Effects of glucose-containing peritoneal-dialysis solutions on rates of lipogenesis *in vivo* in the liver, brown and white adipose tissue of chronic uraemic rats

Rita A. KLIM and Dermot H. WILLIAMSON

Metabolic Research Laboratory, Nuffield Department of Clinical Medicine, Radcliffe Infirmary, Woodstock Road, Oxford OX2 6HE, U.K.

(Received 4 March 1983/Accepted 3 May 1983)

1. Chronic uraemic rats had decreased food intake, and this was accompanied by decreased weight of the epididymal fat-pads and interscapular brown adipose tissue. Normal rats whose food intake was restricted to an amount similar to that of the uraemic rats showed similar decreases in weight of the adipose-tissue depots. In addition, the food-restricted rats had decreased liver weight compared with normal or uraemic rats. 2. The basal rate of lipogenesis was decreased in liver and epididymal fat-pads of food-restricted and uraemic rats and in interscapular brown adipose tissue of uraemic rats. 3. Administration of a low-glucose-containing (1.36%) peritoneal-dialysis solution slightly increased lipogenesis in liver of uraemic rats, but had no significant effect in epididymal fat-pads. For brown fat, the rate of lipogenesis was increased in normal, food-restricted and uraemic groups, but the values for the last group were 4–5-fold lower than for the food-restricted or control groups. 4. A high-glucose-containing (3.86%) peritoneal-dialysis solution gave similar rates of lipogenesis in liver, epididymal fat-pads and brown fat of all three groups, but for brown fat moderately uraemic rats showed a considerably lower rate of lipogenesis than did mildly uraemic rats. 5. The basal plasma insulin concentration was lower in the food-restricted (50%) and uraemic (70%) groups than in the control group. The low-glucose peritoneal-dialysis solution increased plasma insulin to control values in the food-restricted rats, but had no significant effect on plasma insulin in the uraemic rats, despite a significant increase in blood glucose in this group. 6. It is concluded that there is an impairment of the lipogenic response to intraperitoneal glucose loads in interscapular brown adipose tissue of uraemic rats, and that this is not due to the accompanying decrease in food intake. The hypoinsulinaemia may be an important factor. The possible relevance of this finding to the obesity observed in some uraemic patients treated by peritoneal dialysis with glucose-containing solutions is discussed.

Chronic uraemia in the human is characterized by a number of metabolic abnormalities, including carbohydrate intolerance (Hampers *et al.*, 1970; DeFronzo *et al.*, 1973; Bagdade, 1975; DeFronzo & Alverstrande, 1980), insulin resistance (Westervelt, 1969; Roth *et al.*, 1973), raised plasma glucagon (Bilbrey *et al.*, 1974; Sherwin *et al.*, 1976) and hypertriglyceridaemia (Bagdade, 1968, 1970; Bierman, 1970). Uraemic patients undergoing continuous ambulatory peritoneal dialysis for their condition receive large amounts of glucose (125–270 g per day; Lindholm *et al.*, 1979) via the dialysis

solutions. Studies on glucose absorption from the dialysis solution have shown that substantial quantities are absorbed (70–80%; Anderson & Bergström, 1971; Brown *et al.*, 1973; Lindholm *et al.*, 1979; Grodstein *et al.*, 1981), and this excessive absorption of carbohydrate may contribute to the hypertriglyceridaemia and obesity in some patients on long-term continuous ambulatory peritoneal dialysis (Nolph & Prowant, 1979; DeSanto *et al.*, 1979).

Experimentally induced uraemia in the rat has many of the metabolic abnormalities commonly

associated with the condition in humans (Chantler *et al.*, 1974; Soman & Felig, 1977; Bagdade *et al.*, 1978). In the present work we have investigated the effects of glucose-containing peritoneal-dialysis solutions on the rates of lipogenesis *in vivo* in liver, brown and white adipose tissue of control rats and rats made chronically uraemic by surgical removal of five-sixths of the total kidney mass. As uraemic rats show anorexia and decreased growth (Chantler *et al.*, 1974; Diaz *et al.*, 1975; Adelman & Holliday, 1977), the results have also been compared with those for a group of control rats subjected to a similar degree of underfeeding as the uraemic animals.

