Influence of insulin and glucose on pyruvate catabolism in perfused rat hindlimbs

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The effects of insulin and glucose on the oxidative decarboxylation of pyruvate in isolated rat hindlimbs was studied in non-recirculating perfusion with [1-¹⁴C]pyruvate. Insulin increased the calculated pyruvate decarboxylation rate in a concentration-dependent manner. At supramaximal insulin concentrations, the calculated pyruvate decarboxylation rate was increased by about 40% in perfusions with 0.15–1.5 mM-pyruvate. Glucose up to 20 mM had no effect. In the presence of insulin and low physiological pyruvate concentrations (0.15 mM), glucose increased the calculated pyruvate oxidation. This effect was abolished by high concentrations of pyruvate (1 mM). The data provide evidence that in resting perfused rat skeletal muscle insulin primarily increased the activity of the pyruvate dehydrogenase complex. The effect of glucose was due to increased intracellular pyruvate supply.

Oxidative decarboxylation of pyruvate by the mitochondrial pyruvate dehydrogenase complex (EC 1.2.4.1, EC 2.3.1.12, EC 1.6.4.3) is a key reaction in carbohydrate metabolism. A wide variety of factors, including substrate, coenzyme and ion concentrations, have been shown to influence the activity of the complex by either activating or inhibiting the components of the interconversion system and/or by feedback inhibition of the active dephosphorylated form (for reviews, see Denton *et al.*, 1975; Denton & Hughes, 1978; Reed, 1981; Wieland, 1983).

Different experimental approaches have been used for studies on the regulation of pyruvate decarboxylation in intact tissues. Determination of the activation state of the pyruvate dehydrogenase complex primarily yielded information on the actual and total capacity in a tissue (Coore *et al.*, 1971; Siess *et al.*, 1971; Wieland *et al.*, 1971; Hennig *et al.*, 1975; Hagg *et al.*, 1976; Stansbie, 1976). ¹⁴CO₂ release from [1-¹⁴C]pyruvate has been used, e.g. in the perfused rat liver (Häussinger *et al.*, 1975; Scholz *et al.*, 1978; Patel *et al.*, 1982; Zwiebel *et al.*, 1982; Sies *et al.*, 1983), heart (Hiltunen & Hassinen, 1976; Dennis *et al.*, 1979; Waymack *et al.*, 1979; Bünger *et al.*, 1983), and skeletal muscle (Schadewaldt *et al.*, 1983b,c), to investigate the pyruvate flux.

With respect to the hormonal regulation by insulin, there is agreement that the hormone increases the activation state of the pyruvate

dehydrogenase in adipose tissue (Jungas, 1971; for further references see Denton & Hughes, 1978) and the lactating mammary gland (Kankel & Rein-aer, 1976; Baxter & Coore, 1978). Contradictory results have been reported for the rat heart (Kerbey *et al.*, 1976; Ohlen *et al.*, 1978) and liver (Patzelt *et al.*, 1973; Topping *et al.*, 1977).

Skeletal muscle is an important and well-known target tissue of insulin. There is, however, little information on the effect of the hormone on pyruvate decarboxylation in this tissue. The effect of diabetes in decreasing the activation state of pyruvate dehydrogenase in rat gastrocnemius muscle reported by Hagg *et al.* (1976) and Hennig *et al.* (1975), and studies on the putative intracellular mediator of insulin (Jarett & Seals, 1979; Larner *et al.*, 1979), suggest a regulatory role of insulin in skeletal-muscle pyruvate catabolism.

This study was undertaken to gain insight into the significance of insulin action on the regulation of pyruvate oxidation in resting skeletal muscle in the absence and presence of glucose. It has been repeatedly shown that the activation state of the pyruvate dehydrogenase complex need not necessarily parallel changes in pyruvate flux (Hansford & Cohen, 1978; Dennis *et al.*, 1979; Waymack *et al.*, 1979; Bünger *et al.*, 1983). Therefore the effects of insulin and glucose on the metabolism of [1-¹⁴C]pyruvate in isolated perfused rat hindlimbs were investigated. The preparation has been widely used for the study of skeletal-muscle

metabolism (Ruderman *et al.*, 1971) and offers the advantage of metabolic and isotopic steady-state conditions in non-recirculating perfusion. Preliminary results have been presented (Schadewaldt *et al.*, 1983a, 1984a).

