The Regulation of Glyceride Synthesis in Isolated White-Fat Cells

THE EFFECTS OF ACETATE, PYRUVATE, LACTATE, PALMITATE, ELECTRON-ACCEPTORS, UNCOUPLING AGENTS AND OLIGOMYCIN

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1. The incorporation of 5 mM-[U-¹⁴C]glucose into glyceride fatty acids by fat cells from normal rats incubated in the presence of 20 munits of insulin/ml was increased by acetate, pyruvate, palmitate, *NNN'*-tetramethyl-*p*-phenylenediamine, phenazine methosulphate, dinitrophenol, tetrachlorotrifluoromethyl benzimidazole and oligomycin. Lactate did not stimulate glucose incorporation into fatty acids. The effects of these agents were concentration-dependent. 2. In the presence of 5 mM-glucose+insulin, [U-¹⁴C]acetate, [U-¹⁴C]pyruvate and [U-¹⁴C]lactate were incorporated into fatty acids in a concentration-dependent manner, thereby further increasing the total rate of fatty acid synthesis. 3. *NNN'*-tetramethyl-*p*-phenylenediamine decreased the incorporation of [U-¹⁴C]pyruvate into fatty acids in normal cells and increased the incorporation of [U-¹⁴C]lactate into fatty acids. 4. In fat cells from 72 h-starved rats the stimulatory effects of *NNN'*-tetramethyl-*p*-phenylenediamine upon glucose and lactate incorporation into fatty acids were totally and partially abolished respectively whereas the stimulatory effects of acetate upon glucose incorporation were retained. 5. Combinations of the optimum concentrations of the substances that stimulate glucose incorporation into fatty acids were tested and compared. The effects of acetate+*NNN'*-tetramethyl-*p*-phenylenediamine and acetate+palmitate upon normal cells were additive. The effects of *NNN'*-tetramethyl-*p*-phenylenediamine+palmitate were not additive. It was found that total fatty acid synthesis in the presence of glucose was most effectively increased by raising the concentration of pyruvate in the incubation system. 6. The significance of these results in supporting the proposal that fatty acid synthesis from glucose in adipose tissue is a 'self-limiting process' is discussed.

As is discussed in the preceding paper (Saggerson, 1972), the pathway of glucose conversion into fatty acids in rat white adipose tissue *in vitro* is thought to be 'self-limiting' (Flatt, 1970), and is sensitive to the action of agents that increase the utilization of ATP and cytoplasmic NADH in the tissue. Since the effects of such agents are likely to be concentration-dependent, optimum or even significant responses to these agents may not easily be found unless a wide range of concentrations is tested. Isolated fat cells (Rodbell, 1964) therefore provide a better experimental system than incubated adipose tissue segments for investigations of this kind. In this study concentration ranges of acetate (Flatt & Ball, 1966; Del Boca & Flatt, 1969), *NNN'*-tetramethyl-*p*-phenylenediamine (Halperin & Robinson, 1970), phenazine methosulphate (Katz & Wals, 1970), pyruvate, lactate and uncoupling agents (Rognstad & Katz, 1969) have been tested and the optimum concentrations at which these stimulate glucose conversion into fatty

acids determined. Testing certain combinations of these agents at their optimum concentrations provides some information concerning the regulation of this pathway.

Materials and Methods

Chemicals and animals

These were obtained and treated as described by Saggerson (1972). In addition, phenazine methosulphate, oligomycin (15% oligomycin A, 85% oligomycin B) and L(+)-lactic acid (grade L-1) were obtained from the Sigma (London) Chemical Co. Ltd. (London S.W.6, U.K.). Lactic acid was neutralized with NaOH and standardized enzymically (Hohorst, 1963) to give a stock solution of 0.4 M-sodium lactate. *NNN'*-Tetramethyl-*p*-phenylenediamine was obtained from BDH Chemicals Ltd., Poole, Dorset, U.K., and tetrachlorotrifluoromethylbenzimidazole was a gift from Dr. E. J. Harris.

Methods

All experimental methods were as described by Saggerson (1972) with the exception that 1mM-L-ascorbic acid was included in enzymic assays of lactate and pyruvate to eliminate drift due to oxidation of NADH by *NNN'*-tetramethyl-*p*-phenylenediamine. Similar drift in the presence of phenazine methosulphate was corrected for by extrapolation (Bergmeyer, 1963).

Expression of results

Rates of incorporation of substrates are expressed throughout as μg -atoms of substrate carbon/h per 100 μg of fat-cell DNA and are the means $\pm$ S.E.M. of the number of determinations (usually 3–5). In a single determination one measurement was made of

each parameter presented in a particular table. A different fat-cell preparation was used for each determination.