Experimental

Materials

Animals. Male Wistar rats weighing 213 ± 35 g (66) at time of death were used; there were no significant differences between the mean body weights of the experimental groups. They were maintained on a chow diet (PRM diet; E. Dixon and Sons Ltd., Ware, Herts., U.K.) and water *ad libitum* in an animal house with lights on from 08:00 to 20:00h. Chronic uraemia was induced by the five-sixths bilateral-nephrectomy technique (Morrison, 1966). Uraemic rats were allowed approx. 14 days to recover from the last operation before use. Daily food intake was measured for 4–5 days before the experiments in both the control and uraemic rats. A second control group of rats was food-restricted to 14 g per day (about two-thirds of normal intake) for 10–11 days to examine the effects of anorexia on lipogenesis in normal rats.

Peritoneal-dialysis solutions. Two commercial peritoneal-dialysis solutions were used: DIANEAL 1.36% [Travenol Laboratories, Thetford, Norfolk, U.K.; composition (per litre); 13.6 g of glucose, 5.6 g of NaCl, 0.15 g of $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 0.26 g of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ and 5.0 g of sodium lactate] and DIANEAL 3.86% (as above, but containing 3.86% glucose instead of 1.36%). It should be noted that both peritoneal-dialysis solutions, in addition to glucose, contain lactate (0.5%), which can also act as a lipogenic substrate. Thus, any discussion in this paper of the lipogenic effect of these solutions refers to the additive effects of glucose plus lactate, and not just that of glucose alone. In addition, 0.9% NaCl was used as a control dialysis solution.

Biochemicals. All biochemicals were obtained from Boehringer Corp. (London), Lewes, Sussex, U.K.

Methods

Lipogenesis *in vivo*. The rates of lipogenesis *in vivo* were measured by incorporation of $^3\text{H}_2\text{O}$ into saponified tissue lipids (Jungas, 1968). All experiments were performed between 08:00 and 12:00h. A

sample of blood was taken from the tail vein before the start of the experiment for the measurement of basal blood glucose. Rats were given an intraperitoneal injection of 20 ml of either one of the DIANEAL solutions or of 0.9% NaCl. After 60 min $^3\text{H}_2\text{O}$ was injected (4 mCi in 0.4 ml, intraperitoneally) and then 55 min later the rat was anaesthetized with Nembutal (0.1 ml of a 60 mg/ml solution per 100 g body wt.). At 60 min after intraperitoneal $^3\text{H}_2\text{O}$ administration, rats were dissected and the epididymal fat-pads, interscapular brown fat and liver were removed and weighed. Samples of these tissues were taken for determination of ^3H incorporation into saponified lipids (Stansbie *et al.*, 1976). Arterial blood was collected from the abdominal aorta into a heparinized tube for determination of specific radioactivity of plasma. Another sample of arterial blood was deproteinized with HClO_4 and, after removal of denatured protein by centrifugation (3000 g for 5 min) and of excess HClO_4 with KOH, the neutral supernatant fluid was used for determination of metabolites.

Metabolite determinations. Blood glucose (Slein, 1963) was determined enzymically. Blood urea nitrogen was determined colorimetrically with a commercial kit (urea nitrogen, catalogue no. 640; Sigma London Chemical Co., Poole, Dorset, U.K.). Plasma insulin was measured by radioimmunoassay with charcoal phase separation as described by Albano *et al.* (1972).

