Biochem. J. (1971) **124**, 615–621
Printed in Great Britain

Studies on the Conversion of Pyruvate into Fatty Acids in White Adipose Tissue

EFFECTS OF INSULIN, ALLOXAN-DIABETES AND STARVATION

BY MITCHELL L. HALPERIN
University of Toronto School of Medicine, Department of Medicine, Medical Sciences Building, Room 7363, Toronto 181, Ont., Canada

(*Received 6 April 1971*)

The effect of insulin on the conversion of pyruvate into fatty acids in the presence and in the absence of glucose was studied in epididymal adipose tissue of the rat. 1. In adipose tissue from the normal rat, conversion of pyruvate into fatty acids is directly related to its concentration, the maximal rates occurring with 40mM- and the half-maximal rates with approx. 4mM-pyruvate. Insulin treatment did not greatly influence the maximal rates, but the half-maximal rates were at much lower pyruvate concentrations. This effect of insulin could be seen with physiological concentrations of this hormone (50–100μunits/ml). 2. In adipose tissue from acute-alloxan-diabetic and 36h-starved rats the conversion of pyruvate into fatty acids was almost zero until its concentration exceeded 3mM and then increased markedly as the concentration of pyruvate was increased. The lag phase of this S-shaped curve was decreased but not eliminated when insulin was present. This could account for the very low rates of glucose conversion into fatty acids in these metabolic states. Maximum rates of fatty acid synthesis were similar in the presence and in the absence of insulin, but only when 30–40mM-pyruvate was employed. Re-feeding of the starved rats or insulin treatment of the diabetic rats *in vivo* for several days restored these patterns to normal.

Lipogenesis can occur in white adipose tissue at rapid rates. Glucose is the major physiological substrate for this pathway. Its conversion into glyceride fatty acids is controlled primarily by insulin. Insulin seems to act by two mechanisms: (1) by increasing intracellular pyruvate concentrations, presumably as the result of augmented glucose transport (Krahl, 1951; Winegrad & Renold, 1958); (2) by stimulating pyruvate conversion into fatty acid (Jungas, 1970; Halperin, 1970; Halperin & Robinson, 1971). In adipose tissue from starved and 48h-alloxan-diabetic rats, fatty acid synthesis from glucose occurs at very low rates. This rate of lipogenesis is only in small part restored to normal values by treatment with insulin *in vitro*.

The experiments reported here were undertaken to extend our observations that insulin augments pyruvate conversion into fatty acids, and also to investigate the low lipogenic rate in adipose tissue from starved and diabetic rats. At a very high concentration of pyruvate (40mM), the rate of synthesis of fatty acids was rapid and insulin no longer augmented this rate. Insulin increased lipogenesis from pyruvate in adipose tissue from normal rats when lower pyruvate concentrations were employed (<5mM). The concentration of insulin required for this effect (50–100μunits/ml) is similar to that seen in the postprandial state (Yalow & Berson, 1960). When adipose tissue from starved or diabetic rats is incubated with low concentrations of pyruvate (<3mM) the rate of fatty acid synthesis is exceedingly low. Insulin increased this rate almost to normal only at high non-physiological pyruvate concentrations (approx. 5mM). The shape of a graph of the rate of fatty acid synthesis plotted against the pyruvate concentration changes from a hyperbolic to an S-shaped one in starvation and diabetes. The findings reported in this paper suggest an interpretation of the observed low rates of conversion of glucose into glyceride fatty acids during insulin deficiency.

MATERIALS AND METHODS

Rats. Epididymal fat-pads were obtained from male rats (100–150g; Microbiological, Baltimore, Md., U.S.A.) fed on a Purina Lab Chow diet. In the studies with

starved–re-fed rats, the rats were starved for 72h and re-fed for 48h on a high-carbohydrate diet. In all experiments the animals were allowed free access to food before the time of killing (approx. 10a.m.) except in the experiments with starved rats where food was removed for 36h.

