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INHIBITION OF ALDOSE REDUCTASE IN FIVE TISSUES OF THE STREPTOZOTOCIN-DIABETIC RAT

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Abstract—For 22 days, streptozotocin-diabetic and normal rats were intubated once daily with ICI 105552 (1-(3,4-dichlorobenzyl)-3-methyl-1,2-dihydro-2-oxoquinol-4-ylacetic acid, sodium salt: 50 mg/kg body weight) an inhibitor of aldose reductase (EC 1.1.1.21), the first enzyme of the sorbitol pathway. Treatment with ICI 105552 affected neither glycaemia nor tissue glucose nor inositol concentrations yet reduced significantly the abnormal accumulations in diabetes of sorbitol in the lens (70% reduction), sciatic nerve (86%) and seminal vesicles with coagulating glands (S.V.C.G., 55%). ICI 105552 had no effect upon sorbitol accumulated in the diabetic kidney but it reduced the level in controls by 43%. The compound reduced the accumulation of sorbitol in diabetic retina by 58% although variation was too great for the decrease to be significant statistically. Treatment with ICI 105552 produced small (≤ 11%) yet statistically significant increases in the weights of the kidneys, and both liver and kidney weight/100 g residual body weight but did not affect the weights of the body, lens, retina or S.V.C.G. The importance of these findings for the development of potentially chemotherapeutic aldose reductase inhibitors is discussed.

The two enzymes of the sorbitol pathway (Fig. 1), which converts glucose into sorbitol and then fructose, are aldose reductase (A.R.) and sorbitol dehydrogenase (S.D.H.). A.R. [1] has a K_m for glucose of 28.5 mM [2]; thus in diabetes, when the concentration of glucose rises in certain tissues during periods of inadequate glycaemic control, flux through the sorbitol pathway may become increased considerably. The increase may manifest itself in a number of ways, dependent upon the relative activities of A.R., S.D.H. and enzymes which catabolize fructose (Fig. 1).

In rodents, A.R. or S.D.H. have been demonstrated in the seminal vesicles [3, 4], lens [2, 3, 5–7], sciatic nerve [3, 4, 8], kidney [3, 5, 9] and retina [5, 10]. Uncontrolled diabetes results in the accumulation of fructose in seminal fluid [11], sorbitol and fructose in the lens [12, 13] and nerve [14], sorbitol in the kidney [15] and sorbitol [13, 16], fructose [13, 16], fructose-1-phosphate (F1P) [17] and lactate [18] in the retina. In diabetic man there can be an accumulation of fructose in seminal fluid [11] and of both fructose and sorbitol in lens [19] and nerve [20].

Pathologic changes have been demonstrated in the lens [12, 21], retina [22–24], kidney [25, 26] and peripheral nerve [27] of rodents with experimental or idiopathic diabetes. The changes are reminiscent of those found in diabetic-cataract, -retinopathy, -nephropathy and -autonomic neuropathy [28].

Aspects of pathogenesis consequent upon increased flux through the sorbitol pathway can be due to the osmotic effects of accumulated sorbitol [12, 15], or might result from the excessive production of lactate [29].

The present report concerns the effects of ICI 105552, an aldose reductase inhibitor (A.R.I.), upon the sorbitol pathway in five tissues of the rat, the compound's effect upon organ weights, and considers possible implications of the findings towards the development of potential chemotherapy for diabetic complications.

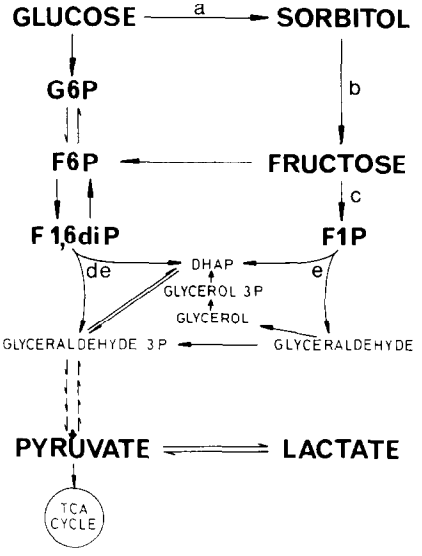

Fig. 1. The relationship of the sorbitol pathway to glycolysis. Key: (a) aldose reductase (EC 1.1.1.21); (b) sorbitol dehydrogenase (EC 1.1.1.14); (c) ketohexokinase (EC 2.7.1.3); (d) fructose-1,6-diphosphate aldolase (EC 4.1.2.13, isoenzyme A); (e) fructose-1-phosphate aldolase (EC 4.1.2.13, isoenzyme B); (DHAP) dihydroxyacetone phosphate and (T.C.A. cycle) tricarboxylic acid cycle.

