Biochemical Pharmacology, Vol. 29, pp. 1103–1108.

0006-2952/80/0415-1103 $02.00/0

MONOAMINE OXIDASE INHIBITORS AND LIVER METABOLISM: STUDIES WITH ISOLATED RAT HEPATOCYTES

FELICITY P. A. CARR,* STEPHEN A. SMITH† and CHRISTOPHER I. POGSON*
Biological Laboratory, University of Kent, Canterbury, Kent, CT2 7NJ, U.K.

(*Received* 22 *July* 1979; *accepted* 14 *November* 1979)

Abstract—Gluconeogenesis from a range of substrates in isolated liver cells from 48 hr starved rats was inhibited by clorgyline, L-deprenyl, pargyline, tranylcypromine, phenelzine and iproniazid. Generally similar results were seen in cells from fed and diabetic rats and in those from starved guinea pigs. These effects were reversed on removal of inhibitor by washing. Ureogenesis from alanine, but not β-oxidation, was also inhibited by similar concentrations of pargyline and tranylcypromine. The cell contents of malate, aspartate, oxoglutarate and phosphoenolpyruvate were decreased 50 per cent by pargyline. ATP was also decreased to a smaller extent. MAOIs‡ increased oxygen uptake in mitochondria incubated with several substrates (state 4), an effect consistent with a degree of uncoupling of respiration and oxidative phosphorylation. These results are discussed in terms of a non-specific interaction with membrane lipids.

Monoamine oxidase [Amine : oxygen oxidoreductase (deaminating) flavin containing; EC 1.4.3.4; MAO] inhibitors have been used widely for many years for treatment of depressive states and hypertension [1]. Such inhibitors may be classified both chemically (e.g. based on hydrazine, phenylcyclopropylamine and proparglyamine) and pharmacologically (i.e. acting on MAO-A or MAO-B). Despite their apparent specificity and potency, a number of MAO inhibitors have been associated with effects not related to MAO function. These include inhibition of serum amine oxidase [2, 3], microsomal enzymes [4, 5], the low K_m aldehyde dehydrogenase [6–8], red cell pyruvate kinase [9] and other enzymes less well characterized [10]. Furthermore, it is clear that MAOIs of various types interact with systems involved in the regulation of glucose metabolism *in vivo*. Insulin secretion is reported to be both increased [11–13] and decreased [14–17] after exposure to MAOIs; the variation in response may be related to species and experimental differences as well as the chemical nature of the inhibitor [18]. Clinically there is evidence that MAOIs may potentiate insulin-dependent hypoglycaemia [19–22]. The precise mechanisms of these effects are unclear, but may involve systems other than MAO [18].

Hydrazine is known to lower blood glucose concentrations in several species *in vivo* [23, 24], and this has been associated with the inhibition of gluconeogenesis seen in perfused rat liver preparations [25]. Other hydrazines, including those with MAOI activity, also inhibit gluconeogenesis in guinea pig liver, although possibly by a different mechanism [25, 26].

In this paper we report on the effects of non-hydrazine MAOIs on gluconeogenesis and other processes in isolated liver cells and compare these results with those from studies with hydrazines.

MATERIALS AND METHODS

Animals. All rats were male, Sprague–Dawley strain CSE/ASH, 150–200 g, inbred in this laboratory. Guinea pigs were male, Dunkin–Hartley strain, 300–400 g. Where appropriate, diabetes was induced in rats by intravenous injection of streptozotocin (60 mg/kg body wt) in 0.9%(w/v)NaCl. These animals were used after 10 days.

Liver cells. Hepatocytes were prepared from rats and guinea pigs by a collagenase-dependent procedure as described elsewhere [27, 28]. No additions were made to the perfusion medium for cell preparations from animals fed *ad lib.*

The procedures for cell incubations, measurement of $^{14}CO_2$ release and assay of medium and intracellular metabolites were as previously [29]. Glycogen was determined by a standard procedure [30].

