Studies on the Metabolism of Adipose Tissue. XIII. The Effect of Anaerobic Conditions and Dietary Regime on the Response to Insulin and Epinephrine*

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Studies have been made of the effects of epinephrine and insulin on the free fatty acid (FFA), glycerol, and lactate production of rat epididymal adipose tissue incubated in a Krebs-Ringer phosphate medium containing no glucose or albumin. Tissue from normally fed, fasted, and fasted-refed animals was employed under both aerobic and anaerobic conditions. Under aerobic conditions tissue from fasted-refed rats produced glycerol at a rate ten times greater than tissue from normally fed animals but had a lower tissue FFA level. Oxygen consumption of this tissue was four times that of normally fed rats, presumably reflecting a high rate of FFA reesterification. Epinephrine, 0.1 μg per ml, increased the accumulation of tissue FFA and medium glycerol with tissue from either normally fed or fasted-refed rats, although the effects were not as dramatic in the latter case. When insulin, 1000 μunits per ml, was also present these effects of epinephrine were sharply reduced. Anaerobic incubation reduced the formation of FFA and glycerol but increased lactate production in both types of tissue. When epinephrine was added to tissue from normally fed rats under anaerobic conditions there was very little change in glycerol or FFA accumulation and a small increase in lactate production. Tissue from fasted-refed animals responded to epinephrine under anaerobic conditions with a three-fold increase in glycerol production but no change in tissue FFA content. Lactate formation was increased 60%. The presence of insulin inhibited these effects of epinephrine on both glycerol and lactate production. Tissue from fasted animals showed little response to either epinephrine or epinephrine plus insulin and was not affected by anaerobic incubation. The activities of the initial enzymes of the Embden-Meyerhof and Warburg-Dickens-Lipmann pathways in adipose tissue from normally fed rats were found to decrease with increasing age of the animal. The tissue content of these enzymes was reduced by fasting and was restored to above normal levels by a subsequent refeeding period.

The rate at which adipose tissue triglycerides are hydrolyzed to FFA[1] and glycerol is markedly stimulated by the addition of epinephrine and certain other hormones to the tissue incubated *in vitro*. This stimulation may be largely prevented by the addition of insulin when glucose is absent from the incubation medium. On the other hand, in the presence of glucose the rate of hydrolysis is pronouncedly enhanced by the addition of insulin and a concomitant reesterification of the FFA to triglycerides occurs (Jungas and Ball, 1963). The need for glucose to permit reesterification is understandable in terms of the glycerophosphate it furnishes for this process. However, the manner by which glucose seemingly reverses the inhibitory action of insulin on the lipolytic process itself is not clear. The possibility that glucose is in some manner involved with furnishing certain energy requirements for the activation or functioning of the adipose tissue lipase appeared to be one explanation worthy of investigation. We therefore undertook a study of the response of this tissue to epinephrine and insulin under anaerobic conditions, where the supply of energy should be more restricted than under aerobic conditions. These studies revealed that no marked stimulation of triglyceride hydrolysis by epinephrine occurs under anaerobic conditions either in the presence or absence of glucose. These results then led us to a study of the behavior of adipose tissue obtained from fasted and refed rats, tissue which contains a much larger store of glycogen as an energy source than tissue from normally fed animals. The metabolic responses of this tissue proved to be both qualitatively and quantitatively different from those displayed by tissue from normally fed rats. The results of these studies constitute the body of the present report.

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[1] The abbreviations used are: FFA, free (unesterified) fatty acids, and Tris, tris(hydroxymethyl)aminomethane. The Warburg-Dickens-Lipmann pathway of glucose-6-phosphate metabolism will be referred to as the "pentose shunt."

METHODS

Rats supplied by the Holtzman Company were employed. Unless otherwise stated they weighed 150–200 g at the time of use. They were given free access to water and Purina laboratory chow and the precautions previously described for their care and handling were observed (Ball and Merrill, 1961). These animals will be designated as "fed normally." It should be mentioned that the feeding pattern of these animals is more or less uniform. The lighting in their quarters is automatically controlled. Lights go on at 6 a.m. and the rats are sacrificed some 4–6 hours later. In this interval food consumption is usually at a minimum. Animals designated as "fasted-refed" were fasted for 3 days and then refed for 2 days on the fat-free test diet distributed by Nutritional Biochemicals Corp. formulated according to Wooley and Sebrell (1945). It contained 21% casein, 16% cellulose, 58% sucrose, and a mixture of inorganic salts and vitamins.

