The protein-sparing effect of carbohydrate

2.* The role of insulin

BY M. F. FULLER, T. E. C. WEEKES,† A. CADENHEAD AND J. B. BRUCE

Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB

(*Received* 16 *February* 1977 – *Accepted* 17 *March* 1977)

1. In five experiments with growing female pigs of 38–63 kg, insulin (2 mU/kg per min) and glucose (9–17 mg/kg per min) were infused continuously for 3–7 d. In three further experiments, glucose (9 mg/kg per min) was infused alone for 5 d.

2. In response to the combined infusion, plasma insulin increased 2·7-fold, plasma glucose decreased, on average, by 50 % and plasma urea concentration was reduced by 40 %. Urinary excretion of urea and nitrogen decreased after the first day of infusion to values averaging 70 % of control levels.

3. The infusion of glucose alone provoked only a small increase in plasma insulin. The reduction of urinary urea and of N excretion were approximately 25 % of those observed with the combined infusions.

Additions of carbohydrate to a diet, even to one already supplying generous amounts, result in an increased retention of nitrogen. This, the protein-sparing effect of carbohydrate, has been the subject of many experiments with man and animals, young and adult, fed and fasting. Many of these were reviewed by Munro (1951, 1964). The previous paper (Fuller & Crofts, 1977) described the response of the N metabolism of the growing pig to additions of carbohydrate in a variety of dietary circumstances. The mechanism by which carbohydrate exerts these effects is not fully understood, but probably involves at least three components, which vary in their relative importance according to nutritional circumstances. Two of the components concern the use of carbohydrate as an energy source for the essential processes of maintenance and for protein synthesis. The third mechanism, Munro (1964) suggested, involves the mediation of insulin, released in response to carbohydrate absorption. Insulin, which has long been known to be essential for normal muscle growth (Munro, 1964), may stimulate muscle protein synthesis by increasing amino acid transport (Kipnis & Noall, 1958) and peptide chain initiation (Jefferson, Rannels, Munger & Morgan, 1974). In addition, insulin decreases the postabsorptive release of amino acids from muscle in vivo (Pozefsky, Felig, Tobin, Soeldner & Cahill, 1969), as well as protein degradation by isolated muscle (Jefferson *et al.* 1974; Fulks, Li & Goldberg, 1975). Glucose was also reported to inhibit protein degradation in vitro, but was unable to stimulate protein synthesis in the absence of insulin (Fulks *et al.* 1975). Further evidence of the role of insulin in the protein-sparing effect of carbohydrate is provided by the demonstration that when carbohydrate is given to alloxan-diabetic rats there is no decrease in plasma amino acid concentrations (Munro, 1956) or in urinary urea or N excretion (Nakano & Ashida, 1975). This mechanism may be especially important in the pig, a species in which the insulin response to oral casein (Anderson, 1974) and intravenous arginine (Hertelendy, Takahashi, Machlin & Kipnis, 1970) is relatively poor.

These observations raise the question of whether the effects on protein metabolism of 'surfeit' feeding with carbohydrate can be mimicked by administration of exogenous insulin. This possibility was examined in the work reported here. The aim was, by

* Paper no. 1: *Br. J. Nutr.* (1977), **38**, 479.

† Present address: Department of Agricultural Biochemistry, The University of Newcastle upon Tyne.

Table 1. *Details of animals and infusions used in the experiments with growing female pigs*

Infusion ...	Insulin + glucose					Glucose alone		
Expt no. ...	1	2	3	4	5	6	7	8
Mean animal wt (kg)	63	43	58	38	57	45	41	48
Daily food intake (kg)	2·36	1·60	1·60	1·60	1·80	1·31	1·40	1·40
Duration of experiment (d):								
Control period	2	3	3	3	3	6	5	6
Infusion period	7	7	5	5	3	7	6	6
Mean daily glucose infusion rate (mg/kg per min)								
Day: 1	9·0*	9·0*	9·0*	9·0*	9·0*	9·0*	9·0*	9·0*
2	9·0	9·0*	9·0	9·0	10·4	9·0	9·0	9·0
3	9·0	9·0	9·0	9·0	11·1	9·0	9·0	9·0
4	9·0	9·0	11·0	14·0	—	9·0	9·0	9·0
5	9·0*	9·0	15·9	16·9*	—	9·0	9·0	9·0
6	9·0	9·0*	15·9*	—	—	9·0	9·0	9·0
7	9·0	—	—	—	—	9·0	—	—