Mitochondria. These were prepared from rat liver [31], and incubated at 37° in a medium containing 20mM Tris–HCl, 120mM KCl and 1.5mM EDTA, pH 7.4 (final volume 3 ml). The respiratory control ratio with 2mM glutamate was always greater than four. In some experiments mitochondrial activity was monitored in digitonin-treated liver cells. Nine millilitres of cell suspension (15–20 mg dry wt/ml) were added to 30 ml of 2mM MOPS, 0.25M sucrose, 3mM EDTA, pH 7.0, containing 0.5 mg/ml digitonin. After mixing, the suspension was left for 2½ min at 4° before centrifugation (3000 g, 30 sec). The pellet was washed once and then suspended in a small volume of buffer for polarographic assays.

Materials. Radiochemicals were from the Radio-

* Present address: Department of Biochemistry, University of Manchester, Manchester, M13 9PL, U.K.

† Present address: Beecham Pharmaceuticals, Bio-Sciences Research Division, Great Burgh, Surrey, U.K.

‡Abbreviations used: MAO, monoamine oxidase: MAOI, monoamine oxidase inhibitor.

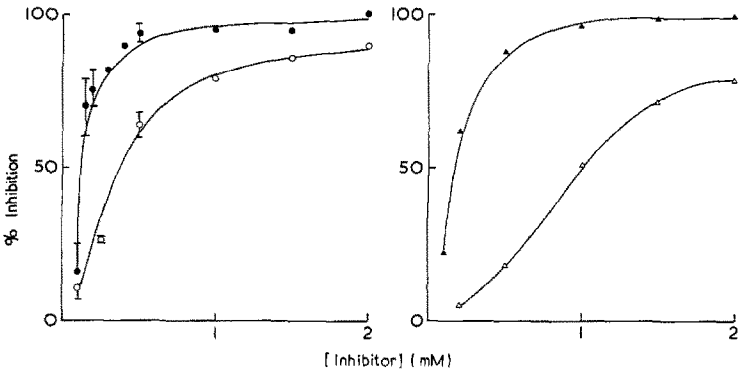

Fig. 1. Effectiveness of monoamine oxidase inhibitors as blockers of gluconeogenesis from 10 mM lactate in isolated liver cells from 48 hr starved rats. The procedures were as described in the Methods section and Table 1. Bars indicate S.E.M. for 3 independent observations. ○, pargyline; ●, clorgyline; △, tranycypromine; ▲, deprenyl.

chemical Centre, Amersham, Bucks, U.K. EDTA (free acid) was from the Sigma Chemical Co., Poole, Dorset, U.K. and oligomycin from the Boehringer Corp. (London) Ltd., Lewes, Sussex, U.K. Pargyline (*N*-benzyl-*N*-methylprop-2-ynylamine) HCl and phenylethylhydrazine sulphate (phenelzine) were from Abbott Labs, Queenborough, Kent, U.K. and K & K (through Kodak Ltd., Kirkby, Lancs, U.K.), respectively. Tranylcypromine (trans-phenyl-2-cyclopropylamine) sulphate, iproniazid (isonicotinic acid 2-isopropylhydrazide) and L-deprenyl (2-phenyl-l-methyl-*N*-methylprop-2-ynylamine) were kindly given by Smith, Kline & French Labs, Roche Products Ltd. and Dr. M. B. Youdim, respectively. All other reagents were from sources given earlier [29] or from standard suppliers.

RESULTS

Gluconeogenesis and glycogenolysis. The effect of varying concentrations of four MAOIs on gluconeogenesis from lactate in liver cells of starved rats is shown in Fig. 1. The half-maximally effective concentrations were, for clorgyline (MAO 'A'-specific [33]) 0.12 mM, for L-deprenyl (MAO 'B'-specific [34]) 0.16 mM, for pargyline (which inhibits both MAO 'A' and 'B' [35] 0.37 mM, and for tranylcypromine (which also inhibits both [35]) 1.0 mM. (The specificities noted in parentheses refer to the activities of drugs at low concentrations—at the concentrations used in this study, no such selectivity is apparent).

Glucose formed in incubations was measured in our earliest experiments both with glucose oxidase/peroxidase and with hexokinase/glucose 6-phosphate dehydrogenase. Although many compounds do interfere with the former method [36], no such interference was found in these experiments, and this assay was therefore used for subsequent work.

