Muscle protein synthesis in the streptozotocin-diabetic rat

A possible role for corticosterone in the insensitivity to insulin infusion *in vivo*

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The effect of insulin infusion *in vivo* on muscle protein synthesis was investigated in rats. In 10-days-streptozotocin-diabetic rats infused *in vivo* with amino acids and glucose, the rate of protein synthesis per unit of RNA (RNA activity) was markedly decreased. Pre-treatment with large doses of insulin at 17 and 1 h before the infusion fully restored RNA activity to normal. Infusion of insulin for 6 h with amino acids and glucose did not restore RNA activity to normal in the diabetic rats. However, in diabetic-adrenalectomized rats similar infusions of insulin fully restored RNA activity to normal. Measurements of plasma corticosterone concentrations indicated a 50% increase in the diabetic rats. Since pre-treatment with corticosterone suppressed the stimulatory effect of insulin infusion on RNA activity in adrenalectomized rats, and since corticosterone treatment for 6 days suppressed RNA activity even though insulin concentrations were elevated, it is suggested that increased concentrations of corticosterone are responsible for the lag in response to insulin in the diabetic rat. This means that the catabolic effects of glucocorticoids must be also considered together with the catabolic effect of insulin lack in diabetes.

Insulin is considered to be one of the major hormones involved in the regulation of protein metabolism in skeletal muscle. Diabetes results in a marked decrease in protein synthesis in skeletal muscle (Hay & Waterlow, 1967), owing to a decrease in both the number of ribosomes as well as their translational efficiency (Wool *et al.*, 1968; Jefferson *et al.*, 1974; Pain & Garlick, 1974; Millward *et al.*, 1976; Flaim *et al.*, 1980). Most of these insulin effects have been studied in isolated perfused preparations of rat heart, skeletal muscle and liver, as well as isolated liver and muscle cells (Jefferson *et al.*, 1972; Jefferson, 1975, 1980; Feldhoff *et al.*, 1977; Frayn & Maycock, 1979; Le Marchand-Brustel & Freychet, 1979). In one study insulin treatment *in vivo* has been shown to increase muscle protein synthesis over 48 h (Pain & Garlick, 1974). However, perfusion with insulin in the hemicorpus preparation from 7-day-alloxan-diabetic rats was much less effective in stimulating protein synthesis than in 2-day-diabetic rats (Flaim *et al.*, 1980). The present study was undertaken to determine whether this acute insensitivity to insulin was observed *in vivo* and whether a constant infusion of insulin into diabetic rats could be used to assess the relationship between muscle protein synthesis and

plasma insulin concentrations *in vivo*. Although physiological concentrations of plasma insulin were produced by infusions of different concentrations of insulin together with amino acids and glucose, the rate of protein synthesis remained depressed. Elevated concentrations of plasma corticosterone were found in these diabetic rats. Further experiments with insulin infusions in adrenalectomized-diabetic rats showed the rate of protein synthesis per unit of RNA (the RNA activity) restored to near-normal values. The inhibitory effect of corticosterone on protein synthesis was demonstrated in adrenalectomized rats treated with corticosterone for 6 days and exhibiting marked hyperinsulinaemia and in adrenalectomized-diabetic rats injected with a single dose of corticosterone before infusions with insulin.

Materials and methods

L-[U-¹⁴C]Tyrosine (10 Ci/mol) was obtained from The Radiochemical Centre (Amersham, Bucks., U.K.) and dissolved in the infusion medium to a final concentration of 1.5 μ Ci/ml. Streptozotocin and corticosterone were obtained from Sigma (London) Chemical Co., Poole, Dorset, U.K. A test kit

(GOD/PEROXIDASE) for glucose determination was obtained from Boehringer Corp. (London) Ltd., Lewes, Sussex, U.K. Uranyl acetate and all other chemicals, unless otherwise stated, were obtained from BDH (Poole, Dorset, U.K.). The protamine zinc insulin (40 units/ml; Wellcome Foundation, Beckenham, Kent, U.K.) and Actrapid mono-component insulin (80 units/ml; Novo Industries, distributed by Farillon Ltd., Bryant Avenue, Romford, Essex, U.K.) were obtained from a pharmacy.

