the glycerol release in the 10 experiments averaged in Figure 1 was 0.46 μmoles per 100 mg of tissue during the 2-hour incubation period. This amount of glycerol should be accompanied by the formation of 1.38 μmoles of free fatty acids. However, only 0.22 μmoles was found in the tissue at the end of the incubation. Since the tissue contains about 0.10 μmole free fatty acids per 100 mg when it is removed from the animal (Jungas, R. L., and Ball, E. G., unpublished results) about 0.12 μmoles accumulated in the tissue during the incubation period. This represents only 9% of the amount produced in the tissue as calculated from the glycerol release. The portion unaccounted for must then have undergone reesterification or oxidation to CO_2. Now the total oxygen consumption measured during this incubation period is sufficient to oxidize completely about 0.10 μmole of oleic acid or only some 8% of the total calculated free fatty acid production. Hence even if we assume that only fatty acids were burned then some 83% of the free fatty acids liberated by complete triglyceride hydrolysis must have been reesterified. Similar calculations may be applied to the situa-

tions where hormones are present. Thus in the presence of epinephrine alone only 35% of the liberated free fatty acid was reesterified. However, when insulin and epinephrine were both present and triglyceride hydrolysis was at its maximum as indicated by glycerol production, then some 97% of the liberated free fatty acids must have been reesterified. It should be noted that such calculations of the extent of reesterification of free fatty acids liberated within the tissue yield minimum values since they do not include the possible reesterification of fatty acids with mono- or diglycerides (see discussion).

The very high rate of reesterification that proceeds in the presence of insulin, epinephrine, and glucose presumably reflects the ability of insulin to stimulate the uptake of glucose by the tissue. This glucose in turn furnishes the glycerophosphate employed in the reesterification process. In view of this key role of glucose it seemed of interest to repeat the experiments just described in the absence of added substrate. These are shown in the right-hand portion of Figure 1. It would be expected that in a glucose-free medium less reesterification would proceed since the store of glycogen in tissue from normally fed rats is low (Wertheimer and Shapiro, 1948). This is indeed found to be the case. Not only is the accumulation of free fatty acids under all conditions greater but the glycerol production is less than that seen with tissue incubated with glucose, *i.e.*, the calculated percentage of reesterification is lower in all cases.[1] For example in the presence of epinephrine the calculated reesterification is less than 10%, in contrast to 35% in the presence of glucose and epinephrine. The most striking effect of the absence of glucose is seen when both epinephrine and insulin are present. Not only does reesterification proceed poorly under these conditions but there is an unexpected decrease in the total glycerol and free fatty acid production over that seen with epinephrine alone. If the sole action of insulin were to facilitate the entrance of glucose into the tissue then one might expect that in a glucose-free medium the stimulation of glycerol and free fatty acid production by epinephrine would be about the same in the presence or absence of insulin. Insulin under these conditions acts as if it were preventing the breakdown of triglycerides, as though it possessed an antilipolytic action. A similar action of insulin though not as dramatic is discernible when insulin is added alone in either the presence or the absence of glucose.

The esterification of each mole of free fatty acid requires one mole of ATP, and an increased rate of free fatty acid esterification should be reflected in an increased oxygen consumption by the tissue. Therefore, the oxygen uptake of the tissue was also measured in these experiments, and the results are shown in Figure 2. In this figure the oxygen uptake during five successive 20-minute periods is shown. Oxygen consumption measurements were begun 5 minutes after the addition of hormones. Hence the first bar of each group represents the period from 5 to 25 minutes in the 2-hour incubation period. Oxygen consumption values for the final 15 minutes of the incubation period are not shown. The untreated tissue respired quite linearly during the entire incubation period, and the addition of glucose had very little effect on this rate. Insulin alone had little influence on the oxygen uptake when glucose was absent but did increase the oxygen consumption by 23% in the presence of glucose. As reported previously (Hagen and Ball, 1961), epinephrine addition produces a transient increase in oxygen consumption which is followed by a severe inhibition of respiration. This inhibition is delayed and somewhat less severe when glucose is present. When glucose, epinephrine, and insulin were added the oxygen consumption was stimulated nearly three-fold, a result consistent with the large amount of free fatty acid esterification calculated to occur in the tissue under these conditions. Of special interest in this figure is the finding that in the absence of glucose insulin prevents the inhibition of oxygen uptake otherwise seen in the presence of epinephrine. The inhibition normally seen in the presence of epinephrine appears to be due to the accumulation of large amounts of free fatty acids within the tissue (Hagen and Ball, 1961). Thus the ability of insulin to prevent this inhibition may be regarded as another manifestation of its ability to prevent accumulation of free fatty acids.