Results

Throughout this study concentrations of 5mM-glucose and 20munits of insulin/ml were used, since these gave maximal rates of fatty acid synthesis under the experimental conditions used unless other agents were added to the incubation medium (Saggerson, 1972). In Table 1 it may be seen that acetate stimulated the incorporation of glucose carbon atoms into fatty acids and that 1.0mM-acetate gave the greatest stimulation of both glucose incorporation and total fatty acid synthesis. The lactate/pyruvate ratio in the cell suspensions after incubation de-

Table 1. *Effect of sodium acetate concentration upon glucose and acetate utilization by fat cells from normal animals*

Fat cells from normal rats were incubated for 1 h in Krebs–Ringer bicarbonate containing 1.0% (w/v) albumin and 20munits of insulin/ml together with either 5mM-[U- ^{14}C]glucose and the required concentration of sodium [^{12}C]acetate or 5mM-[^{12}C]glucose and the required concentration of sodium [U- ^{14}C]acetate. The results are the means $\pm$ S.E.M. of five determinations and are expressed as μg -atoms/h per 100 μg of DNA.

Concn. of sodium acetate (mM)	Incorporation of glucose into glyceride fatty acids	Incorporation of acetate into glyceride fatty acids	Total fatty acid synthesis	Production of lactate+pyruvate	Lactate/pyruvate ratio
0	29.19 $\pm$ 2.02	—	29.19 $\pm$ 2.02	22.58 $\pm$ 0.60	6.29
0.25	34.93 $\pm$ 2.27	5.77 $\pm$ 0.42	40.71 $\pm$ 2.66	26.20 $\pm$ 0.69	6.25
0.50	54.39 $\pm$ 3.12	9.65 $\pm$ 1.00	64.04 $\pm$ 4.18	28.70 $\pm$ 0.76	6.05
1.00	54.76 $\pm$ 3.40	12.09 $\pm$ 1.64	66.83 $\pm$ 5.00	30.93 $\pm$ 1.17	5.72
2.50	51.60 $\pm$ 3.44	15.14 $\pm$ 2.26	66.74 $\pm$ 5.63	30.71 $\pm$ 1.49	5.33
5.00	47.22 $\pm$ 3.06	17.01 $\pm$ 2.45	64.23 $\pm$ 5.30	29.86 $\pm$ 2.04	4.89
10.00	44.12 $\pm$ 2.72	18.70 $\pm$ 2.65	62.82 $\pm$ 4.46	30.11 $\pm$ 2.06	4.37

Table 2. *Effect of sodium pyruvate concentration upon glucose and pyruvate utilization by fat cells from normal animals*

Fat cells from normal rats were incubated for 1 h in Krebs–Ringer bicarbonate containing 1.0% (w/v) albumin and 20munits of insulin/ml together with either 5mM-[U- ^{14}C]glucose and the required concentration of sodium [^{12}C]pyruvate or 5mM-[^{12}C]glucose and the required concentration of sodium [U- ^{14}C]pyruvate. The results are the means $\pm$ S.E.M. of four determinations and are expressed as μg -atoms/h per 100 μg of DNA.

Concn. of sodium pyruvate (mM)	Incorporation of glucose into glyceride fatty acids	Incorporation of pyruvate into glyceride fatty acids	Total fatty acid synthesis	Incorporation of glucose into glyceride glycerol	Incorporation of pyruvate into glyceride glycerol
0	21.70 $\pm$ 2.92	—	21.70 $\pm$ 2.92	3.55 $\pm$ 0.28	—
0.25	31.25 $\pm$ 3.90	5.74 $\pm$ 0.03	36.99 $\pm$ 3.88	4.24 $\pm$ 0.35	0.07 $\pm$ 0.01
0.50	36.99 $\pm$ 4.64	11.20 $\pm$ 0.23	48.20 $\pm$ 4.46	4.25 $\pm$ 0.52	0.14 $\pm$ 0.01
1.00	31.75 $\pm$ 4.95	20.46 $\pm$ 0.40	52.21 $\pm$ 4.95	4.46 $\pm$ 0.47	0.30 $\pm$ 0.03
2.50	20.06 $\pm$ 3.57	31.21 $\pm$ 2.75	51.27 $\pm$ 5.96	4.49 $\pm$ 0.45	0.99 $\pm$ 0.10
5.00	12.14 $\pm$ 2.58	38.27 $\pm$ 2.89	50.40 $\pm$ 5.32	4.28 $\pm$ 0.59	1.76 $\pm$ 0.08

Table 3. *Effect of sodium lactate concentration upon glucose and lactate utilization by fat cells from normal animals*

Fat cells from normal rats were incubated for 1 h in Krebs–Ringer bicarbonate containing 1.0% (w/v) albumin and 20munits of insulin/ml together with either 5mM-[U-¹⁴C]glucose and the required concentration of sodium [¹²C]lactate or 5mM-[¹²C]glucose and the required concentration of sodium [U-¹⁴C]lactate. The results are the means±S.E.M. of four determinations and are expressed as $\mu\text{g-atoms/h}$ per 100 μg of DNA.