Male Wistar rats (weight range 90–100g) were obtained from Charles River (Margate, Kent, U.K.) and housed individually in cages in a humidified temperature-controlled room on a 12h-light/12h-dark cycle. The animals were allowed free access to Oxoid cubed diet and water or 0.9% NaCl (for adrenalectomized animals only). The rats were divided into 13 groups of seven rats each. Ten groups were made diabetic by intravenous injection of streptozotocin (13 mg/100g body wt., dissolved in 0.4 ml of 0.9% NaCl adjusted to pH 4.5 with 50 mM-citric acid) under light diethyl ether anaesthesia (day 1). Plasma glucose concentrations were measured by the glucose oxidase method on a blood sample from the tail, 48h after the administration of streptozotocin. Rats with plasma glucose concentrations of 300–500 mg/100 ml were selected for the experiment. Four groups of the diabetic rats were not further treated before infusion. Insulin was injected subcutaneously [2 units of protamine zinc insulin (Wellcome)/100g body wt.] at 17h and 1h in one group of diabetic rats before infusion. The remaining five groups of diabetic rats were adrenalectomized on day 5 under diethyl ether anaesthesia and their drinking water was replaced with 0.9% NaCl. Four groups of adrenalectomized rats were untreated before infusion. The remaining adrenalectomized–diabetic group was treated with a single dose of corticosterone (5 mg/100g body wt.) 17h before infusion. Of the three groups of non-diabetic rats, one group was untreated and served as controls, and the other two groups were adrenalectomized (day 1). One of the adrenalectomized groups were treated with corticosterone [5 mg/100g body wt. for 6 days (days 4–9)], whereas the other group served as controls. All groups were infused on day 10.

Constant infusion and measurement of muscle protein synthesis

Protein synthesis in rat skeletal muscle was measured by the constant-intravenous-infusion method (Waterlow & Stephen, 1968; Garlick *et al.*, 1973). Except for adrenalectomized rats and adrenalectomized rats treated with corticosterone (5 mg/100g body wt. for 6 days), which were infused with [^{14}C]tyrosine in 0.9% NaCl, all other rats were infused with Vitrum (Vitrum, Stockholm, Sweden)

plus glucose (150 mg/ml) and [^{14}C]tyrosine (1.5 $\mu\text{Ci/ml}$). Vitrum contained the full spectrum of amino acids equivalent to a protein hydrolysate (9 mg of N/ml). The solution was filtered through a Millipore filter before the 10 ml syringes were filled for infusion. Batches of insulin (Actrapid, 80 units) were added directly to the syringes and mixed thoroughly, for delivery at rates of 50, 75 or 100 m-units/h as shown in Table 1.

The infusate entered the rat via a lateral tail vein at the rate of 1.2 ml/h for 6h. To avoid causing undue stress during infusion, the rats were placed individually in Perspex boxes (16.5 cm $\times$ 7.6 cm $\times$ 7.6 cm) with a U-shaped piece cut off at one end from which protruded the cannulated tail. A rubber bung held in place with zinc oxide plaster (B.P.C.) wound around the tail prevented the rat from pulling at the tail or the cannulae. This technique proved to be very useful, since it allowed the animal a certain extent of freedom of movement during the 6h of infusion, as well as access to drinking water. Because the diabetic rats produce a large amount of urine, dehydration in these rats can be a problem.

At the end of infusion, rats were killed by decapitation and blood was collected into heparinized tubes for analysis on plasma. Gastrocnemius, plantaris and soleus muscles were quickly removed from both hindlimbs and frozen in liquid nitrogen until required for analysis. Protein, RNA and tyrosine specific radioactivities were measured as described previously (Garlick & Marshall, 1972; Millward *et al.*, 1974). The fractional rate of protein synthesis was calculated from the specific-radioactivity ratio of protein-bound to free intracellular tyrosine (Garlick *et al.*, 1973). RNA activity (the rate of protein synthesis per unit of RNA) was calculated from the fractional rates of protein synthesis and the RNA/protein ratio (Millward *et al.*, 1973). The rationale for the use of the term 'RNA activity' has been discussed previously (Millward *et al.*, 1976).

