Lipogenesis in Rabbit Isolated Fat-Cells

By E. DAVID SAGGERSON

Department of Biochemistry, University College London, Gower Street, London WC1E 6BT, U.K.

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1. Fat-cells isolated from rabbit perirenal adipose tissue were incubated with the following $U\text{-}^{14}\text{C}$ -labelled substrates: 5mM-glucose (+insulin), 5mM-pyruvate, 5mM-lactate, 5mM-glucose+5mM-acetate (+insulin), and the relative rates of incorporation of these substrates into glyceride fatty acids determined. In general total rates of fatty acid synthesis were similar whatever substrate was supplied to the cells. 2. Rabbit fat-cells incorporated $[U\text{-}^{14}\text{C}]\text{acetate}$ into fatty acids and CO_2 as well in the absence of glucose as in the presence of this substrate. 3. The disposition of the utilization of glucose-derived carbon through various metabolic pathways was determined. 4. Extramitochondrial and mitochondrial activities were determined for 11 enzymes. The cells contained a very low activity of pyruvate carboxylase, undetectable NADP-malate dehydrogenase activity and a high mitochondrial phosphoenolpyruvate carboxylase activity. 5. Various rabbit fat-cell metabolic parameters based on the measurement of ^{14}C incorporation and enzyme activity were compared with the same parameters previously measured in rat and guinea-pig fat-cells. In general guinea pig occupied a position between rat and rabbit with respect to these parameters. 6. The profiles of substrate incorporation into fatty acids and of relative enzyme activities in rabbit fat-cells indicated that the operation of a 'citrate-cleavage' pathway may not be obligatory for the supply of lipogenic acetyl units.

At present there exists a considerable body of knowledge concerning lipogenic processes and their control in rat adipose tissue. Corresponding information for other species is far less. However, the operation and regulation of compartmented processes of such complexity must vary between different mammalian species. In a previous publication (Saggerson, 1974) a study of rat and guinea-pig adipose tissue was presented in which the ability of fat-cells to utilize various substrates for lipogenesis, the disposition of glucose-derived carbon in various metabolic pathways and the intracellular distribution and activities of enzymes involved in these processes, were compared in these two species. The present paper describes a similar study with fat-cells from rabbit white adipose tissue.

Materials and Methods

Chemicals

These were obtained and treated as described by Saggerson (1974).

Animals

Male rabbits were bred in the animal colony at University College and were maintained on diet SG1 (Oxoid Ltd., London S.E.1, U.K.) supplemented with cabbage. All animals had water *ad libitum*. At

death the animals were 12–16-weeks old and weighed 1.7–2.3 kg.

Fat-cell techniques

Preparation. Fat-cells were prepared from rabbit perirenal adipose tissue essentially as described by Rodbell (1964) by shaking the tissue for 30 min in Krebs–Ringer bicarbonate medium (Krebs & Henseleit, 1932) containing 4 mg of collagenase/ml and 2% (w/v) of fatty acid-free albumin.

Incubation of fat-cells and measurement of metabolic products. The procedures for the measurement of the incorporation of ^{14}C -labelled substrates have been described (Saggerson & Tomassi, 1971; Saggerson, 1972*a,b*). Fat-cell DNA was determined as described by Saggerson (1972*a*).

Preparation of fat-cell extracts for measurement of enzyme activities. The methods used were identical with those described by Saggerson (1974) to produce 'supernatant' (S) and 'mitochondrial' (M) fractions. Lactate dehydrogenase (EC 1.1.1.27) and glutamate dehydrogenase (EC 1.4.1.2) were used as marker enzymes in order to correct for cross-contamination of mitochondrial and extramitochondrial enzymes activities. The average percentage activity of lactate dehydrogenase in the 'mitochondrial' fraction was 4.9% and of glutamate dehydrogenase in the 'supernatant' fraction was 7.5%.

Measurement of enzyme activities

ATP-citrate lyase (EC 4.1.3.8), citrate synthase (EC 4.1.3.7), glucose 6-phosphate dehydrogenase (EC 1.1.1.49), glutamate dehydrogenase, lactate dehydrogenase, NAD-malate dehydrogenase (EC 1.1.1.37), NADP-isocitrate dehydrogenase (EC 1.1.1.42), NADP-malate dehydrogenase (EC 1.1.1.40) and 6-phosphogluconate dehydrogenase (EC 1.1.1.44) were assayed spectrophotometrically at 25°C by the methods described by Saggerson (1974). Phosphoenolpyruvate carboxylase (EC 4.1.1.32) and pyruvate carboxylase (EC 6.4.1.1.) were assayed radiochemically at 30°C as described by Saggerson (1974).