* Results for these days were ignored in calculating responses to infusions (see p. 491).

continuous infusion of insulin into a well-nourished animal, to maintain throughout the day blood concentrations of insulin of the same order as those found transiently after meals, and to observe the consequent effects on protein metabolism. Clearly, however, the hypoglycaemic effects of an infusion of insulin alone would tend to activate counter-regulatory responses, such as increased secretion of catecholamine, glucagon and corticosteroid, which would stimulate gluconeogenesis and amino acid breakdown (Felig, 1973). To avoid these consequences, glucose was infused with the insulin at a rate sufficient to maintain plasma glucose concentration within the normal physiological range.

EXPERIMENTAL

Animals

Female Large White × Landrace pigs from the Institute's herd were used. Their weights during the experiment are given in Table 1. Under anaesthesia with trichloroethylene (Trilene; ICI Ltd, Macclesfield, Cheshire) two catheters were introduced into the aorta, one via the superficial saphenous artery of each hind-leg. One catheter, for withdrawing blood, was 100 mm longer than the other which was used for infusion. The free ends of the catheters were led under the skin to emerge on the animal's back. The catheters were filled with saline (9 g sodium chloride/l) containing heparin (100 IU/ml) and stoppered. For urine collection, Foley catheters were introduced into the bladder without anaesthesia. During the experiments the pigs were kept in metabolism cages; at other times they lived in individual pens.

Diet and feeding

A single pelleted diet was used in all experiments. It was composed of (g/kg) ground barley 455, ground maize 100, ground flaked maize 100, oat flakes 100, sucrose 25, dried skimmed milk 50, white fish meal 75, soya-bean meal 75, with a vitamin and mineral supplement. It contained 28·5 g N/kg and the apparent digestibility of N was 0·86. The food was delivered in equal quantities at hourly intervals by automatic feeder, and was usually consumed immediately. Water was available at all times.

Infusions

Insulin and glucose were infused continuously using peristaltic pumps (The Holter Co., Bridgeport, Pa 19405, USA and LKB Produkter, A.B., Bromma, Sweden). Porcine insulin (Novo Actrapid; Martindale Pharmaceuticals, Romford, Essex), diluted in 0·15 M-NaCl containing porcine serum albumin (5 g/l), was infused at a constant rate of 2 mU/kg per min. Preliminary experiments indicated that this rate would produce circulating insulin levels similar to 'peak' postprandial values (Anderson, 1974). Glucose (2 M, sterile solution) was infused at an initial rate of 9 mg/kg per min. This infusion rate was maintained throughout when glucose was infused alone. When insulin and glucose were infused together, blood glucose levels were monitored using the Reflotest-Glucose rapid procedure (Boehringer Corporation (London) Ltd, Ealing, Middx). Glucose infusion rates were increased when necessary to avert extreme hypoglycaemia (blood glucose < 1·1 mmol/l): the mean daily rates of glucose infusion are given in Table 1.

Experimental design and procedures

Experiments consisted of a control period followed by a period of infusion, each lasting between 3 and 7 d. Attempts to conclude with a second control period were abandoned when it was found that the animal's endogenous glucoregulatory mechanisms were evidently disrupted by the combined infusion, taking several days to recover.

Urine, collected into 50 ml 2 M-sulphuric acid as preservative, was weighed and sampled every 24 h at 10.00 hours. Infusions also began and ended at this hour.

Two to seven blood samples (5 ml) were withdrawn each day. Samples were always taken 40 min after feeding. Blood for insulin assay was collected into heparinized tubes and that for glucose and urea analysis into heparinized tubes containing $KF.2H_2O$ (4 mg/ml). Samples were chilled and centrifuged and the plasma was stored at $-20°$ until analysed.