For the metabolic experiments the epididymal adipose tissue from two rats was divided into distal, medial, and proximal portions, and the twelve pieces of tissue so obtained were distributed into six Warburg vessels according to the pattern previously outlined (Jungas and Ball, 1963). Each vessel contained 2.80 ml of a Krebs-Ringer phosphate buffer, *p*H 7.4, modified to contain half the prescribed amount of calcium (Umbreit *et al.*, 1957). No substrate or albumin was added. The vessel center wells contained 0.20 ml of 10% KOH and a folded strip of

filter paper. The six vessels were divided into three pairs; one pair of vessels contained 0.20 ml of 0.15 M NaCl in the side-arm, another pair 0.20 ml of a solution containing epinephrine, and the third pair 0.20 ml of a solution containing both epinephrine and insulin. The vessels were placed in a water bath at 37.3° and shaken at a rate of 120 cycles per minute. One member of each pair of vessels was gassed with oxygen for 5 to 10 minutes while the other member was gassed with nitrogen. The vessels were then sealed off and the contents of the side-arm tipped into the main compartment. Incubation was continued for 120 minutes after the side-arm addition. The oxygen uptake of the aerobic tissues was monitored throughout the incubation period. The gas pressure in the anaerobic vessels was noted at the start and at the completion of the incubation period as a check on the completeness of the anaerobic condition.

At the conclusion of the incubation the tissues were removed immediately, rinsed once in ice-cold 0.15 M NaCl, and assayed for FFA as described earlier (Frerichs and Ball, 1962). The incubation media were assayed for glycerol by the use of glycerokinase and α-glycerophosphate dehydrogenase according to the procedure of Wieland (1957). Medium lactate was measured with lactate dehydrogenase (Scholz *et al.*, 1959). Epinephrine and insulin stock solutions were prepared as previously described (Ball and Merrill, 1961; Hagen and Ball, 1960). These solutions were diluted appropriately with 0.15 M NaCl on the day of their use.

Measurements of tissue enzyme activities were made by preparing approximately 25% homogenates in water of the epididymal adipose tissue obtained from 2–4 rats. Homogenization was performed at room temperature with a Potter-Elvehjem type homogenizer. The homogenate was centrifuged for 20 minutes at 24,000 × *g* in a room maintained at 3°. The aqueous layer appearing between the pellet and the supernatant lipid material was used for the assays without further treatment. The double substrate procedure of Glock and McLean (1953) was used for the assays of the pentose shunt dehydrogenases. The reaction cuvet contained in a final volume of 3.0 ml: 125 μmoles Tris buffer, *p*H 7.6; 50 μmoles $MgCl_2$; 0.5 μmole $NADP^+$; 1.0 μmole 6-phosphogluconate; 1.0 μmole glucose-6-phosphate (when desired); and 0.02 to 0.10 ml of tissue extract. The change in optical density at 340 mμ during the period from 1–2 minutes after initiation of the reaction was used as a measure of enzyme activity.

The rate of $NADP^+$ reduction in the presence of glucose, ATP, and excess pentose shunt dehydrogenases was used to measure hexokinase activity. The reaction cuvet contained in a final volume of 3.0 ml: 125 μmoles Tris buffer, *p*H 7.6; 50 μmoles $MgCl_2$; 0.5 μmole $NADP^+$; 6 μmoles of ATP; 10 μmoles of glucose; 0.2 unit[2] glucose-6-phosphate dehydrogenase; 0.2 unit 6-phosphogluconate dehydrogenase; and 0.05 to 0.10 ml of tissue extract. One half of the change in optical density at 340 mμ during the period 3 to 8 minutes after initiation was used to compute the hexokinase activity.

The ability of tissue extracts to convert glucose-6-phosphate to triose phosphates was used as a measure of the over-all activity of the enzyme sequence glucose-phosphate isomerase, phosphofructokinase, and fructose diphosphate aldolase. Triose phosphate formation was assayed by adding α-glycerophosphate dehydrogenase and following the oxidation of NADH. The reaction cuvet contained in a total volume of 3.0 ml: 100 μmoles Tris buffer, *p*H 8.0; 30 μmoles $MgCl_2$; 2 μmoles ATP; 1.0 μmole NADH; 10 μmoles glucose-6-phosphate; 0.1 unit triose phosphate isomerase; 0.3 unit α-glycerophosphate dehydrogenase; 20 μmoles cysteine; and 0.05 to 0.20 ml of tissue extract. The optical density change at 340 mμ during the interval 4 to 10 minutes after initiation of the reaction was used as a measure of enzyme activity.

The conditions selected for each enzyme measurement were those yielding maximum reaction rates. For example, it was found that homogenates prepared with 0.15 M KCl had a much lower capacity for converting glucose-6-phosphate to triose phosphates than did water homogenates, while 0.25 M sucrose homogenates were intermediate in this regard. The cuvet contents were maintained at 37 ± 1° for all measurements. A small correction was necessary in each assay owing to slow reactions proceeding without substrate addition. Nitrogen analyses were performed on aliquots of the water homogenates by the procedure outlined previously (Frerichs and Ball, 1962).

The enzymes employed in the assays were obtained from the California Corporation for Biochemical Research except for the 6-phosphogluconate dehydrogenase. This enzyme was prepared from rat liver by ammonium sulfate fractionation (Glock and McLean, 1953) and was essentially free of hexokinase and glucose-6-phosphate dehydrogenase activity. $NADP^+$ NADH, glucose-6-phosphate, and 6-phosphogluconate were products of Sigma Chemical Co. The 6-phosphogluconate was recrystallized twice from ethanol (Seegmiller and Horecker, 1951) and contained no glucose-6-phosphate as determined by enzymatic assay. ATP was obtained from Pabst Laboratories.