All four inhibitors, at concentrations close to those giving a half-maximal response in Fig. 1, were effective against substrates entering gluconeogenesis through oxaloacetate (Table 1). Some inhibition was also seen with glycerol and fructose but this was less marked. The percentage inhibition was found to remain similar over a range of lactate concentrations; and there was no evidence of increased potency at

Table 1. Inhibition of gluconeogenesis in rat hepatocytes by monoamine oxidase inhibitors*

Substrate	Rate (nmoles glucose/mg dry wt cells/hr)				
Inhibitor	None	Pargyline	L-Deprenyl	Clorgyline	Tranylcypromine
Concn (mM)		0.5	0.2	0.15	1.3
None	10 ± 2 (18)	1 ± 1 (3)	0 ± 0 (3)	1 ± 1 (5)	1 (2)
L-Lactate	217 ± 10 (30)	86 ± 14 (5)	92 ± 25 (6)	54 ± 18 (6)	36 ± 15 (3)
Pyruvate	182 ± 14 (7)	16	90	28 (2)	80
Alanine	53 ± 6 (5)	26	13	4	19
Glutamine	114 ± 7 (5)	43	100	29 (2)	
Serine	34 ± 9 (5)	12	0	2 (2)	7
Proline	50 ± 8 (4)	15	1	5 (2)	
Propionate	54 ± 8 (5)	7	29	10 ± 1 (3)	
Glycerol	147 ± 11 (3)	102 ± 15 (3)	100 (2)		
Fructose	622 ± 42 (7)	619 ± 29 (3)	574 (2)	424	433

* Cells were incubated for 40 min with or without inhibitors, before addition of substrate (final 10 mM). Rates were measured between 45 and 90 min after the addition of substrate, except for fructose where the period was 45–60 min. Results are means (± S.E.M., where appropriate) with the number of observations in parentheses.

more physiological substrate levels (data not shown).

It is possible that these inhibitors become active only after intracellular transformation or reaction with other cellular constituents. However, when inhibitor (pargyline or tranylcypromine) was added simultaneously with, or 30 min after, the substrate, the effect was immediately apparent and remained linear with time. Furthermore, when the inhibitor was removed from sustained incubations by washing the cells, the rate of gluconeogenesis was restored rapidly to control values.

Haecekel and Oellerich [26] have shown that certain hydrazine-based MAOIs may act through the formation of hydrazones with intracellular oxoacids; such hydrazones are very effective inhibitors when added exogenously. We have examined this possibility by studying the u.v. spectra of mixtures of pargyline, tranylcypromine and oxaloacetate, but have seen no evidence of hydrazone formation by this method.

Haeckel and Oellerich [26] found no effects of non-hydrazine MAOIs on gluconeogenesis in guinea pig liver. This raised the possibility of species-specific differences. Experiments with isolated liver cells from guinea pigs, however, gave results rather similar to those seen with rat-derived cells (Table 2), and are therefore at variance with the earlier claims [26].

Both iproniazid and phenelzine, hydrazine-based MAOIs, inhibited lactate gluconeogenesis in rat and guinea pig cells in the latter to a similar extent to that reported in [26].

Since there was no clear distinction between the effectiveness and apparent modes of action of the several inhibitors used in Table 1, subsequent studies were performed with pargyline and, to a lesser extent, tranylcypromine alone.

Palmitate (1 mM) stimulated gluconeogenesis from lactate in rat cells as expected (362 ± 8 vs 233 ± 31 nmoles glucose/mg dry wt/hr; N = 3). The rates with pargyline (0.6 mM) were, in the presence of palmitate, 97 ± 2, and, with lactate alone, 59 ± 13 nmoles/mg/hr (N = 3); with 1.3 mM tranylcypromine, these were 225 ± 24 and 87 ± 35 nmoles/mg/hr (N = 3), respectively. The extent of the inhibition was very similar whether fatty acid was present or not (in parallel incubations). Both inhibitors remained equally effective in cells derived from a 10-day chronically diabetic rat (data not shown).

In cells from fed rats, glucose output is derived both from glycogen breakdown and gluconeogenesis. The net glucose at the end of the incubation period was similar in all incubations with lactate, whether MAOIs were present or not. With both pargyline and tranylcypromine, however, this was achieved by approximately equal inhibition of gluconeogenesis and stimulation of glycogenolysis (Table 3).