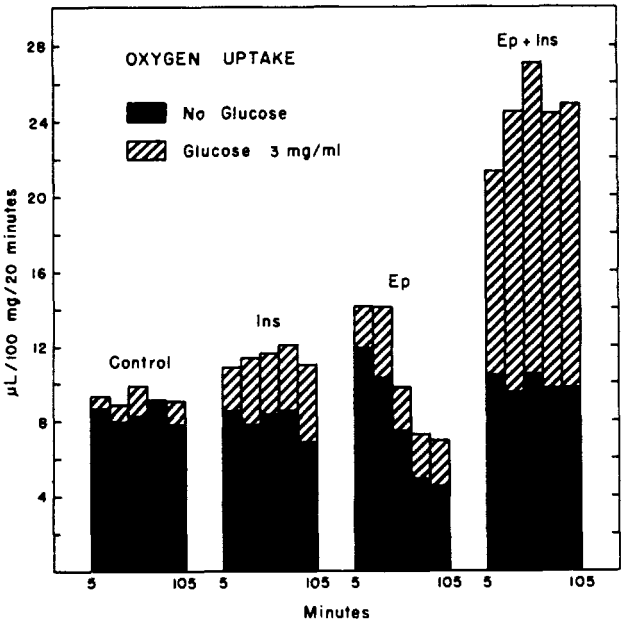


FIG. 2.—The effects of epinephrine and insulin on the oxygen consumption of adipose tissue in the presence and absence of glucose. The experimental conditions are described in the legend to Figure 1.

The experiments just discussed employed 0.1 µg per ml of epinephrine and 1000 µunits per ml of insulin. The effect of varying these concentrations is shown in Figure 3. The results are presented in terms of the ability of various concentrations of insulin to counteract the lipolytic action of epinephrine in a glucose-free medium. Thus a value of 100% on the ordinate means that the accumulation of free fatty acids within the tissue produced by epinephrine has been lowered to the value of the control tissue by the addition of insulin. The experiments were performed in the same manner as those discussed previously except that a special sample of insulin treated to remove zinc and traces of glucagon was employed. In addition gelatin, 2 mg per ml, was included in the incubation medium and in the solutions used to dilute the hormone preparations in order to prevent loss of insulin at low concentrations by adsorption on the glassware (Ball *et al.*, 1959). Several points are of interest. First, the ability of insulin to block the accumulation of free fatty acids produced by epinephrine is readily measurable at concentrations as low as 10 µunits per ml. This is the minimum concentration of insulin to which adipose

[1] The tissue free fatty acid content in the presence of insulin and glucose is not significantly lower than when only insulin is present, *i.e.*, the *P* value of the difference as calculated by Student's *t*-test exceeds 0.01.

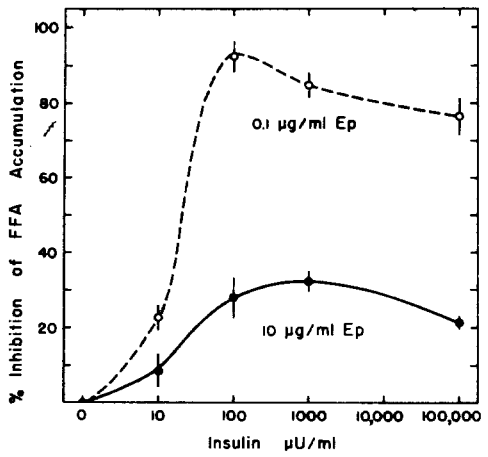


FIG. 3.—The effect of graded concentrations of insulin on the lipolytic action of epinephrine. Five experiments, each employing six pairs of tissue, were performed at each epinephrine concentration. One pair served as control, one pair was exposed to epinephrine alone, and the remaining four pairs were exposed to epinephrine plus graded amounts of insulin. The control tissue free fatty acid content in each set of experiments averaged 0.33 μmoles per 100 mg. Corresponding values in the presence of 0.1 and 10 μg per ml of epinephrine were 2.44 and 3.55 μmoles per 100 mg respectively. Gelatin, 2 mg per ml, was present in all vessels. The vertical lines represent two standard errors of the means.