Analytical

Plasma and urine glucose concentrations were determined by the method of Morley, Dawson & Marks (1968). Urea concentrations in plasma and urine were estimated by the method of Marsh, Fingerhut & Miller (1965), modified for use with an AutoAnalyzer (Technicon Instruments Corp., Tarrytown, New York). N was estimated by the Kjeldahl method. Plasma insulin was measured by a modification of the method of Bassett & Thorburn (1971), using human insulin (24·6 U/mg; Radiochemical Centre, Amersham, Bucks.) as standard. Antiserum (Miles Research Products, Slough) and samples or standards were incubated at room temperature for 4 h before addition of 180 pg ^{125}I-labelled insulin (Radiochemical Centre, Amersham, Bucks.). After further incubation for 20 h, free and antibody-bound hormone were separated using finely ground magnesium silicate (talc) (Bassett & Thorburn, 1971).

RESULTS

Insulin and glucose infusions

The variable length of the combined insulin and glucose infusion period (Table 1) was dictated by the animals' responses to the infusion. Control of blood glucose levels became progressively more difficult as the infusion proceeded in Expts 3–5 and with the increased rate of glucose infusions the animals began to refuse food. These experiments were therefore stopped and the observations on the final day of each of these infusions were ignored when calculating mean values (Table 2). Expt 5 was stopped after only 3 d when the infusion catheter became dislodged. As seen in Figs 1 and 2 the changes produced by the infusions took at least 24 h to stabilize at the start of the infusion. Results for the first

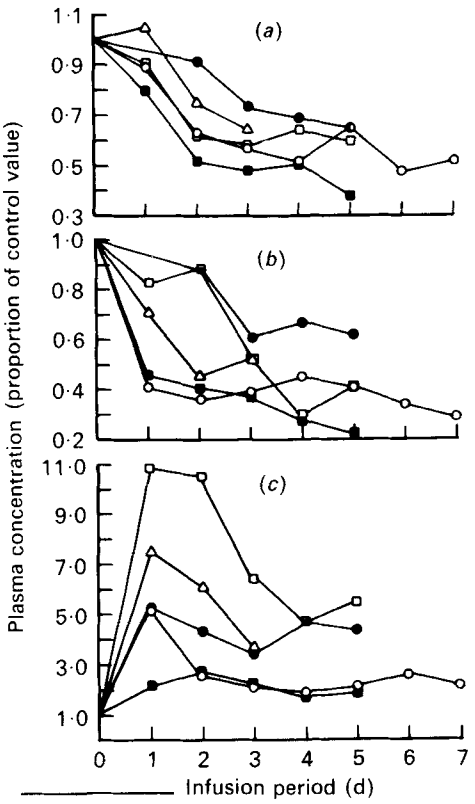

Fig. 1

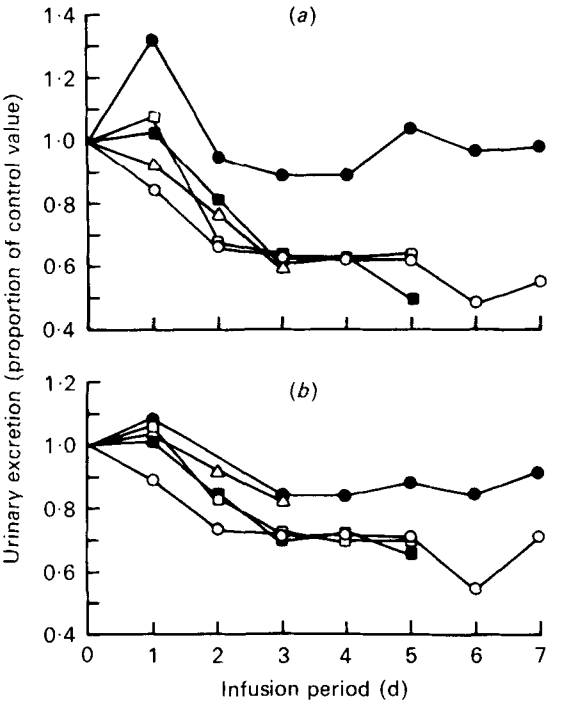

Fig. 2

Fig. 1. Changes in the plasma concentrations of (a) urea, (b) glucose and (c) insulin during the simultaneous infusion of insulin and glucose in growing female pigs. Values are expressed as proportions of the mean value during the previous control period with no infusion. Daily mean values are based on 2–7 samples. (○—○), Expt 1; (●—●), Expt 2; (□—□), Expt 3; (■—■), Expt 4; (△—△), Expt 5. For details of animals and infusions, see Table 1.