Results and discussion

Classification of uraemic rats

The mean blood urea nitrogen of the whole group of uraemic rats was 68.7 ± 37.1 mg % ($\pm$ S.D.; $n = 26$), which was 5 times that of the control animals (13.0 ± 5.7 ; $n = 9$). Uraemic rats are often subdivided on the basis of their blood urea values into mild, moderate or severe groups, with values of 40–80, 100–200 and >200 mg% respectively (Ormerod & Miller, 1980). However, this method of classification makes no allowance for the fact that uraemia decreases food intake (Table 1; Diaz *et al.*, 1975; Adelman & Holliday, 1977), and therefore a more severely ill animal may have a blood urea concentration similar to that of a less severely ill animal with a higher food intake. This view is supported by the negative correlation between the blood urea nitrogen and food intake ($r = 0.65$; $P < 0.001$; $n = 26$) in our group of uraemic rats. To allow for effects of food intake on blood urea we have calculated the ratio of blood urea nitrogen (in mg %) to food intake (g/day) for each rat. Rats with a ratio of above 2.5 were allocated to the 'moderate' group and those below to the 'mild' group. The blood-urea-nitrogen/food-intake ratio of normal rats was 0.63.

Table 1. *Tissue weights and food intake of experimental animals*

For full details see the Experimental section. The uraemic rats were subdivided on the basis of blood urea-nitrogen/food-intake ratio. Rats with a ratio of <2.5 were classified as mild uraemics, and those with a ratio of >2.5 as moderate uraemics. The results are mean values $\pm$ s.d., with the numbers of observations in parentheses. Values that are significantly different from the control rats are indicated: * $P < 0.05$; ** $P < 0.005$.

Experimental group	Blood urea nitrogen (mg %)	Food intake (g/day)	Liver (g)	Epididymal fat-pads (g)	Interscapular brown adipose tissue (g)
Control	13.0 $\pm$ 5.7 (9)	20.7 $\pm$ 2.2 (8)	9.26 $\pm$ 1.42 (35)	1.35 $\pm$ 0.50 (50)	0.17 $\pm$ 0.051 (50)
Food-restricted	14.7 $\pm$ 2.6 (12)	14.0 (18)	7.44 $\pm$ 1.07 (18)*	1.03 $\pm$ 0.30 (18)	0.11 $\pm$ 0.025 (18)**
All uraemics	68.7 $\pm$ 37.1 (26)**	16.5 $\pm$ 3.3 (26)**	9.09 $\pm$ 1.55 (26)	1.07 $\pm$ 0.40 (26)*	0.11 $\pm$ 0.042 (26)**
Mild uraemics	40.2 $\pm$ 7.5 (8)**	19.8 $\pm$ 2.1 (8)	9.45 $\pm$ 1.40 (8)	1.50 $\pm$ 0.43 (8)	0.13 $\pm$ 0.026 (8)*
Moderate uraemics	81.4 $\pm$ 38.1 (18)**	15.0 $\pm$ 2.5 (18)**	8.94 $\pm$ 1.63 (18)	0.88 $\pm$ 0.19 (18)**	0.11 $\pm$ 0.047 (18)**

Food intake and tissue weights

The food intake of the whole uraemic group was decreased by about 20% compared with control rats. Within the entire uraemic group, mildly uraemic rats showed about a 5% decrease, which was not statistically significant, and moderately uraemic rats consumed 30% less than normal rats (Table 1).

To examine the effects of this degree of decreased food intake, a group of normal rats were restricted to 14.0 g of food per day, which was slightly lower (by 1.0 g) than the mean daily food intake of the moderate-uraemic group (Table 1). Uraemia caused no significant change in mean liver weight, but food restriction did (20%; $P < 0.005$). The epididymal fat-pad weights were decreased (21%) in the whole uraemic group, but this effect was entirely due to an even greater decrease in the rats with moderate uraemia (35%; $P < 0.005$). The loss in weight of epididymal fat-pads may in part be due to the depressed activity of lipoprotein lipase reported in acute uraemia (Ransom *et al.*, 1981) and to the lower activity of lipogenesis (Table 2). It has been reported that food restriction (by 35%) causes hypotrophy of interscapular brown adipose tissue of male Sprague-Dawley rats (Rothwell & Stock, 1982). The weight of interscapular brown adipose tissue was decreased to a similar extent in the food-restricted and whole uraemic groups in the present study (Table 1); again the moderate-uraemic group showed the greatest decrease in tissue weight.