Concn. of sodium lactate (mM)	Incorporation of glucose into glyceride fatty acids	Incorporation of lactate into glyceride fatty acids	Total fatty acid synthesis	Incorporation of glucose into glyceride glycerol	Incorporation of lactate into glyceride glycerol
0	21.04±1.33	—	21.04±1.33	3.81±0.15	—
0.4	19.41±1.82	4.76±0.25	24.16±1.95	4.04±0.24	0.23±0.03
0.8	22.49±1.85	8.07±0.22	30.57±1.98	4.22±0.07	0.51±0.07
1.6	18.30±2.28	13.82±0.78	32.29±2.71	4.24±0.19	1.06±0.12
4.0	12.74±1.23	21.80±1.55	34.54±2.13	4.60±0.15	2.72±0.40
10.0	7.40±0.93	27.71±2.79	35.11±3.63	4.01±0.49	7.79±0.77

Table 4. *Effect of phenazine methosulphate upon glucose and lactate incorporation into glyceride fatty acids by fat cells from normal animals*

Fat cells from normal rats were incubated for 1 h in Krebs–Ringer bicarbonate containing 1.0% (w/v) albumin and 20munits of insulin/ml with 5mM-[U-¹⁴C]glucose or 5mM-sodium [U-¹⁴C]lactate together with the required concentration of phenazine methosulphate. The results are the means±S.E.M. of four determinations and are expressed as $\mu\text{g-atoms/h}$ per 100 μg of DNA.

Concn. of phenazine methosulphate (μM)	Incorporation of glucose into glyceride fatty acids	Incorporation of lactate into glyceride fatty acids
0	19.80±0.82	0.96±0.16
1	23.86±0.60	1.25±0.15
5	28.88±1.57	3.46±0.53
10	32.46±0.57	5.68±1.33
30	27.96±1.18	3.77±0.80
100	9.84±2.71	0.84±0.21

creased as the acetate concentration, and hence the acetate incorporation into fatty acids, increased. Changes in the lactate/pyruvate ratio did not appear to relate to changes in glucose conversion into fatty acids.

Table 2 demonstrates that glucose incorporation into fatty acids was also stimulated in the presence of added pyruvate, which was itself incorporated into both fatty acids and glyceride glycerol. Total fatty acid synthesis was maximal above pyruvate concentrations of 0.5–1.0mM. Stimulation of glucose incorporation into fatty acids was maximal at 0.5mM-pyruvate. Higher concentrations of pyruvate decreased the incorporation of glucose-derived carbon atoms into fatty acids, presumably through dilution of the pool(s) of glucose-derived pyruvate. The incorporation into glyceride glycerol and into fatty acids of [¹⁴C]pyruvate relative to [¹⁴C]glucose increased as the pyruvate concentration increased. In

all cases, as found previously, [¹⁴C]pyruvate was incorporated more readily relative to [¹⁴C]glucose into fatty acids than into glyceride glycerol (Reshef *et al.*, 1969; Saggerson & Greenbaum, 1970a).

In Table 3 it may be seen that combinations of 5mM-glucose and various concentrations of lactate resulted in higher total rates of fatty acid synthesis than are encountered with glucose alone. However, unlike pyruvate, lactate did not stimulate glucose incorporation into fatty acids at any of the tested concentrations. Lactate was also incorporated into fatty acids relative to glucose more readily than into glyceride glycerol; however, [¹⁴C]lactate was incorporated into glyceride glycerol relative to [¹⁴C]glucose more readily than was [¹⁴C]pyruvate (Table 2).

The results in Fig. 1 demonstrate that *NNN'*-tetramethyl-*p*-phenylenediamine increased the incorporation of glucose into fatty acids. The optimum

Table 5. *Comparison of the effects of NNN'N'-tetramethyl-p-phenylenediamine and phenazine methosulphate upon glucose utilization by fat cells from normal animals*

Fat cells from normal rats were incubated for 1 h in Krebs-Ringer bicarbonate containing 1.0% (w/v) albumin, 20munits of insulin/ml and 5mM-[U-¹⁴C]glucose together with the required concentrations of NNN'N'-tetramethyl-p-phenylenediamine (TMPD) and phenazine methosulphate. The results are the means±s.e.m. of four determinations and are expressed as µg-atoms/h per 100µg of DNA.

Concn. of TMPD (µM)	Concn. of phenazine methosulphate (µM)	Incorporation of glucose into glyceride fatty acids	Lactate/pyruvate ratio
0	0	14.00±0.90	5.72
60	0	25.10±0.61	1.95
90	0	26.42±0.76	1.60
110	0	27.60±0.52	1.53
140	0	27.84±0.76	1.46
0	5	21.64±0.75	2.68
0	10	26.09±2.17	1.32
0	15	28.14±0.84	0.82
0	20	28.14±1.46	0.74

Table 6. *Combined effects of NNN'N'-tetramethyl-p-phenylenediamine and sodium acetate upon glucose incorporation into glyceride fatty acids by fat cells from normal animals*

Fat cells from normal rats were incubated for 1 h in Krebs-Ringer bicarbonate containing 1.0% (w/v) albumin and 20munits of insulin/ml together with the required additions of 5mM-[¹²C]glucose, 5mM-[U-¹⁴C]glucose, 1.0mM-sodium [¹²C]acetate, 1.0mM-sodium [U-¹⁴C]acetate and 100µM-NNN'N'-tetramethyl-p-phenylenediamine (TMPD). The results are the means±s.e.m. of five determinations and are expressed as µg-atoms/h per 100µg of DNA. Mean ratio $[(B-A)+(C-A)]/(D-A) = 1.02 \pm 0.12$.