Fig. 2. Changes in the urinary excretions of (a) urea and (b) nitrogen during the simultaneous infusion of insulin and glucose in growing female pigs. Values are expressed as proportions of values obtained during a preceding control period with no infusion. (○—○), Expt 1; (●—●), Expt 2; (□—□), Expt 3; (■—■), Expt 4; (△—△), Expt 5. For details of animals and infusions, see Table 1.

day of all infusions and for day 2 of Expt 2 were therefore omitted from further calculations, as were the values for day 5 of Expt 1 when the infusion catheter blocked temporarily.

Insulin. The infusion of insulin increased plasma insulin concentration in all animals but to a varying extent. Fig. 1 shows that after the start of the infusion there was, in all but one animal, a transient increase in plasma insulin to high levels followed by a decrease to levels significantly ($P < 0{\cdot}05$) higher than those during the control period, which appeared to be maintained. Mean values are given in Table 2; on average, plasma insulin increased fourfold during infusion.

Glucose. Plasma glucose concentrations began to decrease immediately the infusions began (Fig. 1). They decreased sharply for 1–2 d, and thereafter tended to decrease more gradually to lower values as the infusion progressed. As with insulin, there were considerable differences between animals in the extent of the decrease (Table 2).

During the infusions the daily loss of glucose in the urine averaged 1·7 g.

Table 2. *Responses of growing female pigs to the infusion of insulin + glucose (Expts 1–5) or of glucose alone (Expts 6–8)*†

Infusion ...	Insulin + glucose				Glucose alone			
	Expt no.	Control period	Infusion period	Difference	Expt no.	Control period	Infusion period	Difference
Plasma insulin (μU/ml)	1	24·9	55·7	+30·8	6	17·5	24·8	+7·3
	2	23·8	98·3	+74·5				
	3	16·9	114·2	+97·3	7	14·7	20·4	+5·7
	4	18·4	39·8	+21·4				
	5	17·9	87·5	+69·6	8	9·6	13·4	+3·8
	Mean ± SEM			+58·7 ± 14·2*				+5·6 ± 1·0*
Plasma glucose (mmol/l)	1	6·28	2·26	−4·02	6	5·47	7·47	+2·00
	2	5·69	3·58	−2·11				
	3	5·29	2·78	−2·51	7	6·29	6·46	+0·17
	4	6·35	2·21	−4·14				
	5	5·68	2·74	−2·94	8	6·33	6·87	+0·54
	Mean ± SEM			−3·14 ± 0·41**				+0·90 ± 0·56
Plasma urea (mmol/l)	1	6·02	3·25	−2·77	6	5·12	5·02	−0·10
	2	6·30	4·35	−1·95				
	3	4·87	2·75	−2·12	7	6·50	4·47	−2·03
	4	5·68	2·83	−2·85				
	5	5·60	3·65	−1·95	8	5·67	4·13	−1·54
	Mean ± SEM			−2·33 ± 0·20***				−1·22 ± 0·58
Urinary urea (g/d)	1	59·0	33·4	−25·6	6	39·3	31·0	−8·3
	2	30·1	28·7	− 1·4				
	3	36·2	23·0	−13·2	7	26·6	24·0	−2·6
	4	27·0	15·6	−11·4				
	5	31·8	19·0	−12·8	8	35·0	27·9	−7·1
	Mean ± SEM			−12·9 ± 3·8*				−6·0 ± 1·7
Urinary nitrogen (g/d)	1	33·6	21·6	−12·0	6	22·9	21·6	−1·3
	2	22·0	18·9	− 3·1				
	3	24·5	17·2	− 7·3	7	16·3	16·5	+0·2
	4	17·3	11·9	− 5·4				
	5	18·5	15·2	− 3·3	8	19·6	16·9	−2·7
	Mean ± SEM			−6·2 ± 1·6*				−1·3 ± 0·8

Statistical significance of differences were: *$P < 0·05$, **$P < 0·01$, ***$P < 0·001$.
† For details of animals and infusions, see Table 1.