Expression of results and calculation of ^{14}C fluxes

Incorporation of substrates by incubated cells. These are expressed throughout as μg -atoms of substrate carbon incorporated/h per 100 μg of fat-cell DNA and are the means of the number of determinations. In a single determination one measurement was made of each parameter presented in a given Table or Figure. A different fat-cell preparation from a different animal was used for each determination. Fluxes of glucose carbon and, where appropriate, acetate carbon through various metabolic pathways were calculated from the yields of ^{14}C in fatty acids, CO_2 and glyceride glycerol and from the measured rates of production of lactate and pyruvate with [$1\text{-}^{14}\text{C}$]glucose, [$6\text{-}^{14}\text{C}$]glucose, [$\text{U-}^{14}\text{C}$]glucose and sodium [$\text{U-}^{14}\text{C}$]acetate as starting substrates (Flatt & Ball, 1964, 1966; Katz *et al.*, 1966).

Enzyme activities. These are expressed throughout as munits/100 μg of fat-cell DNA where 1 munit of enzyme converts 1 nmol of substrate/min at 25° or 30°C in the spectrophotometric and radiochemical assays respectively.

Results

Table 1 shows the ability of rabbit fat-cells to utilize glucose, acetate, lactate and pyruvate as

lipogenic substrates. Insulin stimulated the incorporation of glucose into glycerides. The total rates of fatty acid synthesis using 5 mM concentrations of glucose (in the presence of insulin), glucose+acetate (in the presence of insulin), lactate or pyruvate were similar. In the presence of acetate the incorporation of glucose into fatty acids was greatly decreased. The total rate of fatty acid synthesis as represented by the sum of the incorporation of 5 mM-glucose+5 mM-acetate in the presence of insulin was, however, virtually independent of the acetate concentration over a wide range of acetate concentrations (E. D. Saggerson, unpublished work; see also Table 1 for 5 mM-acetate). In the presence or absence of acetate the incorporation of glucose into glyceride glycerol was high relative to its incorporation into fatty acids. Incorporation of other substrates into glyceride glycerol was low compared with the incorporation of glucose.

In Table 2 the utilization of glucose-derived carbon in various metabolic pathways is more fully examined. Rabbit fat-cells differed from rat and guinea-pig cells (Flatt & Ball, 1966; Saggerson, 1974) in that the formation of glyceride glycerol and tricarboxylic acid-cycle CO_2 constituted a far greater proportion of the utilization of glucose than that found in these other two species. On the other hand the utilization of glucose for fatty acid synthesis, for lactate+pyruvate production and in the hexose monophosphate pathway contributed far less to the overall metabolism of glucose in rabbit cells. The rate of the hexose monophosphate pathway was calculated by method C proposed by Katz *et al.* (1966), which is based mainly on the determination of specific yields of $^{14}\text{CO}_2$ from [$1\text{-}^{14}\text{C}$]glucose and [$6\text{-}^{14}\text{C}$]glucose. These yields could be determined far more accurately than specific yields of ^{14}C in fatty acids which were very low in several experiments. Method D of Katz *et al.* (1966), used previously with rat and guinea-pig fat-cells (Saggerson, 1974), was therefore not used in this study. The main effect of acetate on glucose

Table 1. *Relative utilization of glucose, acetate, pyruvate and lactate for lipogenesis by rabbit fat-cells*

Fat-cells were incubated for 1 h in Krebs-Ringer bicarbonate medium containing 1.0% (w/v) albumin together with one of the following combinations: 5 mM-[$\text{U-}^{14}\text{C}$]glucose+anti-insulin serum or insulin; 5 mM-[$\text{U-}^{14}\text{C}$]glucose+5 mM-sodium [^{12}C]acetate+insulin or 5 mM-[^{12}C]glucose+5 mM-sodium [$\text{U-}^{14}\text{C}$]acetate+insulin; 5 mM-sodium [$\text{U-}^{14}\text{C}$]pyruvate; or 5 mM-sodium [$\text{U-}^{14}\text{C}$]lactate. The results are the means $\pm$ S.E.M. of four experiments (four preparations from four different animals).