Experimental

Hindlimbs of fed male rats (Wistar strain, 180–200g body wt.) were prepared essentially as described by Ruderman *et al.* (1971). Non-recirculating perfusion of one limb with a medium containing 0.15mM-sodium pyruvate was carried out as described by Schadewaldt *et al.* (1983c). Additions to the influent were made with infusion pumps. Insulin, diluted with 0.154M-NaCl, and the other reagents in water solution were infused to give the final arterial concentrations as indicated. Tracer doses of $[1-^{14}\text{C}]$ pyruvate (750–775MBq/mmol) were added to give specific radioactivities of 1.3–2.6Bq/nmol of influent pyruvate.

The concentrations of lactate (Gutmann & Wahlefeld, 1974), pyruvate (Czok & Lamprecht, 1974) and glucose (Bergmeyer *et al.*, 1974) in the perfusate were determined by spectrophotometric methods. $^{14}\text{CO}_2$ production from $[1-^{14}\text{C}]$ pyruvate was used to calculate the $^{14}\text{CO}_2$ release rate on the basis of the specific radioactivity of pyruvate in the perfusate. Likewise, the pyruvate decarboxylation rate in the perfused tissue was calculated on the basis of the specific radioactivity of lactate in the effluent. The specific radioactivity of lactate in the effluent was determined as detailed elsewhere (Schadewaldt *et al.*, 1984b), and largely reflects the intracellular dilution of influent $[1-^{14}\text{C}]$ pyruvate during perfusion (Schadewaldt *et al.*, 1983c).

The glycolytic rate in the perfused tissue was calculated from the lactate-release and pyruvate-decarboxylation rates and was corrected for pyruvate uptake. Insulin concentrations were calculated ($7.2\text{nmol} = 1\text{i.u.}$) from the units added to the perfusate (see Rennie & Holloszy, 1977). The specific radioactivity of lactate was standardized to 1kBq/nmol of influent $[1-^{14}\text{C}]$ pyruvate. Results are means $\pm$ S.D.; statistically significant differences were determined by Student's paired *t* test.

D-Glucose was purchased from Serva, Heidelberg, Germany. Monocomponent pig insulin was obtained from Novo Industrie, Mainz, Germany. The sources for the other materials were as in Schadewaldt *et al.* (1984b).

Results

Effect of insulin

The effect of insulin was first studied in glucose-free perfusion. As shown in Fig. 1, a concentration-

Fig. 1. Concentration-dependent effect of insulin on the $^{14}\text{CO}_2$ release from $[1-^{14}\text{C}]$ pyruvate and on the calculated pyruvate decarboxylation rate in isolated perfused rat hindlimbs

Non-recirculating perfusion was carried out with 0.15mM- $[1-^{14}\text{C}]$ pyruvate as the sole substrate. The effects of insulin are expressed as a percentage of the values during the preceding insulin-free perfusion. Results are means $\pm$ S.E.M. for at least three experiments. The effect of insulin was statistically significant ($P < 0.05$) at concentrations $\geq 145\text{pM}$. The effects of supramaximal insulin concentrations, i.e. $\geq 7.2\text{nM}$, versus 145pM were significant at $P < 0.05$ as determined by Student's non-paired *t* test.

dependent effect of insulin on the $^{14}\text{CO}_2$ release from $[1-^{14}\text{C}]$ pyruvate was observed. No significant effects were measurable with low insulin concentrations (36pM). Addition of physiological insulin concentrations (145pM ; $n = 4$) significantly enhanced the pyruvate uptake by about 7% from 56 ± 20 to $60 \pm 18\text{nmol/min per g}$ of muscle ($P < 0.05$), and the $^{14}\text{CO}_2$ release by $20 \pm 9\%$ ($P < 0.01$). The specific radioactivity of lactate was decreased from 134 ± 27 to $120 \pm 26\text{Bq/nmol}$ ($P < 0.02$). Because this appears as the denominator in the calculation, it has a major effect on the more distinct increase in the calculated pyruvate decarboxylation rate after addition of insulin (Fig. 1).

The effects on the $^{14}\text{CO}_2$ release and the calculated pyruvate decarboxylation rate were more marked at higher insulin concentrations. Above 1.45nM -insulin no further increase was measurable. The results obtained with supramaximal insulin concentration, i.e. 7.2nM , on metabolic rates and $[1-^{14}\text{C}]$ pyruvate metabolism in glucose-free perfusions are included in Tables 1 and 3 respectively.