Results

In Figure 1 the data obtained with normally fed rats are shown. In the presence of oxygen the addition of epinephrine increases the production of both FFA and glycerol. These increases are largely prevented by the addition of insulin. These results are similar to those presented earlier (Jungas and Ball, 1963). Lactic acid production is also increased by epinephrine, and this increase also appears to be inhibited by insulin. If the experiments are carried out under anaerobic conditions then the addition of epinephrine causes only a slight change in the rate of production of these three compounds. The further addition of insulin is without any marked effect. A comparison of the glycerol values for control tissue under aerobic and anaerobic conditions indicates that lipolysis is decreased under anaerobic conditions. Moreover, the ratio of FFA to glycerol also rises under these conditions, indicating less reesterification of the released FFA (see Jungas and Ball, 1963, for a discussion of this aspect). As might be expected, lactic acid production is increased some 6-fold under anaerobic conditions. Experiments similar to the ones shown here have demonstrated that even when the concentration of epinephrine is increased 100-fold to 10 μg per ml there is very little effect on FFA or glycerol production by the tissue under strictly anaerobic conditions. This is also true when 5% albumin is present in the medium, when 3 mg per ml glucose is present in the medium, or when a bicarbonate-buffered medium is employed. Moreover, the lipolytic actions of corticotropin, thyrotropin, and glucagon at concentrations of 1 μg per ml almost disappear under strictly anaerobic conditions.

[2] One unit of enzyme is the amount required to convert one micromole of substrate per minute at 37°.

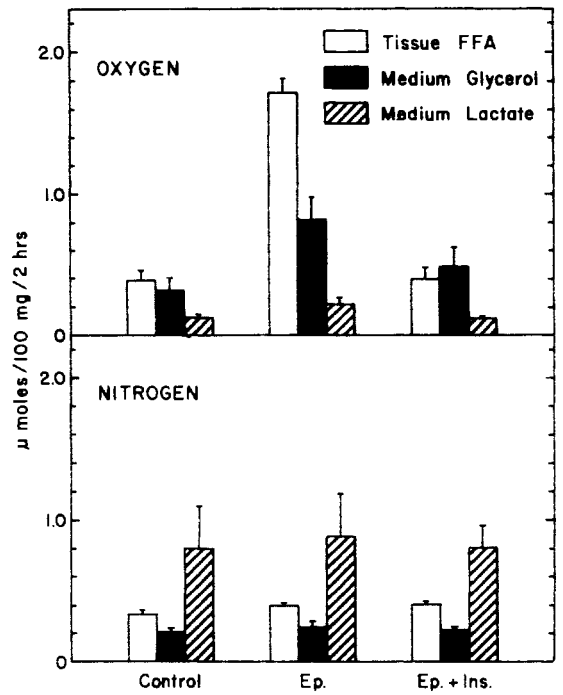

FIG. 1.—The response of adipose tissue from normally fed rats to epinephrine and insulin under aerobic and anaerobic conditions. The concentration of epinephrine (Ep) was 0.1 μg per ml and of insulin (Ins) 1000 μunits per ml. The results represent the averages of four experiments. The vertical lines designate the standard errors of the means.

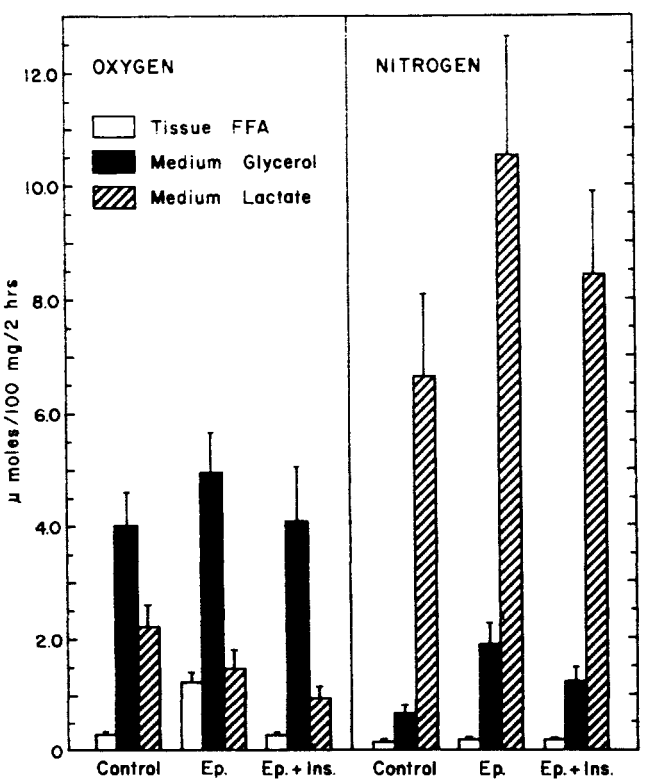

FIG. 2.—The response of adipose tissue from fasted-refed rats to epinephrine and insulin under aerobic and anaerobic conditions. The results represent the averages of six experiments. Other details are described in the legend to Figure 1.