Substrate incorporated into (μg -atoms/h per 100 μg of DNA):	Substrate					
	5 mM-Glucose + anti-insulin serum (10 m-i.u./ml)	5 mM-Glucose + insulin (20 m-i.u./ml)	5 mM-Glucose + 5 mM-sodium acetate + insulin (20 m-i.u./ml)		5 mM-Sodium pyruvate	5 mM-Sodium lactate
			Glucose	Acetate		
Glyceride fatty acids	2.27 $\pm$ 0.09	3.63 $\pm$ 0.22	0.43 $\pm$ 0.03	3.50 $\pm$ 0.39	4.80 $\pm$ 0.73	3.87 $\pm$ 0.13
Glyceride glycerol	6.17 $\pm$ 0.77	9.27 $\pm$ 1.27	9.62 $\pm$ 1.65	0.30 $\pm$ 0.06	0.58 $\pm$ 0.11	0.40 $\pm$ 0.07

Table 2. *Measurement of fluxes of glucose and acetate carbon through metabolic pathways in rabbit fat-cells*

Fat-cells were incubated for 120 min in Krebs-Ringer bicarbonate medium containing 1.0% (w/v) albumin and 20 m-i.u. of insulin/ml. Glucose (5 mM) and 5 mM-sodium acetate were added where indicated. Flasks containing glucose alone contained as isotope either [$1\text{-}^{14}\text{C}$]glucose or [$6\text{-}^{14}\text{C}$]glucose. Flasks containing glucose and acetate contained either [$1\text{-}^{14}\text{C}$]glucose, [$6\text{-}^{14}\text{C}$]glucose, [$1\text{-}^{14}\text{C}$]glucose or sodium [$1\text{-}^{14}\text{C}$]acetate. Flasks containing acetate alone contained sodium [$1\text{-}^{14}\text{C}$]acetate as isotope. The results are expressed in every case as μg -atoms of glucose or acetate carbon incorporated/h per 100 μg of DNA and are the means $\pm$ S.E.M. of four determinations (four preparations from four different animals). n.d. indicates not determined.

Addition to incubation medium	Total glucose utilization	Fatty acid synthesis	Fatty acid synthesis	Glyceride glycerol synthesis	Lactate+ pyruvate production	Lactate/ pyruvate ratio	Glucose-derived tricarboxylic acid cycle	Acetate-derived	Hexose mono-phosphate pathway
		from glucose	from acetate	from glucose	production	ratio	CO ₂	CO ₂	CO ₂
5 mM-Glucose + insulin	24.72 ± 1.27	1.31 ± 0.25	—	8.35 ± 0.79	1.23 ± 0.09	5.59 ± 0.87	4.13 ± 0.43	—	0.81 ± 0.04
5 mM-Glucose + 5 mM-sodium acetate + insulin	29.84 ± 2.29	0.17 ± 0.03	0.75 ± 0.03	7.98 ± 0.31	1.00 ± 0.03	4.54 ± 0.34	7.67 ± 0.45	1.15 ± 0.18	0.80 ± 0.09
5 mM-Sodium acetate + insulin	—	—	0.69 ± 0.07	—	n.d.	n.d.	—	1.20 ± 0.32	—

metabolism in rabbit fat-cells was to decrease the utilization of glucose for fatty acid synthesis as described above and to increase the appearance of glucose-derived carbon in tricarboxylic acid-cycle CO_2 . In the absence of glucose acetate was incorporated into fatty acids and CO_2 as effectively as in the presence of glucose. On the other hand glucose greatly facilitates the utilization of acetate for fatty acid synthesis in rat and guinea-pig cells (Saggerson, 1974).

In Table 3 the intracellular distribution and activities in rabbit fat-cells of several enzymes are presented. Particularly noteworthy were the observations that NADP-malate dehydrogenase was not detectable (sensitivity limit of the assay was 3–4 munits of enzyme activity/100 μg of DNA), that the activity of pyruvate carboxylase was very low and that the mitochondrial activity of phosphoenolpyruvate carboxylase found in the rabbit cells was very high compared with that found previously in rat and guinea-pig fat-cells (Saggerson, 1974).