Urea. Plasma urea invariably decreased in response to the infusions (Table 2). Fig. 1 shows that the decrease was not immediate, 2 or 3 d passing before stable levels were reached. Fig. 2 shows that after the first day of infusion, daily urinary urea output decreased in all but one animal, and after 3 d appeared to stabilize at a value which represented approximately 70 % of that found in the control period. The changes are summarized in Table 2, which shows that on average urea excretion was approximately 13 g/d lower during the infusions.

N. The time-course of response of urinary N excretion to the infusion was similar to that of urea (Fig. 2). Table 2 shows the mean values before and during the infusion. The effect of infusion varied considerably between animals, the mean reduction in N output, 6·2 g/d, was significant ($P < 0·05$), and represented a decrease of approximately 25 %.

Responses after infusion. It was originally intended that each infusion should be followed by a second control period and that the effects of infusion should be assessed by comparison with both control periods. The results of the first experiment, in which this was attempted,

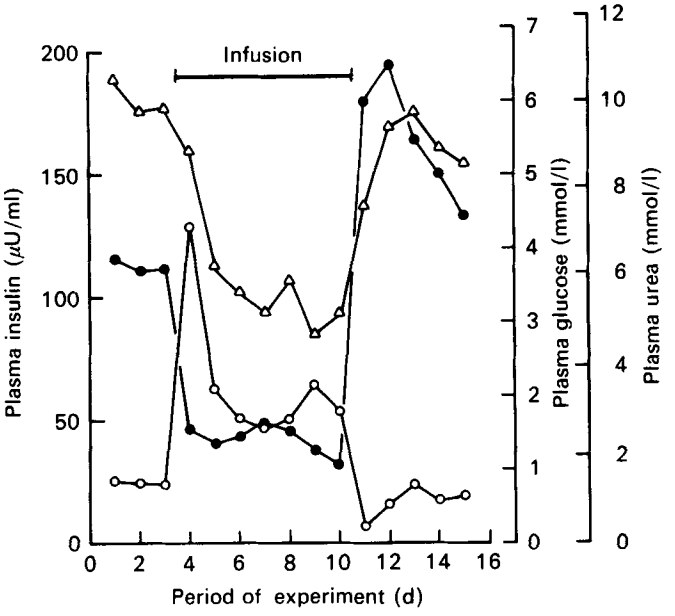

Fig. 3. Plasma concentrations of insulin (○—○), glucose (●—●) and urea (△—△) before, during and after the simultaneous infusion of insulin and glucose in growing female pigs. Daily mean values are based on 4–7 samples. For details of animals and infusions, see Table 1.

are shown in Fig. 3. When the infusion was stopped plasma insulin decreased to levels considerably below those found in the first control period, and even after 5 d had not completely recovered to its former levels. At the same time plasma glucose increased transiently to extremely high levels, then decreased during the following days towards the levels found in the first control period. When plasma glucose was at its highest, there was a considerable glycosuria, a maximum of 272 g glucose being excreted on the day after the infusion was stopped; this decreased to 11 g on the subsequent day and thereafter returned to its former low levels. Plasma urea rapidly returned after the infusion to the levels found in the first control period.

Glucose infusions

The infusion of glucose alone tended to depress the pigs' appetites; two experiments were abandoned when the animals failed to eat all their food. Table 2 gives the results of the remaining three experiments in which the pigs maintained constant food intakes throughout.

Insulin. Plasma insulin concentration of all three animals increased by approximately 40 % in response to the infusion of glucose. Values in the control and infusion periods of Expt 8 were unaccountably low.