Rats were made diabetic by intravenous alloxan injection (50mg/kg) after they had been starved for 36h. The rats were then allowed free access to food and water for an additional 48h. Blood glucose in all diabetic rats was 30–36mM before death.

Chemicals. Enzymes, glycolytic intermediates, adenine nucleotides, sodium pyruvate and triethanolamine were obtained from Boehringer Mannheim Corp., New York, N.Y., U.S.A., collagenase was from Winley Morris Co. Ltd., Montreal, Que., Canada, ^{3}H-labelled water was from New England Nuclear Corp., Boston, Mass., U.S.A., and sodium [U-^{14}C]pyruvate and D-[U^{14}C]glucose were from Amersham/Searle Corp., Don Mills, Ont., Canada. Insulin, alloxan and bovine serum albumin (fraction V) were from Sigma Chemical Co., St Louis, Mo., U.S.A., and protamine zinc insulin (40units/ml) was from Connaught Medical Research Laboratories, Toronto, Ont., Canada.

Media. Fat-pads were incubated in a bicarbonate-buffered medium (Krebs & Henseleit, 1932) containing 20mg of defatted albumin/ml (Denton & Halperin, 1968) gassed with $O_2 + CO_2$ (95:5) in a volume of 2.5ml/fat-pad, unless specified otherwise. The concentrations of the additions are given in the text, figures or tables.

Incubation of epididymal fat-pads. Epididymal fat-pads were prepared and incubated after a 30min preincubation period as described by Winegrad & Renold (1958). After the preincubation period, each fat-pad was cut into five pieces when prepared from diabetic or starved rats, or into eight pieces when normal and starved–re-fed rats were used. The fat-pad pieces were distributed into the corresponding number of flasks, with pad fragments from four rats pooled in each flask. Paired tissues were used at each concentration of substrate.

Isolated cells were prepared as described by Rodbell (1964), except that glucose was absent from the isolation medium.

Extraction and analysis of fat-pads. At the end of the incubation, the fat-pads were blotted lightly and frozen in liquid N_2. The pads or isolated cells plus incubation medium were extracted in 25ml of chloroform–methanol (2:1, v/v) and assayed for ^{14}C and ^{3}H in fatty acids (Denton & Halperin, 1968; Halperin & Denton, 1969). A portion of the chloroform phase was dried and the residue weighed in a tared vessel.

Analysis of the incubation media and tissue. Pyruvate and lactate were measured as described by Denton & Halperin (1968).

Measurement of radioactivity. ^{14}C- and ^{3}H-labelled compounds were assayed in a Beckman model LS230 liquid-scintillation system. For aqueous solutions, the scintillator fluid was 1,4-dioxan containing naphthalene, 2,5-diphenyloxazole, and 1,4-bis-(5-phenyloxazol-2-yl)-benzene (Butler, 1961). For solutions in *n*-heptane the scintillator fluid was toluene containing 2,5-diphenyl-oxazole and 1,4-bis-(5-phenyloxazol-2-yl)benzene (Synder, 1961). Quenching corrections were calculated from the channels-ratio method by using an external standard.

RESULTS

Effect of insulin on [U-^{14}C]*pyruvate conversion into fatty acids.* The conversion of pyruvate into glyceride fatty acids was linear over a 3h period under the experimental conditions used (results not shown). The rate of fatty acid synthesis was increased by pyruvate concentration in the range 0.05–40mM. The maximum rate (35μg-atoms of carbon/2h per g of lipid) was observed with 40mM-pyruvate. The addition of insulin (1000μunits/ml) caused increased pyruvate incorporation into fatty acids when the pyruvate concentrations was less than 10mM, and the maximum rate of fatty acid synthesis (50μg-atoms of carbon/2h per g of lipid) was observed with 10mM-pyruvate. However, with 40mM-pyruvate the rate decreased to that seen in the absence of insulin. The concentration of pyruvate required for half the maximal rate of pyruvate incorporation into fatty acids was approx. 1.2mM in the presence of insulin and more than double this value in its absence (Fig. 1*a*).