Effect of glucose

Some effects of glucose (5 mM) on the metabolism of rat hindlimbs in insulin-free perfusion are shown in Table 1. Under standard perfusion conditions, low amounts of glucose were released into the perfusate, corresponding to about 15% of the glucose arising from muscle glycogen breakdown (cf. Schadevaldt *et al.*, 1983b). After addition of 5 mM-glucose a net uptake was observed. The release of lactate and the glycolytic rate were increased, whereas the pyruvate uptake was slightly decreased.

Glucose affected [1-¹⁴C]pyruvate metabolism in a concentration-dependent manner (Table 2). The ¹⁴CO₂ release and concomitantly the specific radioactivity of lactate were decreased, indicating an increased dilution of label within the perfused tissue. However, the calculated pyruvate decarboxylation rate remained unchanged up to 20 mM-glucose.

Combination of insulin and glucose

The usual experimental procedure and a representative experiment is shown in Fig. 2. Compared

with the insulin-free control perfusions with 5 mM-glucose (cf. Table 1), insulin increased glucose uptake about 3-fold, increased lactate release and glycolytic rate about 2-fold and decreased net pyruvate uptake by about 75% (Table 1). As expected, the effect of glucose in decreasing the ¹⁴CO₂ release from [1-¹⁴C]pyruvate and the specific radioactivity of lactate was more marked in the presence of insulin (Table 3).

In contrast with the lack of effect of glucose on the calculated pyruvate decarboxylation rate in the absence of insulin, an effect of perfusate glucose was observed in the presence of insulin (Fig. 2, Table 3). With respect to the insulin- and glucose-free perfused control, there was an increase amounting to about 20, 30, and 50% at 1 mM-, 5 mM- and 10 mM-glucose respectively. No further increase was seen at 20 mM-glucose (Table 3).

Effect of pyruvate

Compared with the insulin-free control phase of perfusion (cf. Fig. 2), the calculated pyruvate decarboxylation rate was significantly enhanced in perfusions with elevated pyruvate concentrations.

Table 1. *Effect of glucose on metabolic rates in isolated perfused rat hindlimbs in the absence and presence of insulin*
Data are from separate experiments without and with addition of insulin. Non-recirculating perfusion with 0.15 mM-pyruvate in the absence of insulin was carried out for 20–25 min, followed by a 20 min period of perfusion with additional 5 mM-glucose. Perfusions with insulin were performed essentially as shown in Fig. 2. Results are means $\pm$ S.D. for at least four experiments. Perfusate samples for determinations were taken during the metabolic steady state of the respective perfusion period. Statistical significance of differences: versus glucose-free perfusion, * P < 0.05, ** P < 0.005; versus insulin-free control phase of perfusion (results not shown), †† P < 0.005; versus insulin free perfusion (as determined by Student's non-paired t test), ‡ P < 0.05, ‡‡ P < 0.005. Abbreviation: n.d., not determined.

Perfusate glucose concn. (mM) ...	Metabolic rates (nmol/min per g of muscle)			
	No insulin		+ insulin	
	0	5	0	5
Glucose uptake	-31 $\pm$ 9	99 $\pm$ 21**	n.d.	319 $\pm$ 98††
Lactate release	368 $\pm$ 52	516 $\pm$ 119*	343 $\pm$ 68	703 $\pm$ 109**‡
Pyruvate uptake	64 $\pm$ 17	53 $\pm$ 18**	66 $\pm$ 6††	17 $\pm$ 9**‡‡
Glycolytic rate	239 $\pm$ 15	322 $\pm$ 50*	230 $\pm$ 41	432 $\pm$ 51**‡

Table 2. *Effect of glucose on the metabolism of [1-¹⁴C]pyruvate in isolated perfused rat hindlimbs*
Perfusion with 0.15 mM-[1-¹⁴C]pyruvate was carried out as described in the legend of Table 1. ¹⁴CO₂ release and pyruvate decarboxylation rates were calculated as detailed in the Experimental section. The specific radioactivity of lactate was standardized to 1 kBq/nmol of influent pyruvate. Results are means $\pm$ S.D. for four experiments with the glucose concentrations indicated. Statistical significance of differences: versus glucose-free perfusion, * P < 0.05.