Discussion

The metabolic parameters recorded for rabbit fat-cells in this study may be compared with similar measurements made previously in rat and guinea-pig fat-cells. The most noteworthy of these are presented in Table 4 and all relate to white adipose tissue in these three species. Epididymal adipose tissue, which was used for rat and guinea-pig studies, could not be obtained in enough quantity from a single rabbit at the age used; therefore male rabbit perirenal adipose tissue was used. There appears to be no reason to suppose that adipocytes derived from perirenal adipose tissue might have a different metabolic pattern from those derived from epididymal tissue. This was confirmed in a preliminary experiment in which rat fat-cells were prepared from these two anatomical locations in the same animals. Measured rates of ^{14}C -labelled substrate incorporation by epididymal cells were (μg -atoms/h per 100 μg of DNA): 5 mM-glucose (with insulin) 36.61, 5 mM-glucose (with insulin + 1 mM-acetate) 57.01, 1 mM-acetate (with insulin + 5 mM-glucose) 13.88, 5 mM-pyruvate 18.00, 5 mM-lactate 3.11. The corresponding values for perirenal cells were: 36.44, 46.54, 13.77, 19.55 and 1.81 (the results are the means of two separate determinations in each case).

Since the rabbit is a herbivore the observation that the ability to incorporate acetate into fatty acids was comparable with the ability to incorporate glucose was not unexpected. Table 4 shows that there is a graduation from rat to guinea pig to rabbit with respect to the relative incorporation of glucose and acetate into fatty acids, a reverse graduation in the relative oxidation of glucose and acetate-derived carbon in the tricarboxylic acid cycle and a graduation from rat to guinea pig to rabbit with respect to the

Table 3. *Activities and distribution of enzymes in rabbit fat-cells*

Extracts were made and enzyme activities determined in S and M fractions as described in the Materials and Methods section. Mitochondrial and extramitochondrial activities were calculated in each determination by correcting the activities in the M and S fractions respectively with the determined percentage activities of lactate dehydrogenase in the M fraction and of glutamate dehydrogenase in the S fraction. — indicates that the activity in the original S or M fraction was too low to be satisfactorily detected or became zero after applying the above correlation. The results are the means of five determinations $\pm$ S.E.M. (five preparations from five different animals).

Enzyme	Extramitochondrial activity (munits/100 μ g of DNA)	Mitochondrial activity (munits/100 μ g of DNA)	Total activity (munits/100 μ g of DNA)	% activity that is mitochondrial
Glutamate dehydrogenase	—	27.45 $\pm$ 3.12	27.45 $\pm$ 3.12	100
Lactate dehydrogenase	2552.3 $\pm$ 327.3	—	2552.3 $\pm$ 327.3	0
ATP-citrate lyase	9.35 $\pm$ 1.09	—	9.35 $\pm$ 1.09	0
Citrate synthase	—	73.25 $\pm$ 5.48	73.25 $\pm$ 5.45	100
Glucose 6-phosphate dehydrogenase	150.3 $\pm$ 20.5	—	150.3 $\pm$ 20.5	0
NAD-malate dehydrogenase	1834.4 $\pm$ 119.9	292.6 $\pm$ 15.6	2127.0 $\pm$ 127.9	14
NADP-isocitrate dehydrogenase	109.1 $\pm$ 3.5	5.11 $\pm$ 0.53	114.2 $\pm$ 3.6	4
NADP-malate dehydrogenase	—	—	—	—
Phosphoenolpyruvate carboxylase	2.63 $\pm$ 1.19	42.60 $\pm$ 1.89	45.23 $\pm$ 2.22	94
6-Phosphogluconate dehydrogenase	48.91 $\pm$ 2.44	—	48.91 $\pm$ 2.44	0
Pyruvate carboxylase	—	2.75 $\pm$ 0.70	2.75 $\pm$ 0.70	100

Table 4. *Comparison of various metabolic parameters in rat, guinea-pig and rabbit fat-cells*

The rat and guinea-pig data are taken from Saggerson (1972b) and Saggerson (1974). The rabbit data are taken from the Figures and Tables presented in the Results section.