Rates of lipogenesis

The basal rates of lipogenesis (with saline injections) were similar in livers of food-restricted and uraemic rats and were about 50% lower than the hepatic rate for control rats (Table 2). In epididymal fat-pads, food restriction and uraemia also resulted in lower basal lipogenesis (decreases of 85 and 74% respectively). In contrast, food restriction had no significant effect on basal lipogenesis in interscapular brown adipose tissue, whereas the rate was decreased by 80% in uraemic rats.

The low-glucose-containing peritoneal-dialysis solution (DIANEAL 1.36%) slightly increased lipogenesis in liver of uraemic rats, but had no such effect in livers of control or food-restricted rats. In addition, this solution had no significant effect on lipogenesis in epididymal fat-pads of control, food-restricted or uraemic groups, but it approximately doubled the rate in brown fat in all three groups (Table 2). The response of the uraemic group, however, was still 75–80% lower compared with the control and food-restricted groups. A differential sensitivity of the rate of lipogenesis in white and brown adipose tissue to an oral load of glucose has been observed in normal rats (Agius & Williamson, 1980).

With the high-glucose-containing peritoneal-dialysis solution (DIANEAL 3.86%), the rate of hepatic lipogenesis was increased to a similar extent (about 250%) in the food-restricted and uraemic groups compared with the basal rates, whereas there was no significant increase in the control group (Table 2). Similarly, with epididymal fat-pads the DIANEAL 3.86% solution caused no significant change in lipogenesis in the control group, but large increases in both the food-restricted (15-fold) and uraemic (3.5-fold) groups (Table 2); however, the rate for the uraemic group was still 50% below that for the control group. The rate of lipogenesis in interscapular brown adipose tissue was increased to similar values by DIANEAL 3.86% in all three groups of rats. For the uraemic rats this result appeared anomalous because of the apparent impairment of basal lipogenesis and of the response to the low-glucose-containing solution in this tissue. Division of the whole uraemic group into mild and moderate uraemia (see above) gave rates of lipogenesis in brown adipose tissue in response to DIANEAL 3.86% of 92.5 ± 47.7 (5) for mild and 21.1 ± 10.4 (5) μmol of ^3H incorporated into saponified lipid/h per g wet wt. of tissue for moderate uraemics, suggesting a dependence of the impairment in brown adipose tissue on the severity of uraemia. Food restriction alone caused no

Table 2. *Effects of chronic uraemia on rates of lipogenesis in vivo in liver, epididymal fat-pads and interscapular brown adipose tissue*

For full details see the Experimental section. The results are mean values $\pm$ S.D., with the numbers of observations shown in parentheses, and are expressed as μ mol of ^3H incorporated into saponified lipid/h per g wet wt. of tissue. Values that are significantly different from the corresponding value for control rats are shown: * $P < 0.05$; ** $P < 0.01$. Values that are significantly different by Student's t test from those for the saline-treated rats of the same group are shown: † $P < 0.05$; †† $P < 0.01$.