Perfusate glucose concn. (mM)	Sp. radioactivity of lactate (Bq/nmol)	¹⁴ CO ₂ release (nmol/min per g of muscle)	Calculated pyruvate decarboxylation (nmol/min per g of muscle)
0	153 $\pm$ 36	16.0 $\pm$ 2.1	111 $\pm$ 25
1	137 $\pm$ 42	15.8 $\pm$ 1.5	116 $\pm$ 36
5	129 $\pm$ 27*	15.0 $\pm$ 0.6*	117 $\pm$ 17
10	127 $\pm$ 29*	14.2 $\pm$ 1.3*	96 $\pm$ 16
20	121 $\pm$ 27*	13.5 $\pm$ 1.5*	99 $\pm$ 29

At 0.5 mM-, 1.0 mM- and 1.5 mM-pyruvate it was 157 ± 20 , 194 ± 10 and 205 ± 23 nmol/min per g of muscle in the absence and 212 ± 28 , 295 ± 34 and 291 ± 38 nmol/min per g of muscle in the presence of insulin ($n = 4$, $P < 0.01$).

In Fig. 3 the results are plotted by analogy with the method of Hanes (1932). A regression analysis of the data by the method of Wilkinson (1961) yielded pyruvate concentrations with half-maxi-

mal effect on the calculated pyruvate decarboxylation rate of 0.23 ± 0.03 mM in insulin-free perfusion, and of 0.28 ± 0.05 mM after addition of insulin. A maximal pyruvate decarboxylation rate of 235 ± 10 nmol/min per g of muscle in the absence and of 363 ± 28 nmol/min per g of muscle in the presence of insulin was calculated.

In additional perfusions with 1 mM-pyruvate and 7.2 nM-insulin the effect of glucose was examined

Fig. 2. Effect of insulin and glucose on the metabolism of $[1-^{14}\text{C}]$ pyruvate in isolated perfused rat hindlimb. A representative perfusion experiment with 0.15 mM- $[1-^{14}\text{C}]$ pyruvate is shown. Insulin and glucose were added to the influent at the time and concentration indicated. Samples of the effluent were assayed for metabolite concentrations. For details see the Experimental section.

Fig. 3. Linear relationship between the ratio of substrate concentration to calculated pyruvate decarboxylation rate (v) and the arterial pyruvate concentration (s) in rat hindlimb perfusion experiments in the absence ($\circ$) and presence ($\bullet$) of insulin.

Hindlimbs were perfused with $[1-^{14}\text{C}]$ pyruvate at the concentrations indicated: 7.2 nM-insulin was added, in accordance with the experimental procedure shown in Fig. 2. Determinations and calculations were as detailed in the Experimental section. $[s]/v$ was plotted versus $[s]$ by analogy with the method of Hanes (1932). Results are means $\pm$ S.D. for at least four experiments.

Table 3. Effect of insulin and of glucose on the metabolism of $[1-^{14}\text{C}]$ pyruvate in isolated perfused rat hindlimbs. Non-recirculating perfusion with 0.15 mM- $[1-^{14}\text{C}]$ pyruvate was carried out essentially as shown in Fig. 2. Results are means $\pm$ S.D. for at least three experiments with the glucose concentrations indicated. Samples for determinations were taken during the metabolic and isotopic steady state of the respective perfusion period. Statistical significance of differences: versus control phase of perfusion, $\ddagger P < 0.005$; versus glucose-free perfusion after addition of insulin, $*P < 0.02$, $**P < 0.005$.

Condition	Perfusate glucose concn. (mM)	Sp. radioactivity of lactate (Bq/nmol)	$^{14}\text{CO}_2$ release (nmol/min per g of muscle)	Calculated pyruvate decarboxylation (nmol/min per g of muscle)
Control	0	143 ± 26	14.4 ± 4.8	96 ± 20
Addition of insulin	0	$130 \pm 26\ddagger$	$17.7 \pm 5.3\ddagger$	$138 \pm 21\ddagger$
	1	$97 \pm 17^*$	$16.0 \pm 2.6^*$	$150 \pm 22^*$
	5	$71 \pm 7^{**}$	$12.5 \pm 3.2^{**}$	$164 \pm 14^*$
	10	$64 \pm 11^{**}$	$12.2 \pm 1.6^{**}$	$183 \pm 6^{**}$
	20	$69 \pm 11^*$	$10.5 \pm 0.4^*$	$178 \pm 39^*$

as in Fig. 2. In these experiments (results not shown) the calculated pyruvate decarboxylation rate remained essentially unchanged on addition of 5 mM-glucose.