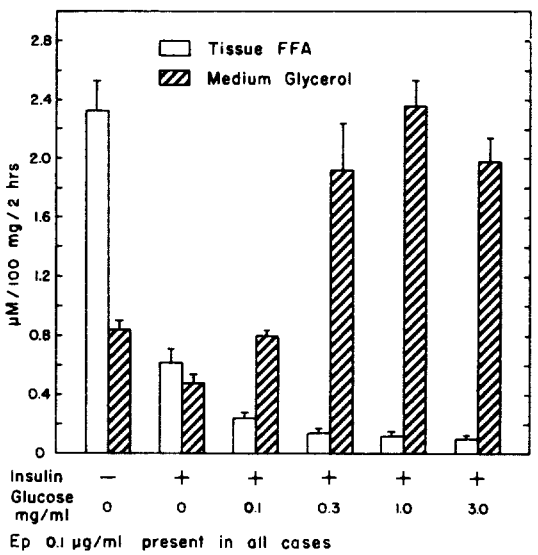


FIG. 4.—The effects of insulin and epinephrine on free fatty acid and glycerol accumulation in the presence of graded concentrations of glucose. When present the insulin concentration was 1000 μunits per ml. Five experiments each employing six pairs of tissue were performed. The vertical lines represent the standard errors of the means.

tissue gives a response as measured by net gas exchange (Ball *et al.*, 1959) or by the metabolic conversion of radioactive glucose to CO_2 (Renold *et al.*, 1960). Second, large amounts of insulin did not appear to be as effective as smaller amounts. For example, when the concentration of epinephrine is 0.1 μg per ml an inhibition of 92% is obtained with 100 μunits per ml of insulin, but only 77% inhibition is seen with 100,000 μunits per ml. The probability that this difference is due to chance may be calculated to be 0.03. Third, when the epinephrine concentration is increased 100-fold to 10 μg per ml the inhibitory effectiveness of insulin diminishes. Moreover, the inhibition is seen not to be dependent on the relative concentrations of epinephrine and insulin.

Additional experiments in the absence of added substrate have been performed to examine the influence of experimental conditions on the ability of insulin to inhibit the accumulation of free fatty acids and glycerol. These experiments have shown that similar results are obtained when a bicarbonate medium is used in place of phosphate, and when 5% bovine serum albumin is included in the incubation medium. In the latter case insulin inhibits the accumulation of free fatty acids in the medium as well as within the tissue. Studies of the time course of the production of glycerol and of free fatty acids have revealed similar effects of insulin whether the incubation period was as short as 10 minutes or as long as 3 hours. Moreover, epinephrine can be replaced by other lipolytic agents. For example, the triglyceride breakdown seen in the presence of thyrotropic hormone or glucagon, 1 μg per ml, is sharply reduced by 1000 μunits of insulin.

Experiments either with no added glucose or with 3 mg per ml glucose have been presented. Results obtained with intermediate glucose concentrations are shown in Figure 4. In these experiments epinephrine, 0.1 μg per ml, was present in all vessels. Insulin alone, 1000 μunits per ml, or insulin plus graded amounts of glucose was added to the vessels as indicated in the figure. In the vessel where insulin was present but not glucose there was a depression of both glycerol and free fatty acid production. As increasing amounts of glucose were provided in the medium in addition to insulin, the tissue content of free fatty acid was progressively lowered until a plateau value of around 0.1 μmole per 100 mg was reached. Glycerol release on the other hand increases as the glucose concentration in the medium increases and reaches a maximum value at physiological concentrations of glucose, *i.e.*, 1 mg per ml. Thus if glycerol production is used as a measure of the breakdown of triglycerides triggered by epinephrine then insulin alone inhibits lipolysis. The addition of glucose not only overcomes this inhibition but further enhances the lipolytic process.

In view of this marked effect of glucose upon the lipolytic process it was of interest to examine the action of several other substrates. Shown in Figure 5 are the effects of pyruvate, succinate, fumarate, and oxalacetate upon oxygen consumption, glycerol release, and free fatty acid accumulation within tissue incubated in the presence of epinephrine and insulin. These substrates all appeared to diminish free fatty acid accumulation to some extent. Pyruvate, however, produced an increase in glycerol production whereas succinate, fumarate, and oxalacetate reduced it. Pyruvate, as noted before (Hagen and Ball, 1961), and oxalacetate stimulate the oxygen consumption of adipose tissue. With these substrates, however, increased oxygen consumption is not accompanied by extensive reesterification.

DISCUSSION

The metabolic processes in adipose tissue under investigation here may be outlined as shown in Scheme 1.