Additions to incubation
medium

TMPD (100µM)	Acetate (1.0mM)	Incorporation of glucose into glyceride fatty acids	Incorporation of acetate into glyceride fatty acids	Total fatty acid synthesis
—	—	25.35±2.92 (A)	—	25.35±2.92
+	—	34.06±2.60 (B)	—	34.06±2.60
—	+	29.81±3.51 (C)	10.84±0.97	40.37±4.12
+	+	38.66±3.05 (D)	8.21±1.12	46.87±3.84

concentration was 100–150µM. The lactate/pyruvate ratio in the cell suspension when cells were incubated with glucose decreased as the NNN'N'-tetramethyl-p-phenylenediamine concentration increased. In Fig. 1 it may also be seen that incorporation into fatty acids of [U-¹⁴C]pyruvate and [U-¹⁴C]lactate decreased and increased respectively with increasing concentration of NNN'N'-tetramethyl-p-phenylenediamine. It is interesting, though possibly fortuitous, that pyruvate and lactate are equally effective as substrates for lipogenesis at a concentration of NNN'N'-tetramethyl-p-phenylenediamine that gives approximately maximal stimulation of glucose incorporation.

In Table 4 it may be seen that incorporation of both glucose and lactate into fatty acids was stimulated by phenazine methosulphate. In both cases optimum effects were found with 10µM-phenazine methosulphate. Incorporation of lactate was stimulated to a greater extent than that of glucose. The experiment shown in Table 5 demonstrates that NNN'N'-tetramethyl-p-phenylenediamine and phenazine methosulphate produced maximal stimulation of glucose incorporation of identical magnitude, though greater changes in the lactate/pyruvate ratio were found with phenazine methosulphate.

The experiments summarized in Tables 6–9 in-

vestigated the relative abilities of acetate, pyruvate, *NNN'*-tetramethyl-*p*-phenylenediamine, lactate and palmitate to stimulate the incorporation of

Fig. 1. Effect of *NNN'*-tetramethyl-*p*-phenylenediamine upon glucose, pyruvate and lactate incorporation into glyceride fatty acids by fat cells from normal animals

Fat cells from normal animals were incubated for 1 h in Krebs–Ringer bicarbonate containing 1.0% (w/v) albumin, 5mm-[U-¹⁴C]glucose+20munits of insulin/ml (●) or 5mm-sodium [U-¹⁴C]pyruvate (○) or 5mm-sodium [U-¹⁴C]lactate (▲) and the required concentration of *NNN'*-tetramethyl-*p*-phenylenediamine (TMPD). Δ---Δ, Lactate/pyruvate ratio at the end of incubations in which cells were incubated with glucose. The results are the means of four determinations.

5mm-glucose into fatty acids in the presence of insulin. The concentrations of acetate, pyruvate and *NNN'*-tetramethyl-*p*-phenylenediamine used were 1.0mm, 0.5mm and 100μM respectively as these produced maximal, or near-maximal, stimulations (Tables 1, 2 and 5; Fig. 1). A concentration of 0.5mm-palmitate produces the maximum stimulatory effect of this compound upon glucose incorporation into fatty acids (Saggerson, 1972).

The stimulatory effects of *NNN'*-tetramethyl-*p*-phenylenediamine and acetate upon glucose incorporation into fatty acids were consistently found to be additive (Table 6). The former compound did not greatly affect acetate incorporation into fatty acids in the presence of glucose. Similarly the stimulatory effects of palmitate and acetate upon glucose incorporation into fatty acids were consistently additive (Table 7). Palmitate considerably decreased the incorporation of acetate into fatty acids. However, additive effects were not seen when *NNN'*-tetramethyl-*p*-phenylenediamine and palmitate were combined (Table 7). Tables 8 and 9 demonstrate that the effect of pyruvate in stimulating glucose incorporation into fatty acids and the effects of acetate, *NNN'*-tetramethyl-*p*-phenylenediamine and palmitate upon this parameter were not additive. Rates of total fatty acid synthesis with glucose+pyruvate, glucose+pyruvate+*NNN'*-tetramethyl-*p*-phenylenediamine, glucose+pyruvate+acetate (Table 8) or glucose+pyruvate+palmitate (Table 9) were approximately the same and, within any one experiment, were always greater than rates of total fatty acid synthesis found with glucose alone, glucose+*NNN'*-tetramethyl-*p*-phenylenediamine, glucose+acetate, glucose+palmitate or glucose+lactate. The total rate of fatty acid synthesis therefore appears to be most effectively increased by raising

Table 7. Combined effects of *NNN'*-tetramethyl-*p*-phenylenediamine, sodium acetate and sodium palmitate upon glucose incorporation into glyceride fatty acids by fat cells from normal animals

Fat cells from normal rats were incubated for 1 h in Krebs–Ringer bicarbonate containing 1.0% (w/v) albumin and 20munits of insulin/ml together with the required additions of 5mm-[¹²C]glucose, 5mm-[U-¹⁴C]glucose, 1.0mm-sodium [¹²C]acetate, 1.0mm-sodium [U-¹⁴C]acetate, 0.5mm-sodium palmitate and 100μM-*NNN'*-tetramethyl-*p*-phenylenediamine (TMPD). The results are the means±S.E.M. of six determinations and are expressed as μg-atoms/h per 100μg of DNA. Mean ratio [(B-A)+(C-A)]/(D-A)=1.00±0.07.