Insulin caused qualitatively similar effects when glucose was in the incubation medium in addition to [U-^{14}C]pyruvate. The maximal rate of incorporation of pyruvate into fatty acid was 100μg-atoms of pyruvate carbon/2h per g of lipid with insulin, but the value was 50 in its absence. The concentration of pyruvate that gave half-maximal rates of fatty acid synthesis was greater than 3mM in the absence of insulin and 1.2mM in its presence (Fig. 1*b*).

Effect of insulin concentration on [U-^{14}C]*pyruvate conversion into fatty acids.* The insulin concentration employed in the above studies (1000μunits/ml) greatly exceeds the physiological value. The study was repeated with a constant pyruvate concentration (1mM) and with insulin concentrations varying from 0 to 1000μunits/ml. Insulin at a concentration of 100μunits/ml caused a 67% increase in pyruvate incorporation into fatty acids. A smaller increase (25%) was observed at an insulin concentration of 50μunits/ml (Fig. 2).

Fatty acid synthesis in adipose tissue from 48h-*alloxan-diabetic rats.* Pyruvate incorporation into fatty acids in adipose tissue from 48h-alloxan-diabetic rats was studied in similar fashion to that reported above for the normal rats. In the absence of glucose, pyruvate incorporation into fatty acids was low until pyruvate concentrations were elevated to high values (40mM). When the rate of fatty acid synthesis was plotted as a function of pyruvate concentration, an S-shaped curve resulted (Fig. 3). In the presence of insulin, pyruvate incorporation into fatty acid was considerably increased. Although low rates of fatty acid synthesis were observed at concentrations of pyruvate less than 1mM, the rate of fatty acid synthesis increased

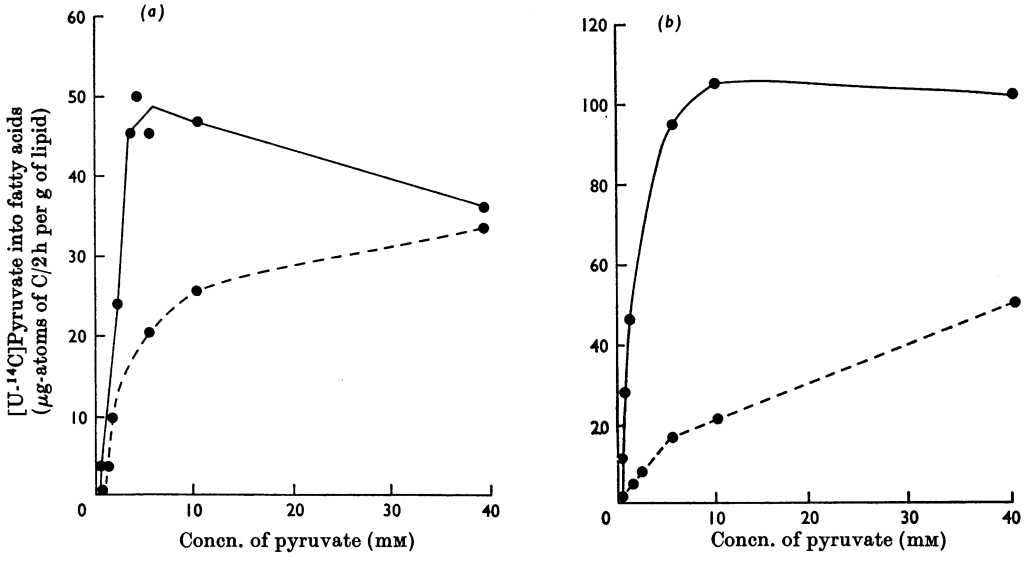

Fig. 1. Effects of insulin on the conversion of [U-^{14}C]pyruvate into fatty acids in adipose tissue from normal rats. (*a*) Fat-pads were preincubated for 30 min in the absence of substrates and then cut into 25–50 mg pieces and distributed into paired flasks. Each flask contained 1 ml of bicarbonate medium, 20 mg of albumin, [U-^{14}C]pyruvate (20 μCi/mmol) (concentration as indicated) and insulin (1000 μunits/ml as indicated), and was gassed with O_2+CO_2 (95:5). All incubations were for 120 min. (*b*) [^{12}C]Glucose (10 mM) was also present. Results are means for 12 paired observations. ----, No insulin; ——, insulin (1000 μunits/ml).