Discussion

Methodology

Under the perfusion conditions used the metabolism of 15–17 g of skeletal muscle was investigated. However, the preparation contains about 1.5 g of adipose tissue (Ruderman *et al.*, 1971), which might interfere with the interpretation of insulin effects on skeletal-muscle metabolism. In adipose tissue incubated with glucose, insulin induced a large increase in the activation state of the pyruvate dehydrogenase complex. However, no insulin effect could be observed in most studies when glucose was omitted from the medium (for references, see Denton & Hughes, 1978; Wieland, 1983). It therefore seems unlikely that adipose tissue contributed significantly to insulin effects in glucose-free perfusions.

In hindlimb perfusions with glucose and supra-maximal concentrations of insulin, adipose-tissue pyruvate catabolism could possibly not exceed 10% of the calculated total pyruvate decarboxylation rate (cf. Table 3), whether estimated on the basis of oxygen consumption (about 3% of total; cf. Ruderman *et al.*, 1971; Schadowaldt *et al.*, 1983c) or on the basis of total pyruvate dehydrogenase in this tissue (Hennig *et al.*, 1975).

Effect of glucose and pyruvate

In perfusions with 0.15 mM-pyruvate, pyruvate was taken up in spite of high glycolytic rate, and hence pyruvate production rate, in the perfused muscle tissue. The uptake was only slightly decreased by addition of glucose. These observations provide evidence that the availability of pyruvate emerging from glycolysis was limited for the pyruvate dehydrogenase reaction and indicate an impaired substrate supply in the insulin-free perfused tissue even in the presence of glucose. That intracellular substrate availability is important for the overall flux through the enzyme is further suggested by the increased pyruvate decarboxylation at elevated perfusate pyruvate concentrations.

Pyruvate is an inhibitor of the pyruvate dehydrogenase kinase (Linn *et al.*, 1969; Cooper *et al.*, 1974), and can thus increase the activation state of the complex. However, in the perfused rat heart the activation state has been found to be decreased with increasing perfusate pyruvate concentrations (Dennis *et al.*, 1979). An increased activation state could only be demonstrated with pyruvate concentrations above 2 mM (Bünger *et al.*, 1983; Dennis *et al.*, 1979). Therefore, and because of the linear relationship shown in Fig. 3, it seems rather

unlikely that pyruvate affected the pyruvate flux in resting perfused rat skeletal muscle to a significant extent by inhibition of the pyruvate dehydrogenase kinase at pyruvate concentrations used in this study.

Whether and to what extent glucose in the presence of insulin might influence the cytosolic and, moreover, the mitochondrial pyruvate concentration in skeletal muscle is actually not known. The concentration-dependent effect of glucose to increase the calculated pyruvate decarboxylation rate and the considerable decrease in net pyruvate uptake by glucose in perfusions with insulin would, however, be reasonably explained by an improvement of intracellular pyruvate supply. The finding that the insulin-dependent effect of glucose was abolished by addition of 1 mM-pyruvate further supports this view.

Effect of insulin

The experiments demonstrate an increasing effect of physiological insulin concentrations on the pyruvate decarboxylation in glucose-free perfused hindlimbs of fed rats. The dose-response relationship is comparable with the effects of insulin on glycogen metabolism in perfused rat hindquarters (Chiasson *et al.*, 1980). From the activation states of the pyruvate dehydrogenase in resting gastrocnemius muscles of normal and insulin-deficient rats reported by Hagg *et al.* (1976) and Hennig *et al.* (1975) and the maximal pyruvate decarboxylation rates in the present study, the total enzyme activity in the perfused tissue can be estimated. It amounted to about 2 units/g of muscle, in both the absence and the presence of insulin. This agrees well with the values given by Hennig *et al.* (1975).

In the concentration range 0.15–1.5 mM-pyruvate, insulin increased the calculated pyruvate flux by about 40% with respect to the insulin-free perfused control. Thus the effect of insulin was apparently independent of the perfusate pyruvate concentration and contrasts with the effect of glucose.