The breakdown of triglyceride to glycerol and free fatty acids, the end-products measured in this study, apparently proceeds through diglyceride and monoglyceride. The presence of these compounds in adipose tissue has been demonstrated by Wadström (1957) and Stein and Stein (1961). This breakdown is catalyzed by a lipase which is apparently activated

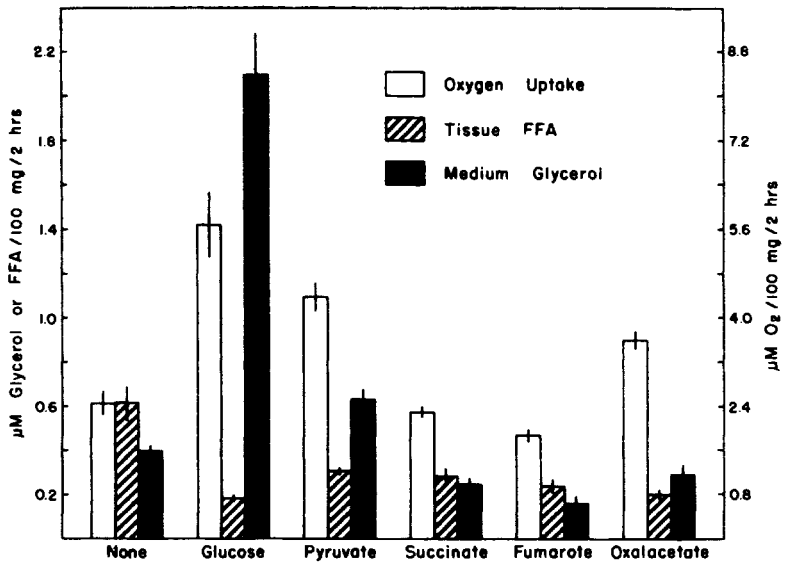
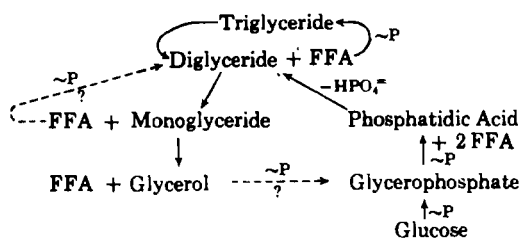


FIG. 5.—The combined action of epinephrine and insulin on oxygen uptake and free fatty acid and glycerol production in the presence of various substrates. Five experiments, each employing six pairs of tissue, were performed. Epinephrine, 0.1 μg per ml, and insulin, 1000 μunits per ml, were present in all vessels. The substrate concentration was 0.01 M. Pyruvate, succinate, fumarate, and oxalacetate were added as their sodium salts. Vertical lines represent two standard errors of the means.

in the presence of epinephrine and a number of other hormones (*cf.* Ball and Jungas, 1961). The first step, the formation of a diglyceride and free fatty acids, may be reversed through the conversion of the latter product to its CoA derivative in an energy-requiring process (Goldman and Vagelos, 1961; Steinberg *et al.*, 1961). Whether the second step, the conversion of diglyceride to monoglyceride, is reversible in a similar manner is doubtful. Studies by Clark and Hübscher (1961) and by Senior and Isselbacher (1962) have shown that this reaction does occur in various tissues, but Steinberg *et al.* (1961) could not demonstrate it in homogenates of adipose tissue. The step monoglyceride to glycerol and free fatty acid appears to be irreversible. As shown in the diagram the free fatty acids released in any one of these breakdown steps may be reconverted to the diglyceride stage through phosphatidic acid. This reaction requires glycerophosphate, which can be formed from glucose. Since glycerokinase is not detectable in adipose tissue homogenates (Wieland and Suyter, 1957) the reaction whereby glycerol is converted to glycerophosphate is indicated in the diagram as questionable.

The data presented here show that glycerol production by adipose tissue incubated *in vitro* in the presence of insulin and epinephrine may be markedly stimulated by the addition of glucose. This glycerol does not appear to be derived directly from this glucose, since it contains no appreciable label when radioactive glucose is employed (Lynn *et al.*, 1960; Flatt and Ball, unpublished). Concomitantly the accumulation of free fatty acids within the tissue is diminished. These metabolic changes would appear to be brought about



Scheme 1

by an accelerated breakdown of triglyceride to free fatty acids and glycerol with a rapid reesterification of the released fatty acids to triglyceride by way of glycerophosphate. That rapid reesterification is occurring is supported by the marked increase in oxygen consumption that occurs concomitantly and by the findings of Cahill *et al.* (1960) that under such conditions a marked incorporation of glucose carbon into glyceride glycerol takes place. The enhanced rate of reesterification in the presence of glucose is understandable in terms of its ability to furnish the glycerophosphate needed for this process. However, the manner by which glucose accelerates the process of lipolysis is not as clear. It is possible to postulate that by furnishing glycerophosphate for the reesterification process glucose lowers the levels of free fatty acids within the tissue to such an extent that lipolysis and hence glycerol production are favored.