Treatment	Experimental group ...	Liver			Epididymal fat-pad			Interscapular brown adipose tissue		
		Control	Food-restricted	All uraemics	Control	Food-restricted	All uraemics	Control	Food-restricted	All uraemics
Saline		9.8 $\pm$ 5.4 (9)	5.0 $\pm$ 2.5* (6)	4.6 $\pm$ 1.1* (7)	6.8 $\pm$ 5.2 (9)	1.0 $\pm$ 0.4** (6)	1.8 $\pm$ 1.7* (7)	23.3 $\pm$ 12.9 (9)	20.4 $\pm$ 9.4 (6)	4.7 $\pm$ 4.0** (7)
DIANEAL 1.36%		7.8 $\pm$ 2.0 (7)	4.8 $\pm$ 0.7** (6)	6.7 $\pm$ 2.2† (8)	8.2 $\pm$ 4.2 (7)	1.5 $\pm$ 0.7** (6)	2.4 $\pm$ 1.7** (8)	45.5 $\pm$ 24.6† (7)	38.2 $\pm$ 17.0† (6)	9.1 $\pm$ 6.0** (8)
DIANEAL 3.86%		12.0 $\pm$ 3.4 (7)	11.7 $\pm$ 7.1† (6)	13.0 $\pm$ 6.8†† (10)	13.0 $\pm$ 14.3 (7)	15.2 $\pm$ 14.1† (6)	6.4 $\pm$ 4.6† (10)	73.6 $\pm$ 50.8†† (7)	55.2 $\pm$ 32.6† (6)	56.8 $\pm$ 49.7†† (10)

significant impairment in the rate of basal lipogenesis in brown fat or its response to the two glucose-containing dialysis solutions. This latter finding is of interest, because Rothwell & Stock (1982) have reported that a similar degree of food restriction in male Sprague-Dawley rats decreased the mitochondrial GDP-binding protein (Nicholls, 1979), suggesting a lower capacity for thermogenesis.

Blood glucose and plasma insulin

The basal blood glucose (before intraperitoneal injection) tended to be lower in the food-restricted and uraemic rats, but the difference was only significant in the latter group (Table 3; $P < 0.05$). No appreciable change in blood glucose occurred in any group after intraperitoneal injection of saline. Only the uraemic rats showed a significant rise in blood glucose compared with the control group after DIANEAL 1.36%. With DIANEAL 3.86% all groups showed an increase in blood glucose, but the increases in the food-restricted and uraemic rats were not significantly different from that of control rats (Table 3).

The mean plasma insulin in the food-restricted rats injected with saline was about 50% lower than that of control rats, and for the uraemic rats it was 70% lower (Table 3). DIANEAL 1.36% increased the plasma insulin of the food-restricted rats to values similar to those for control rats, whereas the mean value for uraemic rats was still 45% lower (Table 3). Thus the uraemic group had inappropriately low plasma insulin concentrations for the prevailing blood glucose concentration after intraperitoneal injection of either saline or DIANEAL 1.36%. In direct contrast with our results, Soman & Felig (1977) reported raised basal values for plasma glucose and insulin in uraemic rats, but their rats were starved for 8–10 h and had lower blood urea nitrogen concentrations than did the uraemic rats studied in the present work. However, decreased insulin secretion after a glucose challenge has been reported in humans with untreated chronic uraemia (Cohen & Lindall, 1969).

Conclusions

The results of the present study indicate that the basal rate of lipogenesis is depressed in liver, epididymal fat-pad and interscapular brown adipose tissue of chronic uraemic rats. Administration of glucose-containing peritoneal-dialysis solutions restores the rate of hepatic lipogenesis to near control values, but the impairment of lipogenesis in epididymal fat-pads and brown fat is only partially reversed. It is difficult to assess how much of this impairment of lipogenesis is due to the decreased food intake that accompanies the uraemia. Only for brown fat is the evidence strong that the impairment

Table 3. *Effects of chronic uraemia on blood glucose and plasma insulin*

For full details see the Experimental section. The basal blood glucose (mM) in tail-vein blood taken before the start of treatment was 5.8 ± 1.0 (18) for control rats, 5.4 ± 0.6 (18) for food-restricted rats and 5.2 ± 0.5 (22) for uraemic rats. Change in blood glucose was calculated as the difference between basal blood glucose and blood glucose measured at the end of the treatment period. The results are mean values $\pm$ S.D., with the numbers of observations in parentheses. Results that are statistically different by Student's *t* test from the corresponding value for control rats are shown: **P* < 0.05.