Pyruvate is taken up by the perfused tissue. Therefore the concentration of pyruvate with half-maximal effect on the pyruvate flux is somewhat overestimated. Nevertheless, the data indicate that half-maximally effective pyruvate concentrations are in the physiological range. When compared with the apparent K_m of pyruvate dehydrogenase, about 0.02 mM (Cooper *et al.*, 1974; Taylor & Halperin, 1973), and with that of the mitochondrial pyruvate carrier, about 0.15 mM (Halestrap, 1975), it appears that the concentration of pyruvate with half-maximal effect on flux was closer to the apparent K_m of the carrier than to that of the enzyme. Whether this indicates a regulatory role of the carrier, as has been proposed for the perfused rat heart (Waymack *et al.*, 1979) and liver (Zwiebel

et al., 1982), is unclear. In any case, it may be deduced that, in perfused skeletal muscle, carrier-mediated transport into the mitochondrial compartment is not a site of hormonal regulation by insulin.

The evidence presented so far suggests that the insulin-induced increase in pyruvate catabolism in glucose-free perfused rat hindlimbs was primarily attributable to an increased activity of the pyruvate dehydrogenase complex. At the present state of knowledge, no reliable conclusion can be drawn on the ultimate mechanism. Whether insulin effects were due to the release of a mediator with direct influence on the interconversion system or were rather a consequence of decreased acetyl-CoA production from endogenous lipid stores (Ruderman *et al.*, 1971) remains to be investigated.

Compared with the marked effects of electrical stimulation on the activation state of the pyruvate dehydrogenase complex in gastrocnemius muscles of normal and diabetic rats (Hagg *et al.*, 1976; Hennig *et al.*, 1975), the influence of insulin on pyruvate catabolism in resting perfused skeletal muscle was only moderate. Therefore factors other than insulin, e.g. Ca^{2+} , seem to be of major importance for the regulation of pyruvate oxidation in skeletal muscle during the transition from rest to contraction.

This communication contains essential parts of the M.D. Thesis of E.L.