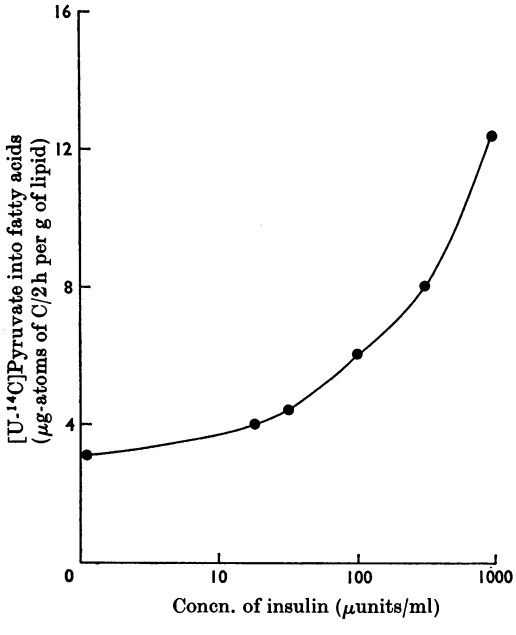

Fig. 2. Effects of insulin concentration on [U-^{14}C]-pyruvate conversion into fatty acids in normal rats. For experimental details see Fig. 1. [U-^{14}C]Pyruvate was present at a concentration of 1 mM. Results are means of 12 observations from pooled fat-pad fragments.

markedly at higher pyruvate concentrations. Although the S-shape of the curve was maintained, in the presence of insulin the horizontal portion was shorter (Fig. 3*a*). The concentration of pyruvate required to give half-maximal rates of fatty acid synthesis was quite variable in fat-pads from diabetic rats, but in the presence of insulin it was as low as 2.5 mM. This value was always higher than that seen in adipose tissue from normal rats. In the absence of insulin the concentration of pyruvate required for half-maximal rates was difficult to calculate, but it always exceeded 9 mM (Fig. 3*a*).

In adipose tissue from diabetic rats the maximal rates of fatty acid synthesis were variable when expressed per g of lipid. Consequently the rates of lipogenesis in the presence and in the absence of glucose cannot be accurately compared. The variability probably resulted from the variable severity and duration of the diabetic state in individual animals. When both [^{12}C]glucose and insulin were present, the lag phase of the S-shaped curve was almost completely abolished. The concentrations of pyruvate required for half-maximal rates of fatty acid synthesis were at least 5–10 mM in the absence of insulin and 2.5–3.0 mM in its presence (Fig. 3*b*).

With shorter duration and less severity of the diabetic state, both the horizontal phase of the S-shaped curve and the concentration of pyruvate