Experimental group ...	Control		Food-restricted		All uraemics	
Treatment	Change in blood glucose (mM)	Plasma insulin (munits/l)	Change in blood glucose (mM)	Plasma insulin (munits/l)	Change in blood glucose (mM)	Plasma insulin (munits/l)
Saline	-0.2 ± 1.1 (6)	46.5 ± 26.8 (7)	$+0.3 \pm 1.0$ (6)	$22.9 \pm 7.5^*$ (6)	-0.7 ± 1.6 (6)	$14.5 \pm 5.0^*$ (7)
DIANEAL 1.36%	-0.4 ± 1.3 (5)	49.2 ± 23.3 (6)	$+0.4 \pm 0.8$ (5)	46.6 ± 3.8 (5)	$+1.3 \pm 0.7^*$ (5)	$26.2 \pm 8.0^*$ (6)
DIANEAL 3.86%	$+0.7 \pm 1.2$ (7)	—	$+1.2 \pm 0.8$ (6)	—	$+1.7 \pm 1.6$ (10)	—

is due to the uraemia (or some associated factor) rather than the accompanying hypophagia. An important factor in the impairment of lipogenesis may be the decreased plasma insulin (both the basal and the response to intraperitoneal glucose) in the uraemic rat. Alternatively, the hyperglucagonaemia reported by others (Soman & Felig, 1977) in the chronic uraemic rat may play a role in the depressed rate of lipogenesis, because glucagon has been shown to inhibit acetyl-CoA carboxylase, a key lipogenic enzyme, in rat adipocytes (Zammit & Corstorphine, 1982). Plasma glucagon concentrations were not measured in the present study, but, if they are also raised in our rats, the combination of low plasma insulin and high glucagon may explain the impairment of lipogenesis in adipose tissue. Intragastric glucose feeding or administration of insulin increases lipogenesis in brown fat of both fed and starved (24 h) rats (Agius & Williamson, 1980, 1981; Sugden *et al.*, 1981). In small mammals, brown adipose tissue plays a key role in diet-induced and non-shivering thermogenesis and therefore in maintenance of the energy balance (Himms-Hagen, 1976; Rothwell & Stock, 1981). Untreated chronic renal failure is characterized by hypothermia (Berman & Rhodes, 1961), and it is possible this is in part due to an impairment of the thermogenic response of brown adipose tissue. If the impairment of brown-adipose-tissue lipogenesis demonstrated in the present work is also present in human uraemics, it may impair their ability to dissipate the excess carbohydrate presented in peritoneal-dialysis solutions (up to 250 g of glucose per day) and thus lead to obesity.

This work was generously supported by Travenol International Services Inc. We thank Mrs. Vera Ilic for performing the assay of plasma insulin. D. H. W. is a

member of the External Scientific Staff of the Medical Research Council, U.K.