Additions to incubation medium			Incorporation of glucose into glyceride fatty acids	Incorporation of acetate into glyceride fatty acids	Total fatty acid synthesis
TMPD (100μM)	Acetate (1.0mm)	Palmitate (0.5mm)			
—	—	—	13.27 ± 1.97 (A)	—	13.27 ± 1.97
—	—	+	19.07 ± 2.69 (B)	—	19.07 ± 2.69
+	—	—	19.92 ± 3.31	—	19.92 ± 3.31
+	—	+	19.85 ± 3.24	—	19.85 ± 3.24
—	+	—	17.16 ± 2.74 (C)	8.40 ± 0.85	25.56 ± 3.12
—	+	+	23.19 ± 3.72 (D)	3.75 ± 0.47	26.94 ± 3.51

Table 8. *Combined effects of NNN'N'-tetramethyl-p-phenylenediamine, sodium acetate and sodium pyruvate upon glucose incorporation into glyceride fatty acids by fat cells from normal animals*

Fat cells from normal rats were incubated for 1 h in Krebs-Ringer bicarbonate containing 1.0% (w/v) albumin and 20munits of insulin/ml together with the required additions of 5mM-[¹²C]glucose, 5mM-[U-¹⁴C]glucose, 1.0mM-sodium [¹²C]acetate, 1.0mM-sodium [U-¹⁴C]acetate, 0.5mM-sodium [¹²C]pyruvate, 0.5mM-sodium [U-¹⁴C]pyruvate and 100μM-NNN'N'-tetramethyl-p-phenylenediamine (TMPD). The results are the means ± s.e.m. of four determinations and are expressed as μg-atoms/h per 100μg of DNA.

Additions to incubation medium			Incorporation of	Incorporation of	Incorporation of	Total fatty acid synthesis
TMPD (100μM)	Pyruvate (0.5mM)	Acetate (1.0mM)	glucose into glyceride fatty acids	pyruvate into glyceride fatty acids	acetate into glyceride fatty acids	
—	—	—	19.35 ± 1.80	—	—	19.35 ± 1.80
+	—	—	25.76 ± 2.56	—	—	25.76 ± 2.56
—	—	+	26.88 ± 1.89	—	7.97 ± 0.92	34.85 ± 2.81
—	+	—	31.31 ± 3.19	11.56 ± 0.66	—	42.86 ± 3.69
+	+	—	31.10 ± 2.35	10.07 ± 0.73	—	41.17 ± 3.02
—	+	+	29.65 ± 2.96	8.96 ± 1.77	5.71 ± 0.62	44.32 ± 4.54

the concentration of pyruvate. In Table 9 it may also be seen that the ability of 0.5mM-palmitate or 100μM-NNN'N'-tetramethyl-p-phenylenediamine to stimulate glucose incorporation into fatty acids is decreased in the presence of 1mM-lactate. The incorporation of lactate into fatty acids in the presence of glucose is also decreased considerably by palmitate and slightly by NNN'N'-tetramethyl-p-phenylenediamine. Palmitate also slightly decreased the incorporation of 0.5mM-pyruvate into fatty acids in the presence of glucose. The rates of glyceride glycerol formation shown in Table 9 indicate that esterification of palmitate is not affected by the presence of lactate or pyruvate.

Fat cells from 72h-starved rats showed a response to NNN'N'-tetramethyl-p-phenylenediamine different from that found with normal cells. In Fig. 2 it may be seen that no stimulation by NNN'N'-tetramethyl-p-phenylenediamine of glucose incorporation into fatty acids was found even though the lactate/pyruvate ratio decreased considerably as the concentration of the compound was increased. In a parallel experiment performed with fat cells from 24h-starved rats (the results are not shown) a stimulation was observed in the presence of NNN'N'-tetramethyl-p-phenylenediamine, but this was smaller in magnitude than observed with normal cells. The stimulatory effects of the compound upon glucose incorporation into fatty acids therefore appear to decrease with increasing periods of starvation. In Fig. 2 it may also be seen that the compound produced a severe decrease in pyruvate incorporation into fatty acids in fat cells from 72h-starved rats, whereas lactate incorporation was only slightly increased by NNN'N'-tetramethyl-p-phenylenediamine. The results shown in Table 10 indicate that the ability of acetate to stimu-

late glucose incorporation into fatty acids is, however, not lost in fat cells from 72h-starved rats.

In the experiment summarized in Table 11, the effects of oligomycin and uncoupling agents upon glucose incorporation into fatty acids were examined. Dinitrophenol and tetrachlorotrifluoromethyl benzimidazole both stimulated glucose incorporation by 20–30%. One extremely low concentration of oligomycin also stimulated fatty acid synthesis; higher concentrations of oligomycin were found to be inhibitory.

Discussion

The results presented in this study, and those in the preceding paper (Saggerson, 1972), are in agreement with previous proposals that fatty acid synthesis from glucose in white adipose tissue under normal conditions does not proceed at the maximum possible rate in the presence of apparently saturating concentrations of glucose and insulin. The results support proposals that fatty acid synthesis from glucose is restrained by the need to utilize ATP and cytoplasmic NADH, which are produced in this process (Flatt & Ball, 1966; Del Boca & Flatt, 1969; Flatt, 1970; Halperin & Robinson, 1970).