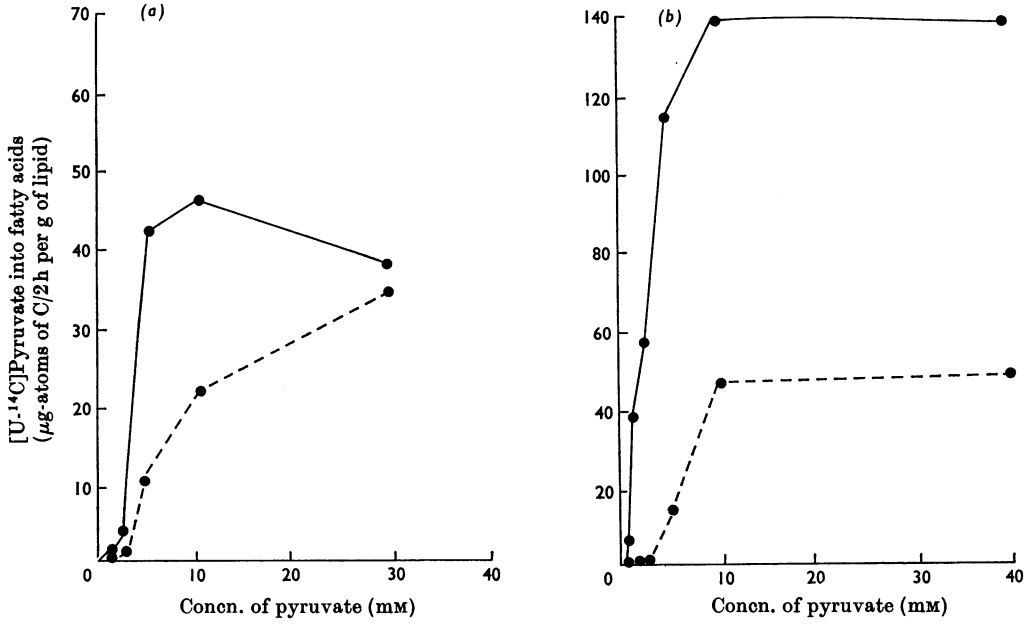

Fig. 3. Effects of insulin on [U-^{14}C]pyruvate conversion into fatty acids in adipose tissue from 48h-alloxan-diabetic rats. For experimental details see Fig. 1. D-[^{12}C]Glucose (10mM) was present in the experiment shown in (*b*). Results are means for 12 paired observations. ----, No insulin; ——, insulin.

required for a half-maximal rate of lipogenesis were decreased, approaching the kinetics seen in the normal postprandial rats (results not shown).

The graph of fatty acid synthesis rate versus pyruvate concentration was no longer S-shaped when pyruvate was substrate in tissues from animals that were diabetic for 48h and subsequently treated with insulin (3 units of protamine zinc insulin/day) for several days. These experimental results were similar to those observed in tissue from normal animals (Fig. 4).

Effect of nutritional state on [U-^{14}C]*pyruvate conversion into fatty acids.* The curve showing the relationship between pyruvate concentration and fatty acid synthesis in adipose tissue from 36h-starved rats was S-shaped, as observed with tissue from diabetic animals. Insulin shortened the lag phase of this curve and increased the conversion of pyruvate into fatty acids at all the pyruvate concentrations used. Half-maximal rates occurred with 10mM-pyruvate in the absence of insulin, but the value was 3.0mM in its presence. Glucose, when present with insulin, nearly abolished the lag phase of this curve (Fig. 5*b*).

In adipose tissue from 72h-starved rats that were then re-fed for 48h, the curves for the conversion of pyruvate into fatty acid were no longer S-shaped (results not shown) and resembled those from the normal animals.

Rate of pyruvate output from adipose tissue of normal, diabetic, starved and starved–re-fed rats. The rate of pyruvate output into the incubation medium should be a function of the intracellular pyruvate concentration. Since intracellular pyruvate concentrations cannot be directly measured with accuracy, I determined the output rates as an indicator of these values (Table 1). The pyruvate output and concentration in the medium were as high or higher in adipose tissue taken from starved and diabetic rats, suggesting that pyruvate concentrations were at least as high in these metabolic states as in tissues from normal fed rats. The rate of conversion of glucose into fatty acids in these states was, however, much lower when compared with that of normal fed animals.