The contents of several metabolites in cells incubated with or without pargyline are given in Table 4. Total malate, aspartate, oxoglutarate and phosphoenolpyruvate contents were all depressed approximately 50 per cent by the inhibitor. ATP is also decreased, suggesting that the action of pargyline may be rather non-specific. ATP contents in the presence of other MAOIs in the same series of experiments were: L-deprenyl (0.16 mM), 7.6 ± 1.0; clorgyline (0.15 mM), 4.2 ± 0.4; tranylcypromine (1.3 mM), 8.8 ± 1.1; N = 3 throughout. Only the fall with tranylcypromine failed to reach significance.

The possibility that the decreased contents of ATP and certain other metabolites might be correlated with an increase in the percentage of 'non-viable' cells in the incubation was investigated. First, the cellular CoASH, acetyl CoA and short-chain acyl CoA contents and the medium lactate/pyruvate and hydroxybutyrate/acetoacetate ratios were found to be unaffected by both pargyline and tranylcypromine. Second, an assessment of metabolic integrity by monitoring succinate oxidation in the presence and absence of digitonin [37] showed that viability remained similar in all incubations whether or not MAOIs were present (results not shown).

Other pathways. β-oxidation, as measured by $^{14}CO_2$ and ketone body production from [1-^{14}C] palmitate was unaffected by pargyline and tranylcypromine at concentrations giving half-maximal inhibition of lactate gluconeogenesis. These results agree with those of Bressler *et al.* [11] working with tranylcypromine alone.

Both pargyline and tranylcypromine blocked urea

Table 2. Inhibition of gluconeogenesis in guinea pig liver cells by monoamine oxidase inhibitors*

Substrate	Inhibitor	Rate (nmoles glucose/ mg dry wt cells/hr)	P (vs control)
Lactate (10 mM)		162 ± 34	
Lactate (10 mM)	Pargyline (1 mM)	29 ± 1	<0.01
Lactate (10 mM)	Tranylcypromine (1.5 mM)	101 ± 6	<0.05
Lactate (10 mM)	Clorgyline (1 mM)	5 ± 1	<0.01
Propionate (10 mM)		87 ± 13	
Propionate (10 mM)	Pargyline (1 mM)	60 ± 2	NS
Propionate (10 mM)	Tranylcypromine (1.5 mM)	91 ± 10	NS
Propionate (10 mM)	Clorgyline (1 mM)	7 ± 6	<0.01

* Cells were incubated for 40 min, with or without inhibitors, before addition of substrate. Rates were measured between 30 and 90 min after addition of substrate. The final concentrations of additions are given in parentheses. Results are means ± S.E.M. of three independent observations; significance between means was tested with the paired *t*-test [32]. NS, not significant.

Table 3. The effect of pargyline and tranylcypromine on glucose metabolism in 'fed' rat liver cells*

Additions	nmoles glucose/mg dry wt cells			
	Net glycogenolysis	Net glucose	Gluconeogenesis	Glycolysis
None	615 ± 18	432 ± 16		183
Lactate (10 mM)	492 ± 20	586 ± 12	94	
Pargyline (0.3 mM)	788 ± 1	584 ± 16		204
Lactate (10 mM) + Pargyline (0.3 mM)	771 ± 4	608 ± 5		163
Tranylcypromine (1.3 mM)	738 ± 4	508 ± 16		230
Lactate (10 mM) + Tranylcypromine (1.3 mM)	701 ± 15	595 ± 10		106

* Values reflect changes over the whole incubation period of 90 min; the initial glycogen content was 804 nmoles glucose units/mg dry wt. All additions were made at the start of the incubation period. Results are means ± S.D. of 3 replicate observations.

formation from alanine (Table 5) in cells from starved rats, the extent of this inhibition being comparable to that of glucose synthesis. Similar results were seen with ornithine plus NH_3, but pargyline was, surprisingly, somewhat less effective with glutamine as a substrate (Table 5). (Tranylcypromine, but not pargyline, interfered with colour development in the urea assay system. It was therefore, necessary to perform internal standards for each measurement involving this MAOI.)

Oxygen uptake. Whole cells exhibited unchanged rates of oxygen uptake in the presence of MAOIs.