Stimulation of glucose incorporation into fatty acids in fat-pad segments in the presence of acetate has been reported previously (Flatt & Ball, 1966; Del Boca & Flatt, 1969; Saggerson & Greenbaum, 1970a). In this study, in which incubations were carried out for 1 h (Table 1), 42% of 1mM-acetate added to the incubations was found to be converted into fatty acids in that time. With addition of 0.5mM-acetate 67%, and with 0.25mM-acetate 80%, was converted into fatty acids in the same time. Since

Table 9. Combined effects of NNN'N'-tetramethyl-p-phenylenediamine, sodium palmitate, sodium pyruvate and sodium lactate upon glucose incorporation into glycerides by fat cells from normal animals

Fat cells from normal rats were incubated for 1 h in Krebs-Ringer bicarbonate containing 1.0% (w/v) albumin and 20 munits of insulin/ml together with the required additions of 5 mM-[14C]glucose, 5 mM-[U-14C]glucose, 100 μM-NNN'N'-tetramethyl-p-phenylenediamine (TMPD), 0.5 mM-sodium palmitate, 0.5 mM-sodium [12C]pyruvate, 0.5 mM-sodium [U-14C]pyruvate, 1.0 mM-sodium [12C]lactate and 1.0 mM-sodium [U-14C]lactate. The results are the means ± S.E.M. of four determinations and are expressed as μg-atoms/h per 100 μg of DNA.

Additions to incubation medium				Incorporation of glucose into glyceride fatty acids	Incorporation of pyruvate into glyceride fatty acids	Incorporation of lactate into glyceride fatty acids	Total fatty acid synthesis	Incorporation of glucose into glycerol	Total glyceride glycerol formation
TMPD (100 μM)	Palmitate (0.5 mM)	Lactate (1.0 mM)	Pyruvate (0.5 mM)						
-	-	-	-	12.58 ± 0.84	-	-	12.58 ± 0.84	3.06 ± 0.19	3.06 ± 0.19
+	-	-	-	17.27 ± 1.02	-	-	17.27 ± 1.02	3.82 ± 0.38	3.82 ± 0.38
-	+	-	-	22.13 ± 1.68	-	-	22.13 ± 1.68	11.26 ± 0.99	11.26 ± 0.99
-	-	+	-	13.06 ± 0.73	7.78 ± 0.11	-	20.84 ± 0.63	3.86 ± 0.54	4.74 ± 0.62
-	-	-	+	15.77 ± 1.13	2.19 ± 0.04	-	17.96 ± 1.17	11.11 ± 1.29	12.17 ± 1.42
+	-	+	-	13.13 ± 0.31	6.21 ± 0.51	-	19.33 ± 0.58	3.63 ± 0.27	4.55 ± 0.43
-	+	-	-	17.97 ± 0.35	-	-	30.75 ± 0.38	3.60 ± 0.23	3.82 ± 0.26
-	-	-	+	18.99 ± 0.64	-	-	28.65 ± 0.35	11.29 ± 0.51	11.60 ± 0.54
-	-	-	-	-	12.78 ± 0.10	-	-	-	-
-	-	-	-	-	9.67 ± 0.71	-	-	-	-

Fig. 2. Effect of NNN'N'-tetramethyl-p-phenylenediamine upon glucose, pyruvate and lactate incorporation into glyceride fatty acids by fat cells obtained from 72h-starved animals

Fat cells from starved animals were incubated for 1 h in Krebs-Ringer bicarbonate containing 1.0% (w/v) albumin, 5 mM-[U-14C]glucose+20 munits of insulin/ml (●) or 5 mM-sodium [U-14C]pyruvate (○) or 5 mM-sodium [U-14C]lactate (▲) and the required concentration of NNN'N'-tetramethyl-p-phenylenediamine (TMPD). Δ---Δ, Lactate/pyruvate ratio at the end of the incubations in which cells were incubated with glucose. The results are the means of four determinations.

[14C]acetate is also converted into 14CO2 (Flatt & Ball, 1966; Saggerson & Greenbaum, 1970a), it is probable that these low concentrations of acetate are totally utilized under the experimental conditions. With smaller concentrations of fat cells, or with shorter periods of incubation, the maximum stimulation of glucose incorporation into fatty acids may therefore occur at very low concentrations of acetate indeed.

Total fatty acid synthesis was increased by addition of pyruvate or lactate to the glucose in the incubation medium (Tables 2 and 3). However, glucose incorporation into fatty acids was stimulated only in the presence of pyruvate. Halperin (1971) has shown that pyruvate incorporation into fatty acids in fat-pad segments is stimulated by glucose; however, the converse, that pyruvate stimulates glucose incorporation, does not appear to have been reported. Reshef *et al.* (1969) have determined the relative rates of incorporation of [14C]glucose in the presence of pyruvate and [14C]pyruvate in the presence of glucose, but did not present their data as absolute rates of incorporation. Presumably pyruvate and lactate, by providing pyruvate, increase total fatty

Table 10. *Effect of sodium acetate upon glucose incorporation into glyceride fatty acids by fat cells from 72h-starved animals*

Fat cells from 72h-starved rats were incubated for 1 h in Krebs–Ringer bicarbonate containing 1.0% (w/v) albumin and 20munits of insulin/ml together with either 5mM-[U-¹⁴C]glucose and the required concentration of sodium [¹²C]acetate or 5mM-[¹²C]glucose+5mM-sodium [U-¹⁴C]acetate. The results are the means±s.e.m. of four determinations and are expressed as µg-atoms/h per 100µg of DNA. n.d., Not determined.