These data indicate that the ability of epinephrine and other hormones to activate adipose tissue lipase (Hollenberg *et al.*, 1961) is lost under anaerobic conditions. This fact suggested to us that the lipase activation process might require high-energy phosphate and thus resemble that occurring in the activation of muscle or liver phosphorylase by epinephrine. A role for ATP in the activation of lipase by epinephrine in homogenates of adipose tissue has already been reported (Rizack, 1961). The availability of ATP in the anaerobic tissue should be much less than in the aerobic tissue, since no substrate was present in the incubation medium and the sole source of anaerobic ATP production would be the conversion of tissue carbohydrate stores to lactic acid. Moreover, since adipose tissue from rats fed laboratory chow *ad libitum* contains only 0.01–0.04% glycogen (Tuerkischer and Wertheimer, 1942; Frerichs and Ball, unpublished), even the amounts of ATP produced by this process would be limited.

One method of investigating this question appeared to be the use of adipose tissue with richer glycogen stores. Adipose tissue containing 2 to 3% of its wet weight as glycogen may be obtained from rats refed a diet rich in carbohydrate subsequent to a fasting period (Frerichs and Ball, 1962). Therefore experiments similar to those described above were carried out with adipose tissue from fasted-refed rats. The results are presented in Figure 2. It is immediately evident that this tissue is far more active metabolically on a wet weight basis than is the tissue from rats fed normally on Purina chow. The untreated aerobic glycogen-rich tissue produced glycerol at a rate more than ten times greater than tissue from normally fed rats under the same conditions. This rate is even greater than that seen with tissue from normally fed rats incubated with glucose, insulin, and epinephrine (Jungas and Ball, 1963). Despite this high rate of glycerol production, the FFA content of the untreated glycogen-rich tissue is not elevated. If most of the glycerol is being produced by the total hydrolysis of triglycerides, as seems most likely (Jungas and Ball, 1963), it follows that the tremendous quantities of FFA liberated are being nearly totally reesterified to triglycerides. Thus an amount of glycerol phosphate nearly equal to that shown for glycerol in the figure must have been provided from the glycogen stores of the tissue. In addition substantial amounts of glycogen were converted to lactic acid, and the extent of this process in the glycogen-rich tissue under aerobic conditions was about sixteen times greater than that seen in the tissue from normally fed rats.

Addition of epinephrine to the aerobic glycogen-rich tissue stimulates both glycerol production and FFA accumulation in a manner similar to that seen with tissue from normally fed rats. In contrast to tissue from normally fed rats lactic acid production is reduced by epinephrine. With both epinephrine and insulin present the increases in glycerol production and tissue FFA accumulation seen with epinephrine alone are eliminated, and lactate production is even further reduced. It is to be noted, however, that insulin does not repress lipolysis below that of the control tissue.

When glycogen-rich tissue is incubated under anaerobic conditions, the rate of lipolysis is markedly reduced, as is evident from the glycerol production, and a tremendous outpouring of lactic acid from the tissue occurs. Addition of epinephrine increases both glycerol and lactic acid production, while insulin in part counteracts this action of epinephrine. Thus epinephrine appears to accelerate lipolysis under anaerobic conditions only when glycogen-rich tissue is used. It should be noted that this conclusion is based upon the assumption that the glycerol released into the medium is derived largely from the hydrolysis of

triglyceride. This assumption appears to be valid for adipose tissue from normally fed rats (Jungas and Ball, 1963) but may not be applicable to tissue from fasted-refed rats.

For purposes of comparison, similar experiments were performed on rats that were fasted 3 days but not refed. These results are given in Figure 3. Several features deserve mention. First, the FFA content found in the tissues at the end of the incubation is almost exactly three times the amount of glycerol released into the medium. There is thus practically no reesterification of FFA occurring in this tissue. Since glycerol phosphate is needed for the reesterification process and the fasted tissue has almost no glycogen from which to produce it, this result is readily understandable. Secondly, the tissue from fasted rats shows almost no response to either epinephrine or epinephrine plus insulin under aerobic or anaerobic conditions. It should be noted that the glycerol release is as great in the untreated fasted tissue as it is in the tissue from normally fed rats incubated in the presence of epinephrine. This finding reflects the greater lipolytic activity known to be associated with tissue from fasted rats (Gordon and Cherkes, 1958; Reshef *et al.*, 1958) and may account for the failure of the fasted tissue to respond to epinephrine addition. Thirdly, in fasted tissue the rate of lipolysis under anaerobic incubation is about the same as that seen under aerobic conditions. This behavior contrasts with that seen (*cf.* Figs. 1 and 2) with tissue from either normally fed or fasted-refed rats. No explanation for this difference in behavior has yet been established. Finally, the fasted tissue does produce some lactic acid. In fact, the aerobic glycolysis in the fasted tissue exceeds that seen in tissue from normally fed rats. Since no detectable glycogen is reported to be present in the fasted tissue (Richter, 1931; Tuerkischer and Wertheimer, 1942), the source of this lactic acid is somewhat puzzling. Studies of the time course of this lactate released revealed that the medium content of lactate was highest at the time of hormone addition, and decreased during the course of the incubation. Thus the lactic acid may be present in the tissue as it is removed from the animal.