Glucose. Plasma glucose increased substantially during glucose infusion in Expt 6, but to only a trivial extent in the other two experiments. There was no significant glycosuria.

Urea. Plasma urea concentration was not altered in Expt 6, but in each of the other experiments there was a distinct decrease. Urea excretion was reduced during all three infusions; the average reduction was 17 %.

N. Urinary N excretion was reduced by an average of 1·3 g/d during the glucose infusions. This was significantly ($P < 0·05$) less than that produced by the combined infusions. In each instance the reduction in urinary N was less than that expected from the decrease in urea excretion, implying some compensatory increase in another nitrogenous constituent of urine; what that was is not known.

Table 3. *Comparison of the protein-sparing effects of carbohydrate in growing female pigs, infused intra-arterially as glucose, alone or with insulin (present study), with that expected had the same carbohydrate addition been given orally as starch (Fuller & Crofts, 1977)*

(Mean values with their standard errors)

	Protein-sparing effect (mg N spared/g carbohydrate added)	
Expt nos. ...	6–8	1–5
Infused glucose:		
Alone	2·0±1·4	—
With insulin	—	8·2±1·9
Oral starch	4·9±0·2	3·7±0·3

DISCUSSION

These results showed that the prolonged infusions of insulin and glucose could reduce the N excretion of normally-fed growing pigs significantly more than glucose alone. This finding lends further support to the view that the secretion of insulin and its effects on protein metabolism are an important component of the protein-sparing effect of carbohydrate. It is therefore of interest to compare these results with those reported in the previous paper (Fuller & Crofts, 1977) when the carbohydrate was given orally as starch. Table 3 shows the specific protein-sparing effects (mg N spared/g carbohydrate added) obtained in the two sets of experiments under comparable conditions of protein and energy intake. This comparison suggests that the protein-sparing effect of oral starch is more than twice that of glucose infused alone but less than half that of glucose infused with insulin. Plasma insulin was not measured in the experiments with oral starch, but it is known that the plasma insulin response to oral glucose in the pig, in common with other species, is greater than that elicited by a similar degree of arterial hyperglycaemia produced by intravenous glucose infusion (Jensen, Nielsen & Kühl, 1976). Certainly, in the present experiments, the response of insulin to the infusion of glucose was modest compared with the normal postprandial hyperinsulinaemia of the pig (Anderson, 1974), but whether the small reduction in N excretion observed could be attributed entirely to this secretion of insulin is not known. These observations are, however, consistent with the hypothesis that a major component of the protein-sparing effect achieved by a surfeit feeding of carbohydrate is mediated by insulin. In these circumstances, when the basal diet supplies a considerable quantity of carbohydrate, any other component, such as that attributable to the role of glucose as an energy source, would be correspondingly small, but with lower energy intakes would assume increasing importance. This hypothesis does not, however, exclude the possibility of a synergism between glucose and insulin. It may be that the response attributed, by difference, to insulin may only be achieved in the situation where there is a continuous flux of glucose into muscle. This was achieved in the present experiments by a continuous replenishment of plasma glucose by infusion to maintain normo-glycaemia. This approach has been used with success for periods of up to 8 h in man (Costrini, Ganeshappa, Whalen & Soergel, 1973; Sherwin, Kramer, Tobin, Insel, Liljenquist, Berman & Andres, 1974), but was less successful in the longer infusions of the present study, when an increasing tendency to hypoglycaemia was encountered in some animals. If this tendency activated counter-regulatory secretion of glucagon or corticosteroids, the potential N-conserving effect of insulin may have been underestimated.

Although insulin was infused at a constant rate, plasma insulin levels showed considerable

variation during the course of the infusion, decreasing from high values on the first day. The activity of the hepatic insulin-degrading enzyme glutathionine-insulin transhydrogenase (*EC* 1.8.4.2) is related to plasma insulin levels in the rat (Thomas, Wakefield & Jones, 1973; Uete, Shimano, Shimizu & Morikawa, 1976). The exogenous insulin probably inhibited endogenous insulin release, either directly (Iversen & Miles, 1971), or as a consequence of hypoglycaemia (Miller, Waid & Joyce, 1976). The rebound increase in blood glucose (Fig. 3) when the infusions were terminated (Somogyi effect) probably also reflects the delayed recovery of the pancreatic β-cell from the effects of exogenous insulin infusion. In man, insulin release was similarly inhibited for 80 min after insulin infusion at 1·67 mU/kg per min for only 60 min (Horwitz, Rubenstein, Reynolds, Molnar & Yanaihara, 1975).