Concn. of sodium acetate (mM)	Incorporation of glucose into glyceride fatty acids	Incorporation of acetate into glyceride fatty acids	Total fatty acid synthesis
0	0.37±0.08	—	0.38±0.08
0.25	0.40±0.07	n.d.	n.d.
0.50	0.42±0.08	n.d.	n.d.
1.00	0.50±0.09	n.d.	n.d.
2.50	0.79±0.15	n.d.	n.d.
5.00	1.09±0.17	1.22±0.12	2.31±0.28

Table 11. *Effects of sodium 2,4-dinitrophenate, tetrachlorotrifluoromethyl benzimidazole and oligomycin upon glucose incorporation into glyceride fatty acids by fat cells from normal animals*

Fat cells from normal rats were incubated for 1 h in Krebs–Ringer bicarbonate containing 1.0% (w/v) albumin, 5mM-[U-¹⁴C]glucose and 20munits of insulin/ml together with the required concentrations of (A) sodium 2,4-dinitrophenate, (B) tetrachlorotrifluoromethyl benzimidazole (TTFB) or (C) oligomycin. The results are the means±s.e.m. of four determinations and are expressed as µg-atoms/h per 100µg of DNA.

A		B		C	
Concn. of 2,4-dinitrophenate (µM)	Incorporation of glucose into glyceride fatty acids	Concn. of TTFB (µM)	Incorporation of glucose into glyceride fatty acids	Concn. of oligomycin (ng/ml)	Incorporation of glucose into glyceride fatty acids
0	14.00±0.90	0	14.00±0.90	0	14.00±0.90
10	14.59±0.48	0.1	15.82±0.98	2.5	16.79±0.56
40	16.10±0.42	0.5	16.95±0.70	10	12.47±1.12
75	16.79±0.43	1.0	17.64±0.42	50	10.78±0.56
150	16.52±0.70	2.0	17.93±0.84		
		5.0	17.64±0.70		

acid synthesis by elevating the intracellular pyruvate concentration and hence increasing the rate of acetyl-CoA formation from pyruvate. Pyruvate also presumably stimulates the conversion of [¹⁴C]glucose into [¹⁴C]pyruvate and hence into fatty acids by utilizing cytoplasmic NADH for formation of lactate from added pyruvate. A more complete flux analysis is required, including the measurement of [¹⁴C]-pyruvate+[¹⁴C]lactate production from [¹⁴C]glucose in the presence of lactate and pyruvate and also the measurement of [¹⁴C]lactate formation from [¹⁴C]-pyruvate and [¹⁴C]pyruvate formation from [¹⁴C]-lactate, before the effects of pyruvate and lactate upon glucose utilization can be exactly defined.

Halperin & Robinson (1970) found that, in fat-pad

segments, 50µM-NNN'-tetramethyl-*p*-phenylenediamine slightly stimulated ³H₂O incorporation into fatty acids in the presence of glucose, doubled ³H₂O incorporation in the presence of lactate and slightly decreased incorporation in the presence of pyruvate. Robinson & Halperin (1970) have shown that the compound enables adipose tissue mitochondria to oxidize external NADH. The results shown in Fig. 1 are in accordance with these observations and indicate that the effects of NNN'-tetramethyl-*p*-phenylenediamine upon normal fat cells may be considerable if a range of concentrations are tested. Stimulation of glucose and lactate incorporation into fatty acids supports the proposal that these processes are limited by the ability to reoxidize cytoplasmic

NADH under normal conditions. Inhibition of pyruvate incorporation into fatty acids is consistent with the evidence of Kneer & Ball (1968) that, when pyruvate is the sole substrate supplied to adipose tissue, reducing equivalents for reductive synthesis are supplied by mitochondrial oxidative processes. With fat cells from starved animals (Fig. 2) *NNN'*-tetramethyl-*p*-phenylenediamine did not stimulate glucose incorporation into fatty acids and only slightly stimulated lactate incorporation, whereas pyruvate incorporation was decreased as in normal fat cells. Decreases in the lactate/pyruvate ratio in cell suspensions from starved animals with increasing concentration of *NNN'*-tetramethyl-*p*-phenylenediamine suggested that this compound was facilitating an oxidation of cytoplasmic NADH in 'starved cells' as well as in normal cells. Halperin & Robinson (1970) have suggested that reductive synthesis of glycerol phosphate from glucose leads to a stimulation of lactate utilization by oxidizing cytoplasmic NADH. Utilization of [14 C]lactate by starved adipose tissues is increased by addition of pyruvate or glucose, but there is no net uptake of lactate, reflecting exchange between [14 C]lactate and unlabelled lactate (Schmidt & Katz, 1969). The results shown in Fig. 2 would suggest that factors other than utilization of cytoplasmic NADH restrict lactate utilization in fat cells from starved animals. The inability of *NNN'*-tetramethyl-*p*-phenylenediamine to stimulate glucose conversion into fatty acids in 'starved cells' is discussed below.