Metabolic parameter	Animal		
	Rat	Guinea pig	Rabbit
Extramitochondrial NADP-linked dehydrogenase relative activities			
Glucose 6-phosphate dehydrogenase	1.00	1.00	1.00
6-Phosphogluconate dehydrogenase	0.93	0.66	0.33
NADP-malate dehydrogenase	2.94	0.14	0
NADP-isocitrate dehydrogenase	0.51	0.33	0.74
Mitochondrial 'oxaloacetate-generating' relative activities			
Pyruvate carboxylase	1.00	1.00	1.00
Phosphoenolpyruvate carboxylase	0.01	0.38	15.5
Citrate/isocitrate 'handling' enzymes, relative activities			
Citrate synthase	100	100	100
NADP-isocitrate dehydrogenase (mitochondrial)	7.9	8.8	7.0
NADP-isocitrate dehydrogenase (extramitochondrial)	22.2	88.9	148.9
ATP-citrate lyase	36.1	22.0	12.8
Effect of 20 μ M-phenazine methosulphate on fatty acid synthesis from			
5 mM-Glucose (+insulin)	Increases	No effect	Decreases†
5 mM-Lactate	Increases	Decreases	Decreases†
Effect of 5 mM-acetate on fatty acid synthesis from 5 mM-glucose (+insulin)	Increases	No effect	Decreases
Ratio of 5 mM-glucose carbon to 5 mM-acetate carbon incorporated into fatty acids	2.78	0.33	0.23
Ratio of glucose carbon to acetate* carbon in tricarboxylic acid-cycle-derived CO ₂	0.80	1.58	6.67
Glucose (+insulin) / pyruvate/lactate incorporation into fatty acids (all at 5 mM)	1:0.90:0.18	1:1.30:2.13	1:1.32:1.07
Ratio of lactate produced to pyruvate produced (in cells incubated with 5 mM-glucose + insulin)	8.41	0.98	5.59
Lactate dehydrogenase activity (munits/100 μ g of DNA)	3309	617	2552

* The rat and guinea-pig values were obtained with 5 mM-glucose and 2.5 mM-acetate; the rabbit value was obtained with 5 mM-glucose and 5 mM-acetate.

† E.D. Saggerson, unpublished work.

effect of acetate on glucose conversion into fatty acids.

The results shown in Table 1 and summarized in Table 4 indicate that glucose (in the presence of insulin), pyruvate and lactate are incorporated into fatty acids by rabbit cells to approximately the same extent. This is essentially the pattern found in guinea-pig cells with the exception that the relative utilization of lactate is greater in this species. The relatively low utilization of lactate by rat cells supports the argument that fatty acid synthesis from lactate and glucose is restricted by the ability to dispose of cytosolic NADH (Schmidt & Katz, 1969; Halperin & Robinson, 1970; Katz & Wals, 1970; Saggerson, 1972*b*). Such a restraint would not appear to operate in guinea-pig and rabbit fat-cells. Further evidence is provided by the observation that phenazine methosulphate does not stimulate the incorporation of lactate or glucose in these two species.

Table 4 shows a correlation between the lactate dehydrogenase activity of the cells and the lactate/pyruvate ratio under the conditions of incubation used. The significance, if any, of this correlation is unclear.

Another trend that is apparent in progression from rat to guinea pig to rabbit is a 53-fold decrease in pyruvate carboxylase activity and a 24-fold increase in mitochondrial phosphoenolpyruvate carboxylase activity. In rabbit fat-cells it is possible that the carboxylation of phosphoenolpyruvate rather than that of pyruvate may be the major route of formation of mitochondrial oxaloacetate from C₃ precursors. A generalization has been suggested by Hanson & Ballard (1968) that tissues which use glucose as a source of carbon for fatty acid synthesis require the 'citrate-cleavage' pathway. ATP-citrate lyase is an essential component of this pathway together with a source of mitochondrial oxaloacetate and a means of disposal of cytosolic oxaloacetate. Tables 1 and 4 show that pyruvate is incorporated into fatty acids as readily as glucose by rabbit cells. When glucose is supplied the cell may have the option of forming mitochondrial oxaloacetate from pyruvate by using the relatively low pyruvate carboxylase activity or from phosphoenolpyruvate by using the relatively high mitochondrial phosphoenolpyruvate carboxylase activity. When pyruvate is the sole substrate, however, the formation of mitochondrial oxaloacetate from phosphoenolpyruvate is presumably not operative and the low pyruvate carboxylase activity must therefore meet the oxaloacetate requirements of the postulated citrate-cleavage pathway. Alternatively, fatty acid synthesis from glucose or pyruvate in rabbit cells may not be wholly dependent on the 'citrate-cleavage' pathway and may use other means of transport of acetyl units from mitochondria to cytosol (Srere, 1965). This hypothesis is supported by the graduation from rat to guinea pig to rabbit