Mitochondrial populations were prepared in two ways. First, a standard procedure using whole liver as starting material was followed [31]. Second, isolated liver cells, prepared as usual, were treated with digitonin to render the plasma and outer mitochondrial membranes permeable to added compounds. Results with the two preparations were similar. Preexposure of cells to pargyline before digitonin treatment did not affect the subsequent oxygen uptake; this is consistent with the 'wash-out' experiments reported above.

When added to mitochondrial suspensions in state 4 (ADP-limited), the MAOIs examined stimulated oxygen uptake with all the substrates tested (Table 6). In further experiments, pargyline relieved the inhibition of ADP-stimulated respiration by oligomycin, although to a lesser extent than did dinitrophenol. These observations are consistent with MAOIs acting as uncouplers of oxidative phosphorylation. The apparently weak nature of this effect may be partly attributable to a degree of inhibition of electron transport itself; this is more apparent at higher concentrations (above 4 mM) of pargyline, where the oxygen uptake is increasingly depressed. This inhibition may be related to the known affinity of this drug for flavins [38].

In preliminary experiments, we found no evidence for specific interaction of MAOIs with mitochondrial anionic transport systems.

DISCUSSION

From the results presented above, it is again clear that the effects seen are not directly related to the specific inhibition of MAO. The concentrations of drugs required for effects on carbohydrate and other metabolism are considerably above those which inhibit MAO activity totally. Furthermore, effects on MAO activity are not, with the drugs in this paper, reversible, i.e. overcome by washing, whereas those on the processes studied here are so reversed. Similarly, there is no reason to link these present

Table 4. Effect of pargyline on metabolite contents in rat liver cells*

Metabolite		nmoles/mg dry wt cells		
		Control	Plus pargyline (0.3 mM)	P
Malate	(4)	1.0 ± 0.2	0.6 ± 0.1	<0.005
Aspartate	(4)	1.7 ± 0.3	0.6 ± 0.1	<0.05
Oxoglutarate	(3)	1.9 ± 0.3	0.9 ± 0.1	<0.05
Phosphoenolpyruvate	(3)	0.33 ± 0.04	0.16 ± 0.03	<0.005
ATP	(3)	10.5 ± 0.8	6.4 ± 0.8	<0.005
Glucose	(3)	206 ± 19	124 ± 26	<0.05

* Cells were preincubated with or without 0.3 mM pargyline for 40 min before addition of lactate (final 10 mM) to all vials. Reaction was stopped after a further 90 min (see Materials and Methods). Values for glucose denote the accumulation in the medium at the end of the incubation; other metabolites were measured in cells only. Results are means ± S.E.M. with the number of independent observations in parentheses. Significance between means was tested with the paired *t*-test [32].

Table 5. Inhibition of ureogenesis in liver cells from starved rats by pargyline and tranylcypromine*

Substrate	Inhibitor	Rate (nmoles urea/mg dry wt/hr)	P (vs control)
Alanine		143 ± 16 (3)	
Alanine	Pargyline (0.6 mM)	71 ± 13 (3)	<0.005
Alanine	Tranylcypromine (1.5 mM)	78 ± 15 (3)	<0.05
Glutamine		174 ±16 (4)	
Glutamine	Pargyline (0.6 mM)	138 ± 18 (4)	NS
Glutamine	Tranylcypromine (1.5 mM)	84 ± 17 (4)	<0.005

* Cells were incubated for 90 min with substrates (10 mM) with or without inhibitors. Rates were measured between 45 and 90 min of incubation, and are expressed as means ± S.E.M. for the number of independent observations shown in parentheses. Significance between means was tested with the paired *t*-test [32]. NS, not significant.

observations with specific effects on other enzymes such as aldehyde dehydrogenase (see introduction for references).

The pattern of the inhibition of glucose synthesis (slight inhibition from glycerol and fructose, decreased contents of aspartate, malate and phosphoenolpyruvate) is reminiscent of that seen with hypoglycin A and its metabolite, methylenecyclopropylpyruvate [39]. In this case, the locus of inhibition appears to be primarily butyryl CoA dehydrogenase, the consequences of this being a block of β-oxidation and derangement of CoA metabolism [39, 40]. The MAOIs tested in our experiments, however, neither inhibited β-oxidation nor altered the distribution of CoA metabolites.