DISCUSSION

The rate of fatty acid synthesis in white adipose tissue of the rat is controlled, in part, by the supply of intracellular pyruvate (substrate). This rate is primarily influenced by insulin-induced augmented glucose transport (Krahl, 1951; Winegrad & Renold, 1958). When the glucose supply is no longer limiting, near-maximal rates of fatty acid synthesis are regulated by the rate of generation of extramitochondrial acetyl-CoA (Flatt & Ball, 1964, 1966; Reshef, Hanson & Ballard, 1969; Schmidt &

Katz, 1969; Del Boca & Flatt, 1969; Robinson & Halperin, 1970; Halperin, 1970). This latter pathway seems to be regulated by the intracellular pyruvate concentration (Halperin, 1970; Halperin & Robinson, 1971), which is in turn controlled by the cytoplasmic redox potential. In addition to these mechanisms, insulin stimulates conversion of physiological concentrations of pyruvate into fatty acids, independently of its action on glucose transport (Jungas, 1970; Halperin, 1970; Halperin & Robinson, 1971). Jungas (1970) and R. M. Denton (personal communication) have demonstrated that insulin *in vitro* can increase the activity of pyruvate dehydrogenase, which could account in part for this effect of insulin.

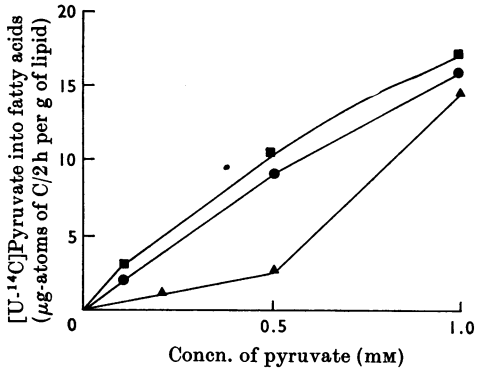

Fig. 4. Effects of duration of alloxan-diabetes and treatment with insulin on the conversion of [U-^{14}C]pyruvate into fatty acids in isolated adipocytes. Adipocytes were isolated from epididymal and perirenal adipose tissue by the method of Rodbell (1964), but in the absence of glucose. Cells from 12 rats were pooled in three separate experiments in the presence of insulin (1000 μunits/ml) and distributed into flasks containing the [U-^{14}C]pyruvate (20 μCi/mmol) (concentrations as indicated) and treated as defined in Fig. 1. All incubations were for 120 min. ■, Normal fed; ●, 48 h diabetes, treated with insulin *in vivo* (see the Materials and Methods section); ▲, 48 h diabetes, not treated.

It has been suggested that the conversion of pyruvate into fatty acids is limited by the ability of white adipose tissue to generate NADPH (Winegrad & Renold, 1958; Kneer & Ball, 1968; Halperin, 1971). Unlabelled glucose markedly increased the conversion of [U-^{14}C]pyruvate into fatty acids and this is further enhanced by insulin (Fig. 1). This occurs despite possible isotope dilution caused by augmented glucose transport.

The kinetics of the conversion of pyruvate into fatty acids are noteworthy. The maximal rates of fatty acid synthesis were not greatly affected by insulin in adipose tissue from either normal, 36 h-starved, or alloxan-diabetic rats (Figs. 1, 3 and 5). In these metabolic states, all the enzymes required for this maximal velocity appear to be present, but in the absence of insulin at least one of these enzymes is inhibited to a major extent. The inhibition seems