Katz & Wals (1970) found that phenazine methosulphate stimulated lactate incorporation into fatty acids in incubated fat-pads but did not significantly stimulate glucose incorporation. With incubated fat cells phenazine methosulphate also stimulated glucose incorporation though lactate incorporation was stimulated to a greater extent (Table 4). Phenazine methosulphate and *NNN'*-tetramethyl-*p*-phenylenediamine stimulated glucose incorporation into fatty acids to almost exactly the same extent (Table 5). This may indicate that these two agents act to relieve the same restraint upon glucose incorporation. The modes of action of the two agents are presumably different, however. *NNN'*-Tetramethyl-*p*-phenylenediamine is reduced by NADH but not by NADPH (Robinson & Halperin, 1970) and is presumably reoxidized by mitochondrial processes. Phenazine methosulphate, however, may be reduced by both NADH and NADPH (McLean, 1960; Katz & Wals, 1970; Saggerson & Greenbaum, 1970a) and is presumably reoxidized by oxygen dissolved in the medium. It is not known whether the free radical of *NNN'*-tetramethyl-*p*-phenylenediamine may uncouple oxidative phosphorylation to any extent in the fat cells (Park *et al.*, 1957).

A stimulation of glucose incorporation into fatty acids in the presence of uncoupling agents is in accord

with the predictions of Flatt (1970). The results shown in Table 11 do not, however, demonstrate conclusively that the effects result from uncoupling of oxidative phosphorylation. A complete flux analysis is necessary to establish this clearly. It is unclear why concentrations of dinitrophenol that stimulated fatty acid synthesis in fat cells in this study have been found to be inhibitory with incubated fat-pads (Rognstad & Katz, 1969).

At their optimum concentrations, the effects upon glucose incorporation into fatty acids of acetate and palmitate or acetate and *NNN'*-tetramethyl-*p*-phenylenediamine were always additive in normal cells whereas the effects of *NNN'*-tetramethyl-*p*-phenylenediamine and palmitate were not. The ability of *NNN'*-tetramethyl-*p*-phenylenediamine (Fig. 2) and of added palmitate (Saggerson, 1972) to stimulate glucose incorporation into fatty acids is lost in 'starved cells'. The ability of acetate to stimulate this process in fat cells or fat-pads from starved animals is, however, retained (Table 10; Del Boca & Flatt, 1969; Saggerson & Greenbaum, 1970b). In fact the degree of stimulation by acetate of glucose incorporation is greater in cells from starved rats than in normal fat cells or adipose tissues. These results would suggest that *NNN'*-tetramethyl-*p*-phenylenediamine and palmitate (and presumably phenazine methosulphate) exert similar effects upon fatty acid synthesis from glucose and that their site(s) of action may be the same. The results also suggest that acetate exerts a different effect or that its site of action is different. These results may be explained if part, or all, of the process of lipogenesis from glucose was carried out in two or more separate compartmented pathways in the fat cell with *NNN'*-tetramethyl-*p*-phenylenediamine and palmitate influencing one or more compartments and acetate others. Starvation may deplete the pathway(s) sensitive to *NNN'*-tetramethyl-*p*-phenylenediamine and palmitate but not the acetate-sensitive pathway(s). It has been suggested that ordered alterations in glycolytic enzyme activities in response to changes in dietary and hormonal states in adipose tissue may reflect alterations in activity of specific compartmented pathways (Saggerson & Greenbaum, 1969). Alternatively, if the effects of *NNN'*-tetramethyl-*p*-phenylenediamine and long-chain fatty acids (palmitate in this case) exclude each other, *NNN'*-tetramethyl-*p*-phenylenediamine may not stimulate glucose incorporation into fatty acids in 'starved cells' because these cells have higher rates of free fatty acid production through lipolysis of tissue glycerides and lower rates of fatty acid esterification (Saggerson, 1972). Stimulation by *NNN'*-tetramethyl-*p*-phenylenediamine in 'starved cells' may therefore not be apparent if endogenous fatty acids produce a degree of stimulation that is reached in normal cells only by

addition of palmitate. Stimulation of glucose incorporation into fatty acids by palmitate also decreases with increasing periods of starvation (Saggerson, 1972).

The results shown in Tables 8 and 9 indicate that the highest rates of total fatty acid synthesis attained in the presence of glucose are those achieved by adding pyruvate to the incubation medium. Since the rate of total fatty acid synthesis in the presence of glucose+pyruvate+acetate (Table 8) was similar to that found with glucose+pyruvate, rates achieved by addition of pyruvate may reflect the maximum fatty acid-synthesizing capacity of the tissue. These rates were more than twice those generally found with saturating concentrations of insulin and glucose alone and were also higher than those found with addition of palmitate, acetate, *NNN'*-tetramethyl-*p*-phenylenediamine, phenazine methosulphate or lactate. The concentration of pyruvate would therefore appear to be an important factor in determining the rate of fatty acid synthesis.

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