The esterification of FFA with glycerol phosphate requires ATP. Hence the different rates of FFA esterification observed for fasted and for fasted-refed rats should be reflected in the tissue's oxygen consumption. Therefore, the oxygen uptake of the aerobic tissues was also measured in the experiments just described. The results are given in Figure 4. The oxygen uptake of the control tissue from fasted-refed rats is greatly elevated compared to that seen with normally fed rats, whereas the fasted tissue's oxygen uptake is reduced below the normals. The presence of epinephrine increased the oxygen uptake only in the glycogen-rich tissue, and insulin reduced this effect. In the case of the tissue from normally fed rats, epinephrine addition produces a transient stimulation of oxygen consumption which is followed by an inhibition (Hagen and Ball, 1961; Jungas and Ball, 1963). When averaged out over the 2-hour incubation period the net result is a slight decrease in oxygen uptake, as shown in Figure 4. When both epinephrine and insulin are added the stimulatory phase of the response is less marked, but the inhibitory phase is absent, with the net result being an increased oxygen uptake during the 2-hour period. Very little effect of the hormones is seen on the oxygen uptake of tissue from fasted rats.

In view of the marked difference in metabolic activity of adipose tissue from rats fed normally with

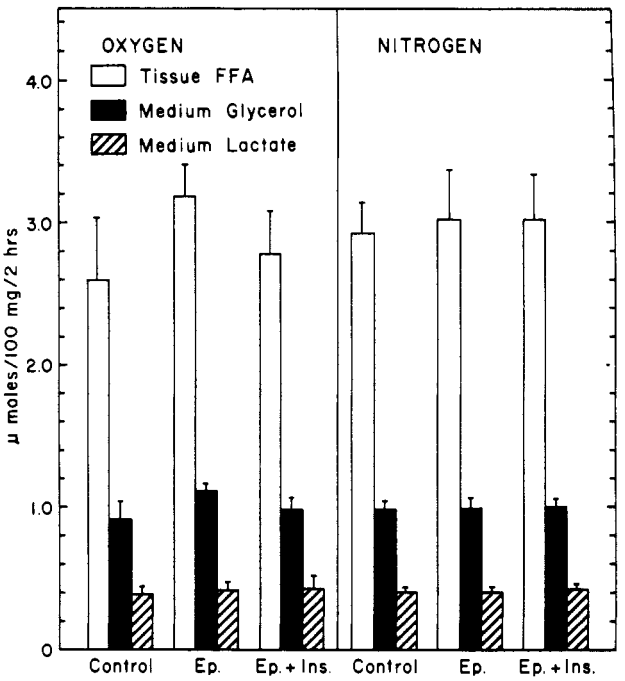

FIG. 3.—The response of adipose tissue from fasted rats to epinephrine and insulin under aerobic and anaerobic conditions. The results represent the averages of four experiments. Other details are described in the legend to Figure 1.

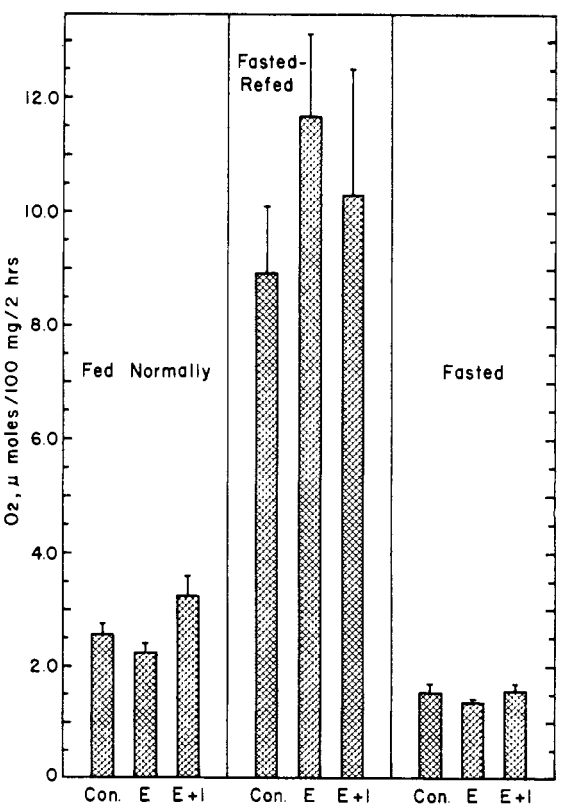

FIG. 4.—The oxygen uptake of adipose tissue from normally fed, fasted-refed, and fasted rats. The measurements were made during the same experiments shown in Figures 1–3. The vertical lines designate the standard errors of the means.

those either fasted or fasted and refed it seemed of interest to study certain enzymatic activities of these tissues along with their nitrogen content. The results of these experiments are given in Figure 5. Values for rats fed normally are presented for three age