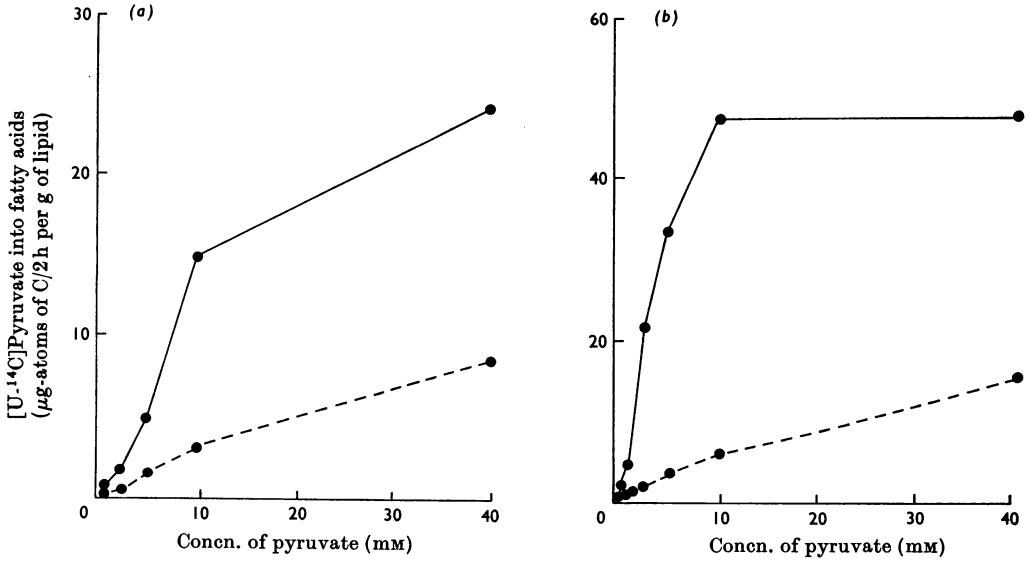

Fig. 5. Effects of starvation on the conversion of [U-^{14}C]pyruvate into fatty acids in adipose tissue. For details of the incubations see Fig. 1. D-[^{12}C]Glucose (10 mM) was present in the experiment shown in (*b*). Results are the means for 12 paired observations. ----, No insulin, 36 h-starved rats; ——, insulin 1000 μunits/ml, 36h-starved rats.

Table 1. *Relationship between pyruvate output and the rate of fatty acid synthesis*

Fat-pads from normal, 36h-starved and 48h acute-alloxan-diabetic rats were preincubated for 30min in bicarbonate medium containing 10mM-glucose and albumin (20mg/ml). They were then transferred into fresh medium that also contained [U-^{14}C]glucose (0.1μCi/ml) and insulin (10munits/ml) where noted, and incubated for an additional 60min. Results are reported as means±S.E.M. for eight paired observations.

Additions to medium	Condition of animal	Pyruvate output rate (μmol/h per g wet wt.)	Concn. of pyruvate in medium (μM)	Fatty acid synthesis rate (μg-atoms of glucose carbon/h per g wet wt.)
Glucose	Normal	0.14±0.03	24±2	1.94±0.30
Glucose+insulin	Normal	0.31±0.04*	54±4*	62.6 ±6.0*
Glucose	Starved (36h)	0.50±0.03	32±2	0.41±0.02
Glucose+insulin	Starved (36h)	1.40±0.10*	82±2*	15.6 ±2.9*
Glucose	Diabetic (48h)	0.23±0.02	24±2	0.43±0.07
Glucose+insulin	Diabetic (48h)	0.66±0.06*	50±4*	6.00±1.10*

* $P<0.01$.

to be of a competitive nature because it is overcome by increasing the concentrations of pyruvate substrate. Pyruvate concentrations inside the adipocyte have been reported to be 0.5mM (Hanson & Ballard, 1970). In adipose tissue from normal rats in the absence of insulin this concentration of pyruvate would be almost an order of magnitude lower than the concentration required for half-maximal rates of fatty acid synthesis. When insulin is present half-maximal rates are seen with approx. 1.5mM-pyruvate. Insulin could markedly increase the rate of fatty acid synthesis, both by increasing the pyruvate concentration and, perhaps more importantly, by lowering the concentration of pyruvate required for rapid lipogenic rates.

The apparent explanation for the low rate of fatty acid synthesis in adipose tissue taken from the acutely diabetic and starved rats may now be considered. When insulin is absent, the maximal velocity can be approximately equal to that achieved when insulin is present, but only at very high pyruvate concentrations (40mM). Half-maximal rates occur with pyruvate concentrations as great as 10mM. Since these pyruvate concentrations are not found in adipose tissue when glucose is the substrate, fatty acid synthesis is inhibited. Even when insulin is present half-maximal rates occur with 2–3mM-pyruvate; this concentration is still considerably higher than that which probably exists *in vivo*. Further, the S-shaped curve accentuates the low rates of fatty acid synthesis at pyruvate concentrations less than 1–2mM. The nature of the major defect in the fatty acid synthesis pathway in diabetes and starvation therefore appears to be indicated by the development of the S-shaped curve as seen in the results of Figs. 3, 4 and 5. A similar phenomenon might also explain the defect in fatty acid synthesis in adipose tissue obtained from animals consuming high-fat diets or from excessively obese or older rats.