Plasma insulin was measured by using a charcoal-separation radioimmunoassay (Herbert *et al.*, 1965). Plasma corticosterone was measured by a modification (M. Cox, unpublished work) of the method described by Few & Cashmore (1971) by a competitive protein-binding assay. The major difference was the use of dextran-coated charcoal instead of Florisil to separate the free and protein-bound corticosterone. A 98% recovery of non-protein-bound corticosterone was obtained with charcoal.

Results

As shown in Table 1, the rate of protein synthesis was depressed in the gastrocnemius muscle of the diabetic rats, as previously reported (Pain &

Table 1. *Rates of protein synthesis and RNA activities in gastrocnemius*

Protein synthesis was calculated from the labelling of intracellular free and protein-bound tyrosine at the end of the infusion. RNA activity was calculated from the rate of protein synthesis divided by the RNA/protein ratio (Millward *et al.*, 1973). Details of pretreatment are described in the text. Infusion medium was [^{14}C]tyrosine in either 0.9% NaCl or Vitrum, which contains a complete amino acid mixture corresponding to a protein hydrolysate (9 mg of N/ml), electrolytes and glucose (150 mg/ml). This was infused at 1.2 ml/h for 6 h. Insulin was added directly to the infusion syringes to give infusion rates as shown. Values shown are means $\pm$ S.D. for six rats.

Pretreatment	Infusion medium	Fractional rate of protein synthesis (%/day)	RNA activity (g of protein/day per g of RNA)	Plasma insulin (μ units/ml)
None	Amino acids + glucose	14.3 $\pm$ 0.9	15.1 $\pm$ 0.9	35.3 $\pm$ 4.6
Diabetic	Amino acids + glucose	5.7 $\pm$ 1.1	7.2 $\pm$ 1.4	2 $\pm$ 1.6
Diabetic + insulin (2 units/100 g)	Amino acids + glucose	14.8 $\pm$ 0.2	15.5 $\pm$ 0.8	>160
Diabetic	Amino acids + glucose + insulin (50 munits/h)	6.7 $\pm$ 1.1	8.8 $\pm$ 0.7	23.2 $\pm$ 3.4
Diabetic	Amino acids + glucose + insulin (75 munits/h)	7.2 $\pm$ 1.0	9.5 $\pm$ 0.9	37.8 $\pm$ 11
Diabetic	Amino acids + glucose + insulin (100 munits/h)	7.6 $\pm$ 1.7	10.9 $\pm$ 1.4	52.5 $\pm$ 18
Diabetic-adrenalectomized	Amino acids + glucose	7.1 $\pm$ 0.6	9.2 $\pm$ 1.0	3 $\pm$ 2.1
Diabetic-adrenalectomized	Amino acids + glucose + insulin (50 munits/h)	11.04 $\pm$ 1.1	15.0 $\pm$ 1.6	43 $\pm$ 4.6
Diabetic-adrenalectomized	Amino acids + glucose + insulin (75 munits)	11.1 $\pm$ 0.8	14.9 $\pm$ 0.8	65.2 $\pm$ 19
Diabetic-adrenalectomized	Amino acids + glucose + insulin (100 munits/h)	11.5 $\pm$ 1.2	15.3 $\pm$ 2.8	75.5 $\pm$ 8.2
Diabetic-adrenalectomized + corticosterone (5 mg/100 g body wt.)	Amino acids + glucose + insulin (100 munits/h)	7.7 $\pm$ 0.5	12.6 $\pm$ 1.6	82.7 $\pm$ 8.4
Adrenalectomized	0.9% NaCl	11.5 $\pm$ 2.0	15.8 $\pm$ 3.2	74.5 $\pm$ 14
Adrenalectomized + corticosterone (5 mg/100 g body wt.) for a period of 6 days	0.9% NaCl	5.72 $\pm$ 0.7	8.26 $\pm$ 1.6	144 $\pm$ 21

Garlick, 1974; Millward *et al.*, 1976). This results from a fall in the capacity for protein synthesis, indicated by a loss of RNA, and decreased RNA activity (Millward *et al.*, 1976). The present results emphasize the importance of the hormonal regulation of muscle protein synthesis, since, even with infusions of amino acids and glucose, the RNA activity in the diabetic rat remained low, at 48% of the control values in the normal rats. In accordance with previous results (Pain & Garlick, 1974), pretreatment with insulin by injection 17 h and 1 h before the infusion restored the RNA activity fully to control values.