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METHODS

Materials. The enzyme inhibitor, 1-(3,4-dichlorobenzyl)-3-methyl-1,2-dihydro-2-oxoquinol-4-ylacetic acid (ICI 105552, sodium salt) and a placebo, L-glutamic acid (sodium salt) were generously provided by ICI Pharmaceuticals Division, Alderley Park, Cheshire, U.K.

Animals and treatment. Male Wistar rats (250 g) were housed in wire-bottomed cages with a standard diet (MRC 41B) and water available *ad lib.* Rats were assigned randomly to 1 of 4 treatments: diabetic dosed with ICI 105552 (designated as treatment DA), diabetic dosed with the placebo (DB), control dosed with ICI 105552 (CA) and control dosed with placebo (CB).

Diabetes was induced by the injection of streptozotocin (75 mg/kg body weight) into the tail vein and was confirmed if 6 days later the fed-state blood glucose concentration exceeded 20 mM.

Daily dosage at 50 mg/kg body weight by gastric intubation with a solution (25 g/l) of either ICI 105552 or placebo as appropriate was begun on day 1, a week after the induction of diabetes. Rats were dosed for 22 days.

Preparation of tissues. At least 2 hr after their 22nd daily dose, two rats per day were anaesthetized deeply (Sagatal, May & Baker, Dagenham, U.K.). Tissues were processed separately for each rat. A tail-vein blood sample was deproteinized with $ZnSO_4$ then $Ba(OH)_2$ solutions prior to analysis for glucose (GOD-Perid, Boehringer Mannheim GmbH). Aqueous extracts were prepared from each of the five tissues to be analysed by GLC. for the concentrations of glucose, sorbitol, fructose and inositol. The extracts were prepared in a boiling-water bath (approx 20 min) from the following approximate amounts of tissue in the specified volume of distilled water containing α-methyl-D-mannoside (α-mm; Sigma, Poole, U.K.) as an internal standard for the GLC analyses: lens (30 mg), or sciatic nerve (70 mg) with 30 nmole α-mm in approx 1 ml; retinas (17 mg) with 6 nmole α-mm in approx 1 ml; part of both kidneys (500 mg) with 1.3 μmole α-mm in 5 ml; whole S.V.C.G. from controls (1.2 g) with 10.3 μmole α-mm in 2 ml; whole S.V.C.G. from diabetics (0.6 g) with 5.15 μmole α-mm in 1 ml. The resulting extracts were centrifuged briefly to sediment debris, deproteinized with $ZnSO_4$ then $Ba(OH)_2$ solutions, then stored at −20° [30].

GLC analysis. Aliquots of the stored extracts (lens or nerve 0.3 ml; retinas ≥ 0.6 ml; kidney 1 ml; S.V.C.G. 0.5 ml) were lyophilized and the residues were treated with Tri.Sil 'Z' (Pierce and Warriner, Chester, U.K.; 0.1 ml, 24 hr, room temp) to prepare trimethylsilyl (TMS) derivatives. Excess Tri.Sil 'Z' was hydrolysed on addition of water (2 ml) then the TMS-sugars were extracted into cyclohexane (0.25 ml, research grade, BDH, Poole, U.K.) and concentrated, by the evaporation of most of the cyclohexane in a stream of oxygen-free nitrogen. 1 μl of these cyclohexane extracts was injected into a column of SE-52 (2.5% w/w) on diatomite-C (J.J.'s (Chromatography) Ltd., King's Lynn, U.K.) at 190° in a Pye-Unicam GCD chromatograph. Sugars were identified by their retention times and quantitated against the internal standard by analysis of peak heights. Methods for the preparation and use of this system have been described previously [30, 31].

Statistics. Results were evaluated by two-way analysis of variance following a satisfactory result from Bartlett's test with either raw or appropriately transformed data [32]. Results are given as the mean ± S.E. with the No. of observations in parentheses and are described as significant only if $P < 0.05$ with a two-tailed test.