When adipose tissus from diabetic or starved rats are incubated for 60min *in vitro*, pyruvate concentrations in the incubation medium (and presumably intracellularly as well) are as high as or higher than those of normal animals (Table 1). Rapid rates of fatty acid synthesis could only be achieved at high non-physiological pyruvate concentrations because of the nature of the S-shaped curve, in both diabetes and starvation (Figs. 3 and 4). It therefore appears that, although glucose transport may be impaired in diabetes, sufficient pyruvate was formed to maintain an adequate concentration of this metabolite. However, the altered kinetics, even when insulin is present, resulted in very low lipogenic rates at physiological pyruvate concentrations.

In summary, insulin augments the conversion of glucose into fatty acids by two mechanisms: (1) by increasing the intracellular pyruvate concentrations secondarily to the augmented glucose transport; (2) by increasing the conversion of pyruvate into fatty acids secondarily to the activation of a regulatory step in the pathway. Inhibition of this latter step seems to be a major method of regulating fatty acid synthesis during starvation and diabetes. The site at which insulin exerts this effect, and the mechanisms involved, remain to be elucidated.

The author is very grateful to Dr B. H. Robinson, Dr F. S. Rolleston and Dr I. B. Fritz for helpful advice and discussions and to Miss B. Kukan, Miss G. McGill and Mrs Linda Richardson for skilled technical assistance. The research was supported by Grant MRC MA 3363 from the Medical Research Council of Canada and the St Michael's Hospital Research Society.

REFERENCES

Butler, F. E. (1961). *Analyt. Chem.* **33**, 409.
Del Boca, J. & Flatt, J. P. (1969). *Eur. J. Biochem.* **11**, 127.

Denton, R. M. & Halperin, M. L. (1968). *Biochem. J.* **110**, 27.
Flatt, J. P. & Ball, E. G. (1964). *J. biol. Chem.* **239**, 675.
Flatt, J. P. & Ball, E. G. (1966). *J. biol. Chem.* **241**, 2862.
Halperin, M. L. (1970). *Can. J. Biochem.* **48**, 1228.
Halperin, M. L. (1971). *Can. J. Biochem.* **49**, 736.
Halperin, M. L. & Denton, R. M. (1969). *Biochem. J.* **113**, 207.
Halperin, M. L. & Robinson, B. H. (1971). *Metabolism*, **20**, 78.
Hanson, R. W. & Ballard, F. J. (1970). *Biochim. biophys. Acta*, **201**, 196.
Jungas, R. L. (1970). *Endocrinology*, **87**, 1368.
Kneer, P. & Ball, E. G. (1968). *J. biol. Chem.* **243**, 2863.
Krahl, M. E. (1951). *Ann. N.Y. Acad. Sci.* **54**, 649.
Krebs, H. A. & Henseleit, K. (1932). *Hoppe-Seyler's Z. physiol. Chem.* **210**, 33.
Reshef, L., Hanson, R. W. & Ballard, F. J. (1969). *J. biol. Chem.* **244**, 1994.
Robinson, B. H. & Halperin, M. L. (1970). *Biochem. J.* **116**, 229.
Rodbell, M. (1964). *J. biol. Chem.* **239**, 375.
Schmidt, K. & Katz, J. (1969). *J. biol. Chem.* **244**, 2125.
Synder, J. (1961). *J. Lipid Res.* **2**, 195.
Winegrad, A. I. & Renold, A. E. (1958). *J. biol. Chem.* **233**, 267.
Yalow, R. S. & Berson, S. A. (1960). *J. clin. Invest.* **39**, 1157.