The MAOI effects on gluconeogenesis were found to be consistent in cells from fed, fasted and diabetic rats and in those incubated with fatty acid. This similarity extended both to rats and guinea pigs and across the spectrum of inhibitors, irrespective of their pharmacological MAO specificity.

These rather generalized effects are consistent with the lowering of cellular ATP contents and with the apparent, but somewhat limited, uncoupling of mitochondrial respiration. The mechanism of this effect is a matter for conjecture but a few points may be made. First, it is unlikely that the inhibitors need to be metabolized to be effective. The reversibility of their actions and the slowness of their known metabolism [10] are evidence of this. Second, recent studies have shown that MAOIs may interact with membranous systems, and that this interaction with lipid and the consequent changes in membrane fluidity can result in changes in membrane-bound enzyme activities [41, 42] and in general membrane protein conformation [43]. Such effects occur at concentrations higher than those which inhibit MAO and similar to those in the experiments reported here, and are readily reversible [43]. Both clorgyline [44, 45] and pargyline [46] bind to mitochondrial membranes and this binding may well result in changes in the coupling efficiency of oxidative phosphorylation.

The concentrations used in our experiments are generally considerably higher than those encountered in patients treated with MAOIs. Nevertheless, the known hepatotoxicity of these drugs may be, at least in part, explicable by the effects observed here and produced by local high concentrations and accumulations.

Acknowledgements—This work was supported by the British Diabetic Association and the Medical Research Council. We are indebted to Dr. M. B. Youdim, Roche Products Ltd. and Smith, Kline & French Labs for their gifts of drugs.

REFERENCES

1. L. S. Goodman and A. Gilman (Eds.), *The Pharmacological Basis of Therapeutics*, 4th edn. MacMillan, London (1970).
2. R. R. Rando and J. De Mairena, *Biochem. Pharmac.* **23**, 463 (1974).

Table 6. Mitochondrial oxidation in the presence of pargyline, tranylcypromine and phenelzine*

Substrate	Inhibitor	Relative rate of O_2 uptake	P (vs control)
Malate plus pyruvate	Pargyline	1.95 ± 0.37 (5)	<0.05
Malate plus pyruvate	Tranylcypromine	1.70 ± 0.12 (3)	<0.05
Malate plus pyruvate	Phenelzine	2.01 ± 0.40 (3)	<0.05
Glutamate	Pargyline	2.53 ± 0.54 (5)	<0.05
Glutamine	Pargyline	1.69 , 2.70 (2)	
Succinate	Pargyline	1.83 ± 0.14 (3)	<0.005

* Mitochondria were prepared from fed rat liver and incubated as described in Materials and Methods. All substrates were 2 mM and inhibitors 1 mM. The results are the ratios of the rates (as nmoles O_2 uptake/mg protein) in the presence and absence of inhibitor. Values are means ± S.E.M. for the number of independent observations given in parentheses. Statistical significance was assessed by the paired *t*-test [32].