RESULTS

Terminal body and organ weights (Table 1)

22 days of dosage with ICI 105552 did not affect significantly the weight of the body, left lens, combined recoverable retina, liver or S.V.C.G. but increased the weight of the kidneys by 5–11% ($P < 0.02$). Expression of liver or kidney weight as a proportion of the residual body weight (R.B.W., e.g. non-liver body weight), thus reducing variation, revealed that the compound had increased both the liver weight/100 g R.B.W. ($P < 0.0002$) and kidney weight/100 g R.B.W. ($P = 0.010$). The differences were of less than 11%.

Diabetic rats had lower body weight ($P < 0.0001$) and S.V.C.G. weight ($P < 0.0001$) and non-significantly lower lens- and recoverable retina-weights. The livers of diabetic rats were not significantly lighter than those of controls and expressed as the proportion liver weight/100 g R.B.W. they were heavier ($P < 0.0001$). The absolute and proportional kidney weights of diabetic rats were increased (both $P < 0.0001$) above control values.

Blood glucose concentrations

The terminal blood glucose concns (mM) were: CA 7.0 ± 0.47 (6), CB 6.3 ± 0.32 (6), DA 26.9 ± 0.96 (7), DB 28.7 ± 1.27 (7). The A.R.I. had no significant effect upon the level of glycaemia.

GLC analyses (Table 2a–e)

Dosage with ICI 105552 did not affect significantly the concentrations of either glucose or inositol in any of the five tissues studied.

In the lens (Table 2a) diabetes effected a 16.7-fold increase (D vs C, $P < 0.0001$) in the concn of glucose, a 76-fold increase (DB vs CB, $P < 0.0001$) in the concn of sorbitol and a 9.6-fold increase ($P < 0.0001$) in that of fructose. Treatment with ICI 105552 gave a 70% reduction of the accumulation of sorbitol (DA vs DB, $P < 0.0001$) resulting in a concentration still 23 fold the normal level; no significant decrease in the concn of fructose occurred. Diabetes reduced the concentration of inositol in the lens by 85% (D vs C, $P < 0.0001$).

In the retina (Table 2b) diabetes effected a 7.9-fold increase (D vs C, $P < 0.0001$) in the concn of glucose, a 2.2-fold increase (D vs C, $P < 0.0002$) in the concn of sorbitol and a 2.1-fold increase (D vs C, $P < 0.0001$) in that of fructose. Treatment with ICI 105552 gave a 58% reduction of the accumulation of sorbitol in diabetic retina but variation was too great for the decrease to be significant statistically. This compound did not affect the levels of fructose in diabetic or normal retinas significantly although

Table 1. The effects of 22 days of dosage with ICI 105552 upon body and organ weights of normal and streptozotocin-diabetic rats

Treatment*	Terminal weights (means ± S.E.) Body (g)	Left lens (mg)	Retina† (mg)	Liver (g)	Liver/100 g R.B.W. (g)‡	Kidney (g)	Kidney/100 g R.B.W. (g)‡	S.V.C.G. (g)§
CA	361 ± 14.6	36 ± 2.4	19 ± 2.3	14.5 ± 0.77	4.18 ± 0.084	2.40 ± 0.087	0.67 ± 0.012	1.16 ± 0.142
CB	364 ± 18.2	36 ± 1.4	19 ± 1.5	13.2 ± 0.56	3.77 ± 0.110	2.28 ± 0.109	0.63 ± 0.030	1.25 ± 0.085
DA	270 ± 11.2	30 ± 3.6	15 ± 1.3	12.5 ± 0.62	4.87 ± 0.185	3.23 ± 0.111	1.22 ± 0.061	0.65 ± 0.079
DB	261 ± 10.7	30 ± 2.2	15 ± 1.3	11.8 ± 0.60	4.73 ± 0.132	2.91 ± 0.138	1.13 ± 0.025	0.53 ± 0.107

* Abbreviation of treatment: CA = control dosed with ICI 105552 (No. of observations = 6); CB = control dosed with placebo (6); DA = diabetic dosed with ICI 105552 (7) and DB = diabetic dosed with placebo (7).
† Combined recoverable weight.
‡ Residual body weight.
§ Seminal vesicles with coagulating glands.

the mean levels were lower in each case. Diabetes did not affect the concentration of inositol in the retina significantly.