References

- Adelman, R. D. & Holliday, M. A. (1977) *Clin. Nephrol.* **8**, 298–303
- Agius, L. & Williamson, D. H. (1980) *Biochem. J.* **190**, 477–480
- Agius, L. & Williamson, D. H. (1981) *Biochim. Biophys. Acta* **666**, 127–132
- Albano, J. D. M., Ekins, R. P., Maritz, G. & Turner, R. C. (1972) *Acta Endocrinol. (Copenhagen)* **70**, 487–509
- Anderson, G. & Bergström, J. (1971) *Scand. J. Urol. Nephrol.* **5**, 77–79
- Bagdade, J. D. (1968) *Am. J. Clin. Nutr.* **21**, 426–429
- Bagdade, J. D. (1970) *Arch. Intern. Med.* **126**, 875–881
- Bagdade, J. D. (1975) *Nephron* **14**, 153–162
- Bagdade, J. D., Yee, E., Wilson, D. E. & Shafir, E. (1978) *J. Lab. Clin. Med.* **91**, 176–186
- Berman, L. B. & Rhodes, P. G. (1961) *Clin. Res.* **9**, 34
- Bierman, E. L. (1970) *Arch. Intern. Med.* **126**, 790–792
- Bilbrey, G. L., Faloona, G. R., White, M. G. & Knochel, J. P. (1974) *J. Clin. Invest.* **53**, 841–847
- Brown, D. J., Adam, W. R. & Dawborn, J. K. (1973) *Aust. N.Z. J. Med.* **3**, 1–5
- Chantler, C., Lieberman, E. & Holliday, M. A. (1974) *Pediatr. Res.* **8**, 109–113
- Cohen, S. L. & Lindall, A. W. (1969) *Clin. Res.* **17**, 544
- DeFronzo, R. A. & Alverstrande, A. (1980) *Am. J. Clin. Nutr.* **33**, 1438–1445
- DeFronzo, R. A., Andres, R., Edgar, P. & Walker, W. G. (1973) *Medicine (Baltimore)* **52**, 469–481
- DeSanto, N. G., Capodicasa, G., Senatore, R., Cicchetti, T., Cirillo, D., Damiano, M., Torella, R., Giugliano, D., Improta, L. & Giordano, C. (1979) *Int. J. Artif. Organs* **2**, 119–124
- Diaz, M., Kleinknecht, C. & Broyer, M. (1975) *Kidney Int.* **8**, 349–354

- Grodstein, G. P., Blumenkrantz, M. J., Kopple, J. D., Moran, J. K. & Coburn, J. W. (1981) *Kidney Int.* **19**, 564–567
- Hampers, C. L., Lowrie, E. G., Soeldner, J. S. & Merrill, J. P. (1970) *Arch. Intern. Med.* **126**, 870–874
- Himms-Hagen, J. (1976) *Annu. Rev. Physiol.* **38**, 315–351
- Jungas, R. L. (1968) *Biochemistry* **7**, 3708–3717
- Lindholm, B., Ahlberg, M., Alvestrand, A., Fürst, P., Karlander, S. G. & Bergström, J. (1979) *Proc. Int. Symp. Paris—CAPD*, 199–206
- Morrison, A. B. (1966) *Methods Achiev. Exp. Pathol.* **1**, 455–475
- Nicholls, D. G. (1979) *Biochim. Biophys. Acta* **549**, 1–29
- Nolph, K. D. & Prowant, B. (1979) *Proc. Int. Symp. Paris—CAPD*, 258–262
- Ormrod, D. & Miller, T. (1980) *Nephron* **26**, 249–254
- Ransom, J., Garfinkel, A. S., Nikazy, J., Schotz, M. C. & Kurokawa, K. (1981) *Metab. Clin. Exp.* **30**, 1165–1169
- Roth, D. A., Meade, R. C. & Barboriak, J. J. (1973) *Diabetes* **22**, 111–114
- Rothwell, N. J. & Stock, M. J. (1981) *Annu. Rev. Nutr.* **1**, 235–256
- Rothwell, N. J. & Stock, M. J. (1982) *Biosci. Rep.* **2**, 543–549
- Sherwin, R. S., Bastl, C., Finkelstein, F. O., Fisher, M., Black, H., Hendler, R. & Felig, P. (1976) *J. Clin. Invest.* **57**, 722–731
- Slein, M. W. (1963) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), pp. 117–123, Academic Press, New York and London
- Soman, V. & Felig, P. (1977) *J. Clin. Invest.* **60**, 224–232
- Stansbie, D., Brownsey, R. W., Crettaz, M. & Denton, R. M. (1976) *Biochem. J.* **160**, 413–416
- Sudgen, M. C., Watts, D. I. & Marshall, C. E. (1981) *Biosci. Rep.* **1**, 469–476
- Westervelt, F. B. (1969) *J. Lab. Clin. Med.* **74**, 79–84
- Zammit, V. A. & Corstorphine, C. G. (1982) *Biochem. J.* **208**, 783–788