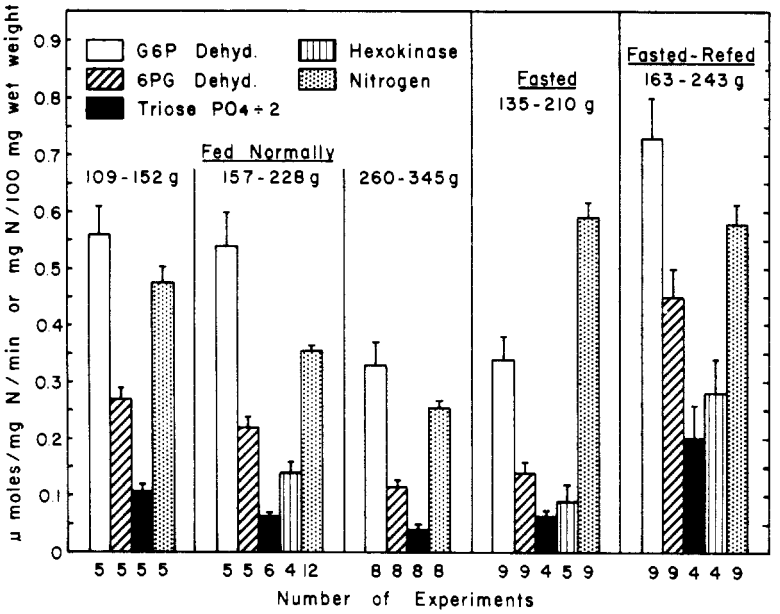

FIG. 5.—The tissue content of certain enzymes and of nitrogen as a function of the animals' weight and dietary regime. The ordinate gives the enzyme activities of tissue homogenates in terms of μmoles of substrate converted per mg of tissue nitrogen per minute. The abbreviations employed are: G6P Dehyd., glucose-6-phosphate dehydrogenase; 6PG Dehyd., 6-phosphogluconate dehydrogenase; Triose $PO_4 \div 2$, one half of the rate of production of triose phosphates from glucose-6-phosphate *via* glucose-phosphate isomerase, phosphofructokinase, and fructose diphosphate aldolase. The range of the weights of the rats employed for the assays is given directly below the diet designations. The vertical lines designate the standard errors of the means.

groups, since it has been found that the metabolic activity of rat adipose tissue expressed per unit of wet weight diminishes rather strikingly after the weight of the rat exceeds 200 g (Ruska and Quast, 1935; Hagen *et al.*, 1959; Benjamin *et al.*, 1961). Expressed in terms of activity per mg N there is a decrease in enzymatic activity as the rat ages for glucose-6-phosphate dehydrogenase, for 6-phosphogluconate dehydrogenase, and for the enzymes involved in the conversion of glucose-6-phosphate to triose phosphates. The value for the two pentose shunt dehydrogenases drops about 50% in going from the youngest age group to the oldest. Triose phosphate production by the glycolytic pathway falls even more than this. Fasting for 3 days appears to lower the activity of all enzymes studied with the exception of those producing triose phosphate from glucose-6-phosphate. The most striking change is seen after 2 days of refeeding of rats fasted 3 days. Here the enzymatic activity may double or triple as a result of the 2 days' refeeding and exceeds in all cases the values for even the youngest control rats. This change from the starved to the refed state occurs with little change in the nitrogen content of the tissue. Thus the results are the same whether expressed as in Figure 5 or in terms of activity per unit wet weight. Thus changes in metabolic activity of intact adipose tissue are mirrored in the activities of certain enzymes extracted from the tissue.

DISCUSSION

The results presented here demonstrate that the ability of epinephrine to increase the production of FFA and glycerol by adipose tissue from normally fed rats is nearly completely lost when the tissue is incubated under strictly anaerobic conditions. These findings differ from those reported by Engel and White (1960), who found that both epinephrine and corticotropin at concentrations of 10 μg per ml markedly stimulated lipolysis under 95% N_2–5% CO_2 in Krebs-Ringer bicarbonate, but agree with earlier reports by these same authors. We have been unable to obtain any appreciable stimulation with epinephrine at concentrations ranging from 0.1 to 10 μg per ml in either phosphate buffer under nitrogen or in Krebs-Ringer bicarbonate gassed with 95% N_2–5% CO_2. The addition of albumin or glucose with or without insulin to the media has had little effect upon these results. Moreover, we have found that the lipolytic action of corticotropin, glucagon, or thyrotropic hormone is negligible under anaerobic conditions at concentrations (1 μg/ml) which yield marked effects under aerobic conditions. We are at a loss to explain this difference in our results from those reported by Engel and White. It should be pointed out, however, that strictly anaerobic conditions are necessary if no stimulation of lipolysis by these agents is to be obtained.