Equally puzzling is the manner by which insulin inhibits the formation of glycerol and the accumulation of free fatty acids within the tissue. This effect of insulin may be seen in the presence or absence of glucose when no lipolytic agent has been added to the tissue. It is seen most strikingly in the absence of glucose and in the presence of a lipolytic agent such as epinephrine (*cf.* Fig. 1), but it is not seen under conditions where both glucose and a lipolytic agent are present. Since the completion of our work, Perry and Bowen (1962) have reported that they also have observed an inhibitory action of insulin in the absence of glucose upon the production of free fatty acids induced by the addition of epinephrine, corticotropin, or growth hormone preparations to adipose tissue *in vitro*. These workers did not study glycerol production or oxygen consumption. In the light of Scheme 1 the inhibitory action of insulin upon glycerol and free fatty acid accumulation may be interpreted as occurring in a number of ways. First, the hormone could be pictured as having an inhibitory action on the tissue lipase. Such an inhibition could be either a direct one upon the enzyme itself or upon a process whereby an interconversion of the enzyme from an inactive to an active form occurs in a manner similar to that seen for phosphorylase (Sutherland and Rall, 1960). In this

regard, evidence has been presented that ATP is needed for the activation of the tissue lipase by epinephrine (Rizack, 1961). Second, insulin could conceivably accelerate the steps whereby diglyceride (or monoglyceride) recombines with free fatty acids. Third, insulin might accelerate the conversion of glycerol to glycerophosphate by activating a latent glycerokinase. This would permit a resynthesis of diglyceride by way of phosphatidic acid. In either of these last two cases a reesterification of free fatty acids would be promoted. This would require additional energy, and some increase in oxygen consumption might be expected to result. An examination of the data from this viewpoint unfortunately permits no clear-cut decision to be reached. This is because a comparison of the oxygen uptake of tissue incubated with epinephrine and insulin with that of tissue incubated with epinephrine alone is difficult because of the progressive inhibition of oxygen consumption seen in the latter case. Thus the present data do not permit a decisive choice among these possible explanations for the inhibitory action of insulin. Results of experiments currently under way favor the concept that insulin acts by inhibiting the lipase or its activation.

The fact that insulin has any action whatsoever on adipose tissue in the absence of glucose demonstrates that all of the processes initiated or accelerated by insulin cannot be simply sequellae resulting from an increased glucose uptake. It should be pointed out that the dramatic effects which insulin exerts on the structure of the adipose cell membrane as seen with the electron microscope (Ball, 1962) and on its electrical potential (Beigelman and Hollander, 1962) can also be observed in the absence of glucose. The consequences to the cellular metabolism of such structural alterations could be quite far reaching. It is therefore not inconceivable that the effects of insulin observed here could be closely related to its effects on the cell membrane. These considerations prompted us to test another substance capable of initiating pinocytosis in adipose tissue, namely, protamine (Ball, 1962). It was found to possess an action similar to that of insulin in that glycerol and free fatty acid production were depressed in its presence. The addition of glucose in this case, however, did not result in the marked enhancement of glycerol production seen with insulin.

In this connection it may be of interest to recall the observation of Sutherland *et al.* (1962) that the enzyme system in liver forming cyclic 3′,5′-AMP from ATP which is responsive to epinephrine may be located in the cellular membrane. Although cyclic 3′,5′-AMP has not yet been shown to be involved in the activation of adipose tissue lipase by epinephrine, this possibility remains attractive.

The fact that the effects of large amounts of epinephrine cannot be blocked by large amounts of insulin suggests that the two hormones are not reacting with each other or competing with each other for a single site of action. Thus the maximum capacity of the lipolytic system would appear to exceed that of the system acted on by insulin. Also the fact that the lipolytic action of other hormones is likewise blocked by insulin indicates that the reaction is not one specifically limited to insulin and epinephrine. These observations are most easily explained by the assumption that the hormones are acting on separate counterbalancing processes which regulate the tissue lipase activity.

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