with respect to the decreasing activities of ATP-citrate lyase, NADP-malate dehydrogenase and pyruvate carboxylase (Saggerson, 1974; also Tables 3 and 4), suggesting that the 'citrate-cleavage' pathway becomes progressively less important in the lipogenic process coupled with a decreasing importance of glucose compared with acetate as a lipogenic carbon source. The role of the extraordinarily high mitochondrial phosphoenolpyruvate carboxylase activity in rabbit fat-cells remains unclear at present. The abundance of this activity does not appear to confer any extra ability to carry out 'glyceroneogenesis' (Reshef *et al.*, 1970) since the incorporation of pyruvate or lactate carbon into glyceride glycerol shown in Table 1 was no higher than that found in rat cells (Saggerson & Tomassi, 1971) and was very low compared with the incorporation of glucose into glyceride glycerol.

In Table 4 the relative activities of four extramitochondrial NADP-linked dehydrogenases are compared in rat, guinea pig and rabbit. In all three species glucose 6-phosphate dehydrogenase showed the highest activity. On progressing from rat to guinea pig to rabbit there is a gradual decline in the relative activity of 6-phosphogluconate dehydrogenase and a drastic decline in NADP-malate dehydrogenase. No particular trend in the relative activity of the extramitochondrial NADP-isocitrate dehydrogenase is discernible on this basis. The absence of NADP-malate dehydrogenase suggests that rabbit fat-cells may be able to meet all demands for NADPH for fatty acid synthesis through the hexose monophosphate pathway. However, a possible involvement of NADP-isocitrate dehydrogenase in the supply of NADPH must be considered. When the activities of the mitochondrial and extramitochondrial NADP-isocitrate dehydrogenases are expressed relative to that of citrate synthase (Table 4), there is a more or less 'constant-proportion group' between citrate synthase and the mitochondrial isocitrate dehydrogenase activity in the three species whereas there is a graduation from rat to guinea pig to rabbit with respect to the increasing relative activity of the extramitochondrial isocitrate dehydrogenase. ATP-citrate lyase activity decreases relative to citrate synthase on progressing from rat to guinea pig to rabbit. Provided extramitochondrial aconitase is sufficiently active, this change in enzyme pattern should ensure that in rabbit cells extramitochondrial NADP-isocitrate dehydrogenase should more effectively compete against ATP-citrate lyase for cytosolic citrate than in rat cells. Guinea pig should again occupy an intermediate position in this respect. These observations also support the hypothesis that rabbit fat-cells may normally perform lipogenesis from substrates (presumably mainly acetate) without an obligatory involvement of the 'citrate-cleavage' pathway.

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References

- Flatt, J. P. & Ball, E. G. (1964) *J. Biol. Chem.* **239**, 675–685
Flatt, J. P. & Ball, E. G. (1966) *J. Biol. Chem.* **241**, 2862–2869
Halperin, M. L. & Robinson, B. H. (1970) *Biochem. J.* **116**, 235–240
Hanson, R. W. & Ballard, F. J. (1968) *Biochem. J.* **108**, 705–713
Katz, J. & Wals, P. A. (1970) *J. Biol. Chem.* **245**, 2546–2548
Katz, J., Landau, B. R. & Bartsch, G. E. (1966) *J. Biol. Chem.* **241**, 727–740
Krebs, H. A. & Henseleit, K. (1932) *Hoppe-Seyler's Z. Physiol. Chem.* **210**, 33–66
Reshef, L., Hanson, R. W. & Ballard, F. J. (1970) *J. Biol. Chem.* **245**, 5979–5984
Rodbell, M. (1964) *J. Biol. Chem.* **239**, 375–380
Saggerson, E. D. (1972a) *Biochem. J.* **128**, 1057–1067
Saggerson, E. D. (1972b) *Biochem. J.* **128**, 1069–1078
Saggerson, E. D. (1974) *Biochem. J.* **140**, 211–224
Saggerson, E. D. & Tomassi, G. (1971) *Eur. J. Biochem.* **23**, 109–117
Schmidt, K. & Katz, J. (1969) *J. Biol. Chem.* **244**, 2125–2131
Srere, P. A. (1965) *Nature (London)* **205**, 766–770