The reason for the difference in action of epinephrine and other hormones upon lipolysis under aerobic and anaerobic conditions is not clear. One possible explanation is that the activation of lipase by these agents requires some form of high-energy phosphate. When adipose tissue is incubated under anaerobic conditions the sole source of this energy will be the glycogen stores of the tissue. In tissue from normally fed rats this source is limited, as is indicated by the small amount of lactic acid that is produced under anaerobic conditions (*cf.* Fig. 1). On the other hand when tissue from fasted-refed rats is used much more energy is available from glycogen stores. The lactic acid production by this tissue under anaerobic conditions is 10-fold or more than that seen with tissue from normally fed rats (*cf.* Fig. 2). Thus the finding that epinephrine can triple the glycerol production and hence presumably lipolysis in glycogen-rich tissue under anaerobic conditions lends some support to the premise that a high energy source is needed for the activation of lipase. It must be noted, however, that no increase in fatty acids is observed concurrently with the increased glycerol production. It seems reasonable to assume that this reflects the ex-

tremely high capacity of this tissue for FFA esterification which is shown under aerobic conditions. On the other hand it cannot be ruled out that the glycerol measured is not derived from triglycerides and hence does not reflect enhanced lipolysis. It does emphasize that conclusions concerning the rate of lipolysis based solely on production of FFA need careful scrutiny.

Along these same lines it is also of interest to note that when tissue from either normally fed or fasted-refed rats is placed under anaerobic conditions, there is a fall in the rate at which glycerol and fatty acids are produced. This is seen most markedly in the case of tissue from fasted-refed rats (Fig. 2). It is as if the lipase present in the tissue upon its removal from the animal becomes repressed or inactivated by being placed under anaerobic conditions. All these findings are suggestive of a pattern for the activation and inactivation of lipase which is not unlike that seen for phosphorylase (Sutherland and Rall, 1960). Thus activation of the lipase would be a process requiring high-energy phosphate. The possible role of adenosine-3′,5′-phosphate in the activation of both lipase and phosphorylase by various hormones has been discussed previously (Ball and Jungas, 1961; Frerichs and Ball, 1962).

It should be noted that no change in the lipase activity of adipose tissue from starved rats occurs when it is placed under anaerobic conditions (*cf.* Fig. 3). Whether this signifies that a different lipase is present in this tissue or that a different mode of activation of the enzyme occurs are matters for further explanation. It may be recalled however in this connection that there is evidence in the literature to indicate that the mobilization of fat in the intact animal in response to fasting may be effected without the intermediation of hormones such as epinephrine (*cf.* Engel and White, 1960; Goodman and Knobil, 1961). It should also be noted that the addition of epinephrine to tissue from starved rats causes only a small increase in the rate of lipolysis. This may be due to the fact that the unstimulated rate is already near the maximum capabilities of the tissue. However of greater interest is the fact that though insulin represses the small effect exerted by epinephrine it does not affect the high inherent rate of lipolysis shown by this tissue.

There are several points with regard to the production of lactic acid that have been observed here that deserve comment. First, the addition of epinephrine to tissue from normally fed animals stimulates both the production of lactic acid and the process of lipolysis if the tissue is incubated under aerobic conditions (*cf.* Fig. 1). This dual action of epinephrine and other hormones on phosphorylase and lipase action has been commented on previously by Ball and Jungas (1961). However in the case of tissue from fasted-refed rats the addition of epinephrine diminishes lactic acid production though lipolysis is still enhanced (*cf.* Fig. 2). At first glance this might suggest that epinephrine is not stimulating glycogen breakdown in this tissue. However the possibility must be considered that the glucose-6-phosphate derived from glycogen is being preferentially channeled into pathways other than lactic acid. As indicated by the relative production of glycerol and FFA as well as the oxygen consumption, lipolysis and reesterification of the liberated FFA is proceeding at a very rapid rate in this tissue. This rate may be further increased by the addition of epinephrine. Thus a sizeable amount of the carbohydrate stores of the tissue are being formed into glycerol phosphate which is utilized for the resynthesis of triglyceride from the liberated FFA. Stated another way, both pyruvate and dihydroxy acetone phosphate are competing for the NADH formed during the oxidation of glyceraldehyde phosphate. When the tissue is placed under anaerobic conditions lipolysis is repressed. Lactic acid production then proceeds at a very rapid pace, indicating that the failure of the tissue to produce more lactic acid under aerobic conditions is not due to a deficiency of the enzymes involved in the production of this metabolite.

The other point of interest with regard to lactic acid production is that the addition of insulin decreases the amount of this metabolite which is formed in the presence of epinephrine. This is true under both aerobic and anaerobic conditions and with tissue from either normally fed or fasted-refed rats. It is not evident with tissue from fasted rats. Insulin thus appears to repress the stimulatory action of epinephrine on both glycogen and fat breakdown. This suggests that a common mechanism may be involved in this dual action of epinephrine. The diminution in lactate production may also reflect the stimulation by insulin of the pathway of glycogen formation, thus counteracting any enhanced phosphorylase activity. Villar-Palasi and Larner (1961) have shown that there is an increased activity of UDPG-glycogen transglucosylase in insulin-treated rat diaphragm.