3. M. D. Houslay and K. F. Tipton, *Biochem. Pharmac.* **24**, 429 (1975).
4. D. M. Valeriano, E. S. Vesell, J. T. Stevens and S. L. Rudnick, *Pharmacology* **17**, 113 (1978).
5. S. D. Nelson, J. R. Mitchell, W. R. Snodgrass and J. A. Timbrell, *J. Pharmac. exp. Ther.* **206**, 574 (1978).
6. D. Dembiec, D. MacNamee and G. Cohen, *J. Pharmac. exp. Ther.* **197**, 332 (1976).
7. M. E. Lebsack, D. R. Petersen, A. C. Collins and A. D. Anderson, *Biochem. Pharmac.* **26**, 1151 (1977).
8. A. I. Cederbaum and E. Dicker, *Archs Biochem. Biophys.* **193**, 551 (1979).
9. M. L. Aizenstein, L. F. Medeiros, L. O. Medeiros and A. C. Zanini, *Gen. Pharmac.* **8**, 217 (1977).
10. A. Pletscher, K. F. Gey and W. P. Burkard, in *Handbook of Experimental Pharmacology* (Ed. V. Erspamer), Vol. 19, p. 38. Academic Press, London (1966).
11. R. Bressler, M. Vargas-Cordon and H. E. Lebovitz, *Diabetes* **17**, 617 (1968).
12. J. J. Gagliardino, R. E. Hernandez, R. R. Rodriguez and H. C. Lauri, *Am. J. Physiol.* **219**, 314 (1970).
13. L. A. Frohman, *Diabetes* **20**, 266 (1971).
14. W. Z. Potter, D. S. Zaharko and L. V. Beck, *Diabetes* **18**, 537 (1969).
15. I. Lundquist, R. Ekholm and I. E. Ericson, *Diabetologia* **7**, 414 (1971).
16. H. Aleyassine and S. H. Lee, *Endocrinology* **89**, 125 (1971).
17. H. Aleyassine and S. H. Lee, *Am. J. Physiol.* **222**, 565 (1972).
18. J. M. Feldman and B. Chapman, *Diabetologia* **11**, 487 (1975).
19. P. I. Adnitt, *Diabetes* **17**, 628 (1968).
20. L. Wickstrom and K. Pettersson, *Lancet* **2**, 995 (1964).
21. A. M. Barrett, *J. Pharm. Pharmac.* **17**, 19 (1965).
22. A. J. Cooper and G. W. Ashcroft, *Lancet* **1**, 407 (1966).
23. F. P. Underhill, *J. biol. Chem.* **10**, 159 (1911).
24. S. R. Fortney, D. A. Clark and E. Stein, *J. Pharmac. exp. Ther.* **156**, 277 (1967).
25. P. D. Ray, R. L. Hanson and H. A. Lardy, *J. biol. Chem.* **245**, 690 (1970).
26. R. Haeckel and M. Oellerich, *Eur. J. clin. Invest.* **7**, 393 (1977).
27. K. R. F. Elliott, R. Ash, D. M. Crisp, C. I. Pogson and S. A. Smith, in *Use of Isolated Liver Cells and Kidney Tubules in Metabolic Studies* (Eds. J. M. Tager, H.-D. Söling and J. R. Williamson), pp. 139–143. North Holland, Amsterdam (1976).
28. K. R. F. Elliott and C. I. Pogson, *Molec. cell. Biochem.* **16**, 23 (1977).
29. S. A. Smith, K. R. F. Elliott and C. I. Pogson, *Biochem. J.* **176**, 817 (1978).
30. P. J. Randle, E. A. Newsholme and P. B. Garland, *Biochem. J.* **93**, 652 (1964).
31. J. B. Chappell and R. G. Hansford, in *Subcellular Components* (Ed. G. D. Birnie and S. M. Fox), p. 43. Butterworths, London (1969).
32. R. G. D. Steel and J. H. Torrie, *Principles and Procedures of Statistics*. McGraw-Hill, New York (1960).
33. J. P. Johnston, *Biochem. Pharmac.* **17**, 1285 (1968).
34. J. Knoll and K. Magyar, *Adv. Biochem. Psychopharmac.* **5**, 383 (1972).
35. N. H. Neff and H.-Y. T. Yang, *Life Sci.* **14**, 2061 (1974).
36. D. R. Nelson and A. K. Huggins, *Analyt. Biochem.* **59**, 46 (1974).
37. J. P. Mapes and R. A. Harris, *Fedn. Eur. biochem. Soc. Lett.* **51**, 80 (1975).
38. L. Oreland, H. Kinemuchi and B. Y. Yoo, *Life Sci.* **13**, 1533 (1973).
39. E. A. Kean and C. I. Pogson, *Biochem. J.* **182**, 789 (1979).
40. D. Billington, H. Osmundsen and H. S. A. Sherratt, *Biochem. Pharmac* **27**, 2879 (1978).
41. M. D. Houslay, *J. Pharm. Pharmac.* **29**, 664 (1977).
42. M. D. Houslay, R. W. Palmer and R. J. S. Duncan, *J. Pharm. Pharmac.* **30**, 711 (1978).
43. S. P. Baker and B. A. Hemsworth, *Biochem. Pharmac.* **27**, 2327 (1978).
44. C. H. Williams and J. Lawson, *Biochem. Pharmac.* **24**, 1889 (1975).
45. C. J. Fowler and B. A. Callingham, *J. Pharm. Pharmac.* **30**, 304 (1978).
46. R. McCauley, *Biochem. Pharmac.* **25**, 2214 (1976).