In the sciatic nerve (Table 2c) diabetes effected a 6.3-fold increase (D vs C, $P < 0.0001$) in the concentration of glucose, a 10.3-fold increase (DB vs CB, $P < 0.0001$) in the concentration of sorbitol and a 10.2-fold increase (DB vs CB, $P < 0.0001$) in that of fructose. Treatment with ICI 105552 gave highly significant (DA vs DB, $P < 0.0001$) reductions in the accumulations of both sorbitol (86% reduction) and fructose (70% reduction) resulting in concentrations respectively 2.3-fold and 3.8-fold the normal (CB) levels. Diabetes did not affect the concentration of inositol in the nerve significantly.

In the kidney (Table 2d) diabetes effected a 7.4-fold increase (D vs C, $P < 0.0001$) in the concentration of glucose, a 3.2-fold increase (DB vs CB, $P < 0.0001$) in the concentration of sorbitol and an 8.1-fold increase (D vs C, $P < 0.0001$) in that of fructose. Treatment with ICI 105552 had no significant effect upon the accumulations of either sorbitol or fructose, yet in the control rats the A.R.I. reduced the basal level of sorbitol by 43% (CA vs CB, $P < 0.05$). Diabetes did not affect the concentration of inositol in the kidney significantly.

In the S.V.C.G. (Table 2e) diabetes effected a 10.7-fold increase (D vs C, $P < 0.01$, Studentized range [33]) in the concentration of glucose, a 2-fold increase (DB vs CB, $P < 0.0005$) in the concentration of sorbitol and a 2.1-fold increase (DB vs CB, $P < 0.0001$) in that of fructose. Treatment with ICI 105552 gave 55% and 54% reductions respectively of the accumulations effected by diabetes; for sorbitol the decrease was significant (DA vs DB, $P < 0.05$) and for both sorbitol and fructose the A.R.I.-treated diabetic levels were not significantly greater than normal (CB). Diabetes reduced the concentration of inositol in the S.V.C.G. by 14% (D vs C, $P < 0.05$).

DISCUSSION

The observations in the diabetic lens that ICI 105552 gave a 70% reduction of sorbitol accumulation but no significant decrease in fructose concentration and that the compound did not reduce the weight of the diabetic rat lens (which swells during cataractogenesis) might well explain why in an 11 month experiment [30*], cataractogenesis was retarded considerably, rather than prevented completely, by ICI 105552 at 50 mg/kg body weight daily.

The magnitude of the effects of diabetes upon the sorbitol pathway of the rat retina were very similar to those reported by other workers using GLC [13] or enzymatic [16] analyses. The variation in the concentration of sorbitol in the retina was too great for the apparent 58% reduction by ICI 105552 of the accumulation of sorbitol in diabetic retina to be of statistical significance. It remains unknown whether flux through the sorbitol pathway to lactate (Fig. 1) may have been reduced in the inhibitor-treated diabetic retina although a subsequent experiment indicated that this might occur [30]. Hutton *et al.* [34]

* Submitted for publication.

Table 2. The effects of 22 days of dosage with ICI 105552 upon the concentrations of sugars and polyols in tissues of normal and streptozotocin-diabetic rats

	Treatment†	Concentration of substance* Glucose	Sorbitol	Fructose	Inositol
(a)					
Lens	CA (5)	310 ± 63	290 ± 77	1080 ± 245	1050 ± 240
	CB (6)	310 ± 20	360 ± 50	780 ± 72	1310 ± 99
	DA (7)	5140 ± 274	8390 ± 1030	7980 ± 542	200 ± 31
	DB (7)	5230 ± 667	27,350 ± 2180	9640 ± 847	150 ± 12
(b)					
Retina	CA (6)	930 ± 179	210 ± 43	460 ± 164	670 ± 67
	CB (4)	1000 ± 171	290 ± 59	530 ± 126	660 ± 77
	DA (6)	8010 ± 720	430 ± 51	900 ± 95	740 ± 80
	DB (6)	7170 ± 773	620 ± 110	1130 ± 85	910 ± 143
(c)					
Nerve	CA (6)	1560 ± 131	140 ± 27	620 ± 60	2390 ± 170
	CB (4)	1570 ± 122	150 ± 37	630 ± 75	2510 ± 111
	DA (7)	9380 ± 725	340 ± 33	2400 ± 250	2150 ± 86
	DB (7)	10,240 ± 367	1550 ± 71	6430 ± 410	2340 ± 101
(d)					
Kidney	CA (5)	4150 ± 364	160 ± 22	830 ± 194	6720 ± 786
	CB (6)	4780 ± 384	280 ± 58	870 ± 60	8090 ± 644
	DA (7)	32,200 ± 2060	880 ± 89	5710 ± 509	7630 ± 427
	DB (6)	34,000 ± 2750	907 ± 79	8210 ± 1130	7540 ± 470
(e)					
S.V.C.G.‡	CA (6)	1340 ± 168	220 ± 44	2040 ± 405	30,400 ± 1430
	CB (6)	1420 ± 175	300 ± 56	2610 ± 449	24,300 ± 2140
	DA (5)	16,200 ± 6630	430 ± 74	3950 ± 817	23,000 ± 2640
	DB (7)	13,700 ± 3070	590 ± 52	5540 ± 309	23,800 ± 2070