The comparisons of metabolic activity made in this study on tissues from rats kept on different dietary regimes were all made on the basis of the wet weight of the tissue. In Figure 5 the nitrogen content per unit wet weight of tissue from animals under these different regimes is given. The most marked difference is seen between rats fed normally and those either fasted or fasted-refed, there being little difference between the latter two. There is, however, no essential difference in the conclusions to be drawn from the data if they are expressed in terms of nitrogen content rather than on a wet weight basis.

Recent measurements (Flatt and Ball, unpublished) with radioactive glucose indicate that in adipose tissue from normally fed rats the amount of hexose phosphate oxidized by glucose-6-phosphate dehydrogenase is approximately equal to the amount cleaved by fructose diphosphate aldolase. It is therefore of interest to compare the maximum capacity under optimal conditions of the pentose shunt dehydrogenases with the capacity of the glycolytic enzymes for producing triose phosphate. As shown in Figure 5, the activity of the shunt dehydrogenases is 2- to 3-fold higher not only in normally fed rats but in fasted or fasted-refed rats as well. These results suggest that at least in tissue from normally fed rats the dehydrogenases of the pentose shunt are not working at their maximum capacity. Thus the rate of NADPH production by the pentose shunt enzymes is probably governed by the amount of glucose flowing through the glycolytic pathway. The possibility that the rate of reoxidation of NADPH, whether by fatty acid synthesis or by other means, might limit the rate of the pentose shunt reactions in the cell has already been suggested (*cf.* Masoro, 1962). The present results are in accord with this hypothesis.

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Characterization of Lipids from Canine Adrenal Glands*

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Neutral lipids, which represented approximately 90% of total adrenal lipids, were isolated by silicic acid chromatography. Methyl esters obtained from cholesterol esters and triglycerides by methanolysis were determined by gas-liquid chromatography. The cholesterol esters were mainly unsaturated, containing high proportions of 8,11,14-eicosatrienoic acid, arachidonic acid, and adrenic acid (7,10,13,16-docosatetraenoic acid) in addition to oleic acid, which was the major component. Over 90% of the cholesterol in canine adrenal gland existed in the esterified form. Adrenal phosphatides were partially purified by silicic acid chromatography. The methyl esters liberated from individual phosphatides by mild alkaline hydrolysis were studied by gas-liquid chromatography. Arachidonic and adrenic acids were distributed in all of the fractions, with relatively higher concentrations in phosphatidylethanolamine. Phosphatidylethanolamine and phosphatidylcholine showed a marked specificity for stearic and palmitic acids, respectively. Lysophosphatidylcholine was surprisingly rich in unsaturated fatty acids. More complete characterization of the phosphatides was provided by anion-exchange chromatography of the water-soluble products of mild alkaline hydrolysis. Under the conditions of Hawthorne and Hübscher (1959), modified for microanalyses and with an extended range of eluting solvents, phosphorus-containing products related to cardiolipin, phosphatidic acid, phosphatidylserine (and lysophosphatidylserine), phosphatidylcholine (and lysophosphatidylcholine), phosphatidylinositol, diphosphoinositide, and triphosphoinositide were observed. The major components were glycerylphosphorylethanolamine and glycerylphosphorylcholine, which accounted for more than 60% of the phosphorus. In addition, the mixture of phosphatides contained traces of sphingomyelin and plasmalogens. Further characterization of cardiolipin, the polyphosphoinositides, and the serine-containing phosphatides will be required before exact structures can be assigned.

It has been recognized for a number of years that adrenal tissue contains high concentrations of polyenoic acids (Sinclair, 1958; Klenk and Eberhagen, 1960), but systematic investigations of the nature of these acids were not available until Dailey *et al.* (1960) reported the fatty acid composition of adrenal cholesterol esters from a variety of species and a number of pure polyenoic acids isolated from bovine adrenal phosphatides were fully characterized (Klenk and Eberhagen, 1960). In confirmation of the structural identifications made by Klenk and Eberhagen, we recently established the structures of three polyenoic acids, isolated from canine adrenal lipids, as 8,11,14-eicosatrienoic acid, 5,8,11,14-eicosatetraenoic (arachidonic) acid, and 7,10,13,16-docosatetraenoic acid (Chang and Sweeley, 1962).

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The occurrence of relatively high proportions of 7,10,13,16-docosatetraenoic acid, for which we wish to propose the name "adrenic acid,"[1] in canine adrenal lipids was surprising, since extensive analyses of other mammalian tissues and body fluids by a number of investigators have indicated little if any of this component. Medium to high levels of adrenic acid[2] were found in the cholesterol esters from adrenal glands of rat, dog, and man, but the cholesterol esters from blood of these

[1] We were reluctant to increase the body of trivial nomenclature but wished to avoid repetitive use of the more awkward generic name. The choice of adrenic acid rested on the proportions of this polyenoic acid in adrenal glands from rat, dog, and man, in which it occurs in much higher concentration than any other mammalian tissue.

[2] Identification by these authors was based on retention times observed on gas chromatography, which indicated a C_{22}-tetraenoic acid. The component which they so designated was almost certainly adrenic acid.