References

- Baxter, M. A. & Coore, H. G. (1978) *Biochem. J.* **174**, 553–561
- Bergmeyer, H. U., Bernt, E., Schmidt, F. & Stork, H. (1974) in *Methoden der enzymatischen Analyse* (Bergmeyer, H. U., ed.), 3rd edn., vol. 2, pp. 1241–1250, Verlag Chemie, Weinheim
- Bünger, R., Permanetter, B. & Yaffe, S. (1983) *Pflügers Arch.* **397**, 214–219
- Chiasson, J.-L., Dietz, M. R., Shikama, H., Wotten, M. & Exton, J. H. (1980) *Am. J. Physiol.* **239**, E69–E74
- Cooper, R. H., Randle, P. J. & Denton, R. M. (1974) *Biochem. J.* **143**, 625–641
- Coore, H. G., Denton, R. M., Martin, B. R. & Randle, P. J. (1971) *Biochem. J.* **125**, 115–127
- Czok, R. & Lamprecht, W. (1974) in *Methoden der enzymatischen Analyse* (Bergmeyer, H. U., ed.), 3rd edn., vol. 1, pp. 785–791, Verlag Chemie, Weinheim
- Dennis, S. C., Padma, A., DeBuysere, M. S. & Olson, M. S. (1979) *J. Biol. Chem.* **254**, 1252–1258
- Denton, R. M. & Hughes, W. A. (1978) *Int. J. Biochem.* **9**, 545–552
- Denton, R. M., Randle, P. J., Bridges, B. J., Cooper, R. H., Kerbey, A. L., Pask, H. T., Severson, D. L., Stansbie, D. & Whitehouse, S. (1975) *Mol. Cell. Biochem.* **9**, 27–53
- Gutmann, I. & Wahlefeld, A. W. (1974) in *Methoden der enzymatischen Analyse* (Bergmeyer, H. U., ed.), 3rd edn., vol. 2, pp. 1510–1514, Verlag Chemie, Weinheim
- Hagg, S. A., Taylor, S. I. & Ruderman, N. B. (1976) *Biochem. J.* **158**, 203–210
- Halestrap, A. P. (1975) *Biochem. J.* **148**, 85–96
- Hanes, C. S. (1932) *Biochem. J.* **26**, 1406–1421
- Hansford, R. G. & Cohen, L. (1978) *Arch. Biochem. Biophys.* **191**, 65–81
- Häussinger, D., Weiss, L. & Sies, H. (1975) *Eur. J. Biochem.* **52**, 421–431
- Hennig, G., Löffler, G. & Wieland, O. H. (1975) *FEBS Lett.* **59**, 142–145
- Hiltunen, J. K. & Hassinen, I. E. (1976) *Biochim. Biophys. Acta* **440**, 377–390
- Jarett, L. & Seals, J. R. (1979) *Science* **206**, 1407–1408
- Jungas, R. L. (1971) *Metab. Clin. Exp.* **20**, 43–53
- Kankel, K.-F. & Reinauer, H. (1976) *Diabetologia* **12**, 149–154
- Kerbey, A. L., Randle, P. J., Cooper, R. H., Whitehouse, S., Pask, H. T. & Denton, R. M. (1976) *Biochem. J.* **154**, 327–348
- Larner, J., Galasko, G., Cheng, K., De Paoli-Roach, A., Huang, L., Daggy, P. & Kellog, J. (1979) *Science* **206**, 1408–1410
- Linn, T. C., Pettit, F. H., Hucho, F. & Reed, L. J. (1969) *Proc. Natl. Acad. Sci. U.S.A.* **64**, 227–234
- Ohlen, J., Siess, E. A., Löffler, G. & Wieland, O. H. (1978) *Diabetologia* **14**, 135–139
- Patel, T. B., DeBuysere, M. S., Scholz, R. & Olson, M. S. (1982) *Arch. Biochem. Biophys.* **213**, 573–584
- Patzelt, C., Löffler, G. & Wieland, O. H. (1973) *Eur. J. Biochem.* **33**, 117–122
- Reed, L. J. (1981) *Curr. Top. Cell. Regul.* **18**, 95–106
- Rennie, M. J. & Holloszy, J. O. (1977) *Biochem. J.* **168**, 161–170
- Ruderman, N. B., Houghton, C. R. S. & Hems, R. (1971) *Biochem. J.* **124**, 639–651
- Schadewaldt, P., Lammers, E. & Staib, W. (1983a) *Hoppe-Seyler's Z. Physiol. Chem.* **364**, 1204
- Schadewaldt, P., Münch, U. & Staib, W. (1983b) *Biochem. J.* **216**, 761–764
- Schadewaldt, P., Münch, U., Prengel, M. & Staib, W. (1983c) *Biochem. Biophys. Res. Commun.* **116**, 456–461
- Schadewaldt, P., Lammers, E. & Staib, W. (1984a) *Aktuel. Endokrinol. Stoffwechsel* **5**, 117
- Schadewaldt, P., Oelers, R., Radeck, W. & Staib, W. (1984b) *Anal. Biochem.* **143**, 308–315
- Scholz, R., Olson, M. S., Schwab, A. J., Schwabe, U., Noell, C. & Braun, W. (1978) *Eur. J. Biochem.* **86**, 519–530
- Sies, H., Graf, P. & Crane, D. (1983) *Biochem. J.* **212**, 271–278
- Siess, E. A., Wittmann, J. & Wieland, O. (1971) *Hoppe-Seyler's Z. Physiol. Chem.* **352**, 447–452
- Stansbie, D. (1976) *Clin. Sci.* **51**, 445–452
- Taylor, W. M. & Halperin, M. L. (1973) *J. Biol. Chem.* **248**, 6080–6083
- Topping, D. L., Goheer, M. A., Coore, H. G. & Mayes, P. A. (1977) *Biochem. Soc. Trans.* **5**, 1000–1001
- Waymack, P. P., DeBuysere, M. S. & Olson, M. S. (1979) *Arch. Biochem. Biophys.* **194**, 258–264
- Wieland, O. H. (1983) *Rev. Physiol. Biochem. Pharmacol.* **96**, 123–170
- Wieland, O., Siess, E. A., Schulze-Wethmar, F. H., von Funcke, G. & Winton, B. (1971) *Arch. Biochem. Biophys.* **143**, 593–601
- Wilkinson, G. N. (1961) *Biochem. J.* **80**, 324–332
- Zwiebel, M., Schwabe, U., Olson, M. S. & Scholz, R. (1982) *Biochemistry* **21**, 346–353