* nmole per wet weight g of tissue: mean ± S.E.

† Abbreviation of treatment: CA = control dosed with ICI 105552; CB = control dosed with placebo; DA = diabetic dosed with ICI 105552 and DB = diabetic dosed with placebo.

No. of observations given in parentheses.

‡ Seminal vesicles with coagulating glands.

dosed diabetic rats orally with a different A.R.I. (3,3'-tetramethylene glutarate) at approximately 5 g/kg/day and showed it to be ineffective in retina and lens because of its poor ability to penetrate biological membranes.

The fact that in the present experiment ICI 105552 had considerable effects upon the lens of diabetic rats is evidence that this compound was available and active within the eye and suggests that the lower mean level of sorbitol in the retina of ICI 105552-dosed rats may have been due to the treatment. The development of retinal lesions over 11 months in similar diabetic rats could however, not be shown to be affected by this treatment [30].

Treatment with ICI 105552 for 22 days clearly (Table 2d) had no significant effect upon the accumulation of sorbitol in the diabetic rat kidney; supporting a similar finding in an 11 month experiment [30] although interpretation of that experiment was complicated by the development of long-term pathologic changes in diabetic kidneys. The fact that the A.R.I. reduced the concentration of sorbitol in the kidneys of control rats but not diabetics might be connected with the presence of marked polyuria in the diabetic rats.

The effects of ICI 105552 upon organ weights (Table 1) were specific to the liver and kidneys, thus it is possible that drug metabolizing enzyme systems had to some extent been induced [35]. The efficacy of treatment with this compound was however, not noticeably impaired in the long-term; the percentage reductions of the accumulations of sorbitol and fructose in diabetic sciatic nerve were the same after 8–11 months [30] as at 22 days.

Inhibition of aldose reductase *in vivo* with ICI 105552 was much more effective, as assessed by sorbitol and fructose levels, in the sciatic nerve, lens and S.V.C.G. than in the retina or kidney. It is interesting to speculate that these differences might in part be due to dissimilarities between the target enzyme in certain tissues. Aldose reductases isolated from bovine retina [36] and rat kidney [9] are reported not to be activated by sulphate ions whereas those from bovine lens [37], sheep seminal vesicles [1, 38], sheep [38] and human [39] placenta, and rat pancreas [40] are. This dissimilarity might indicate the presence of different sites for modulation of the enzyme's activity and so it is conceivable that aldose reductase from one tissue may not be as sensitive to a particular inhibitor as aldose reductase from another tissue. It is known that certain A.R.I.s are more effective against rat lens A.R. than against human placental A.R. and vice versa [41]. It may therefore be prudent to ensure that the more effective inhibitors under development are effective against the appropriate human enzyme wherever possible.