The hormonal regulation of net protein synthesis in a growing animal involves the interaction of many hormones (Turner & Munday, 1976). Thus the manipulation of just one of these hormones is a relatively crude approach to understanding the subtle control mechanisms that exist. However, we have shown, by increasing insulin levels within the physiological range, that there is a considerable potential for increasing N retention, at least for 3–7 d. This action of insulin can account for a large part of the protein-sparing action of dietary carbohydrate.

REFERENCES

Anderson, D. M. (1974). *J. agric. Sci., Camb.* **82**, 29.
Bassett, J. M. & Thorburn, G. D. (1971). *J. Endocr.* **50**, 59.
Costrini, N. V., Ganeshappa, K. P., Whalen, G. E. & Soergel, K. H. (1973). *J. Lab. clin. Med.* **82**, 179.
Felig, P. (1973). *Metabolism* **22**, 179.
Fulks, R. M., Li, J. B. & Goldberg, A. L. (1975). *J. biol. Chem.* **250**, 290.
Fuller, M. F. & Crofts, R. M. J. (1977). *Br. J. Nutr.* **38**, 479.
Hertelendy, F., Takahashi, K., Machlin, L. J. & Kipnis, D. M. (1970). *Gen. comp. Endocr.* **14**, 72.
Horwitz, D. L., Rubenstein, A. H., Reynolds, C., Molnar, G. D. & Yanaihara, N. (1975). *Horm. Metab. Res.* **7**, 449.
Iversen, J. & Miles, D. W. (1971). *Diabetes* **20**, 1.
Jefferson, L. S., Rannels, D. E., Munger, B. L. & Morgan, H. E. (1974). *Fedn Proc. Fedn Am. Socs exp. Biol.* **33**, 1098.
Jensen, S. L., Nielsen, O. W. & Kühl, C. (1976). *Diabetologia* **12**, 617.
Kipnis, D. M. & Noall, M. W. (1958). *Biochim. biophys. Acta* **28**, 226.
Marsh, W. H., Fingerhut, B. & Miller, H. (1965). *Clin. Chem.* **11**, 624.
Miller, R. E., Waid, T. H. & Joyce, P. M. (1976). *Am. J. Physiol.* **230**, 1090.
Morley, G., Dawson, A. & Marks, V. (1968). *Proc. Ass. clin. Biochem.* **5**, 42.
Munro, H. N. (1951). *Physiol. Rev.* **31**, 449.
Munro, H. N. (1956). *Scott. med. J.* **1**, 285.
Munro, H. N. (1964). In *Mammalian Protein Metabolism*, vol. 1, p. 381. [H. N. Munro and J. B. Allison, editors.] London and New York: Academic Press.
Nakano, K. & Ashida, K. (1975). *J. Nutr.* **105**, 906.
Pozefsky, T., Felig, P., Tobin, J. D., Soeldner, J. S. & Cahill, G. F. Jr (1969). *J. clin. Invest.* **48**, 2273.
Sherwin, R. S., Kramer, K. J., Tobin, J. D., Insel, P. A., Liljenquist, J. E., Berman, M. & Andres, R. (1974). *J. clin. Invest.* **53**, 1481.
Thomas, J. H., Wakefield, S. M. & Jones, R. H. (1973). *Biochem. Soc. Trans.* **1**, 1179.
Turner, M. R. & Munday, K. A. (1976). In *Meat Animals, Growth and Productivity*, p. 197. [D. Lister, D. N. Rhodes, V. R. Fowler and M. F. Fuller, editors.] New York and London: Plenum Press.
Uete, T., Shimano, N., Shimizu, S. & Morikawa, M. (1976). *Metabolism* **25**, 375.

Printed in Great Britain