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REFERENCES

1. H. G. Hers, *Biochim. biophys. Acta* **37**, 120 (1960).
2. P. K. Pottinger, *Biochem. J.* **103**, 663 (1967).
3. R. S. Clements, J. P. Weaver and A. I. Winegrad, *Biochem. biophys. Res. Commun.* **37**, 347 (1969).
4. M. A. Ludvigson and R. L. Sorenson, *Diabetes* **29**, 438 (1980).
5. M. A. Ludvigson and R. L. Sorenson, *Diabetes* **29**, 450 (1980).
6. S. Hayman, M. F. Lou, L. O. Merola and J. H. Kinoshita, *Biochim. biophys. Acta* **128**, 474 (1966).
7. J. G. Collins and C. N. Corder, *Invest. Ophthal.* **16**, 242 (1977).
8. K. H. Gabbay and J. B. O'Sullivan, *Diabetes* **17**, 239 (1968).
9. C. N. Corder, J. G. Collins, T. S. Brannan and J. Sharma, *J. Histochem. Cytochem.* **25**, 1 (1977).
10. Y. C. Hamlett, Ph.D. thesis, University of London (1978).
11. T. Mann and V. Parsons, *Biochem. J.* **46**, 440 (1950).
12. J. H. Kinoshita, *Invest. Ophthal.* **13**, 713 (1974).
13. J. C. Hutton, P. J. Schofield, J. F. Williams and F. C. Hollows, *Aust. J. exp. Biol. med. Sci.* **52**, 361 (1974).
14. K. H. Gabbay, L. O. Merola and R. A. Field, *Science, Wash.* **151**, 209 (1966).
15. K. H. Gabbay, *A. Rev. Med.* **26**, 521 (1975).
16. H. Heath and Y. C. Hamlett, *Diabetologia* **12**, 43 (1976).
17. Y. C. Hamlett and H. Heath, *IRCS Med. Sci.* **5**, 510 (1977).
18. H. Heath, S. S. Kang and D. Phillipou, *Diabetologia* **11**, 57 (1975).
19. A. Pirie and R. Van Heyningen, *Exp. Eye Res.* **3**, 124 (1964).
20. J. D. Ward, R. W. R. Baker and B. H. Davis, *Diabetes* **21**, 1173 (1972).
21. S. D. Varma, A. Mizuno and J. H. Kinoshita, *Science, Wash.* **195**, 205 (1977).
22. J. Babel and P. Leuenberger, *Albrecht v. Graefes Arch. Ophthal.* **189**, 191 (1974).
23. D. Papachristodoulou, H. Heath and S. S. Kang, *Diabetologia* **12**, 367 (1976).
24. R. Boot-Handford and H. Heath, *Metabolism* **29**, 1247 (1980).
25. R. P. Boot-Handford and H. Heath, *Br. J. exp. Path.* **62**, 398 (1981).
26. J.-W. Yoon, M. M. Rodrigues, C. Currier and A. L. Notkins, *Nature, Lond.* **296**, 566 (1982).
27. W. W. Schlaepfer, G. C. Gerritsen and W. E. Dulin, *Diabetologia* **10**, 541 (1974).
28. H. Keen and R. J. Jarrett (Eds.), *Complications of Diabetes*. Edward Arnold, London (1975).
29. G. Imre, *Albrecht v. Graefes Arch. Ophthal.* **201**, 263 (1977).
30. R. Poulsom, Ph.D. thesis, University of London (1982).
31. R. Boot-Handford and H. Heath, *Biochem. Pharmac.* **30**, 3065 (1981).
32. G. W. Snedecor and W. G. Cochran, *Statistical Methods*, 6th edition, pp. 299–338. Iowa State University Press, Iowa (1969).
33. P. Armitage, *Statistical Methods in Medical Research*, pp. 205–206. Blackwell, Oxford (1971).
34. J. C. Hutton, P. J. Schofield, J. F. Williams and F. C. Hollows, *Biochem. Pharmac.* **23**, 2991 (1974).
35. A. H. Conney, *Pharmac. Rev.* **19**, 317 (1967).
36. R. N. Frank, S. S. Chang and K. P. Mikus, *Diabetes* **27**, Suppl 2, Abstract 180 (1978).
37. S. Hayman and J. H. Kinoshita, *J. biol. Chem.* **240**, 877 (1965).
38. T. Håstein and W. Velle, *Biochim. biophys. Acta* **178**, 1 (1969).
39. R. S. Clements and A. I. Winegrad, *Biochem. biophys. Res. Commun.* **47**, 1473 (1972).
40. K. H. Gabbay and W. J. Tze, *Proc. natn. Acad. Sci. U.S.A.* **69**, 1435 (1972).
41. J. H. Kinoshita, S. Fukushi, P. Kador and L. O. Merola, *Metabolism* **28**, Suppl 1, 462 (1979).