Somewhat surprisingly, insulin infusions at up to 100 munits/h, which resulted in plasma insulin concentrations higher than in the normal controls, were not very effective in increasing the RNA activity. Previously Flaim *et al.* (1980) showed in perfused hemicorpus preparations that the addition of insulin to the perfusion media (25 units/ml at 35 ml/min) did not fully restore RNA activities to normal in 7-day-alloxan-diabetic rats. In contrast, in 2-day-alloxan-diabetic rats RNA activity was restored to normal during the perfusion by the insulin.

It appears therefore that there is a lag in the effect of insulin in the long-term diabetic rat, the hormone requiring between 6 and 17 h to exert its effect.

It has previously been shown that, in the presence of elevated concentrations of glucocorticoids, insulin concentrations are very elevated but are unable to activate protein synthesis in muscle (Millward *et al.*, 1976; Odedra *et al.*, 1980). As shown in Table 1, in adrenalectomized rats treated with corticosterone, insulin concentrations are well above normal physiological values, yet protein synthesis is markedly depressed, and this results in part from a depressed RNA activity. This effect is not due to lack of amino acids and glucose in this group, since well-fed adrenalectomized rats perfused with 0.9% NaCl have normal RNA activities. The possibility that insulin resistance in the diabetic rat may reflect elevated corticosterone concentrations was therefore investigated. Infusions of insulin in adrenalectomized-diabetic rats were able to activate fully the translational phase of protein synthesis, as judged by the RNA activities. At the lowest rate of infusion (50 munits/h), which resulted in plasma insulin concentrations similar to those in the normal

Table 2. *Effect of insulin in infusions on protein synthesis in plantaris and soleus muscle*

Explanation for the different treatments before infusion are as described in the legend to Table 1. Values shown are means $\pm$ S.D. for six rats.

Pretreatment	Infusion medium	Fractional rate of protein synthesis (%/day)	
		Plantaris	Soleus
None	Amino acids + glucose	13.9 $\pm$ 0.2	17.2 $\pm$ 1.2
Diabetic	Amino acids + glucose	4.6 $\pm$ 1.2	13.5 $\pm$ 2.1
Diabetic + insulin (2 units/100 g)	Amino acids + glucose	16.6 $\pm$ 0.7	18.2 $\pm$ 2.9
Diabetic	Amino acids + glucose + insulin (50 munits/h)	7.2 $\pm$ 1.2	14.5 $\pm$ 2.3
Diabetic	Amino acids + glucose + insulin (75 munits/h)	9.3 $\pm$ 0.6	14.9 $\pm$ 2.2
Diabetic	Amino acids + glucose + insulin (100 munits/h)	10.1 $\pm$ 0.9	15.5 $\pm$ 1.6
Diabetic-adrenalectomized	Amino acids + glucose	7.9 $\pm$ 1.7	14.9 $\pm$ 1.8
Diabetic-adrenalectomized	Amino acids + glucose + insulin (50 munits/h)	14.6 $\pm$ 0.5	15.2 $\pm$ 1.4
Diabetic-adrenalectomized	Amino acids + glucose + insulin (75 munits/h)	13.0 $\pm$ 2.1	18.4 $\pm$ 2.6
Diabetic-adrenalectomized	Amino acids + glucose + insulin (100 munits/h)	12.9 $\pm$ 2.4	18.6 $\pm$ 2.5
Diabetic-adrenalectomized + corticosterone (5mg/100 g body wt.)	Amino acids + glucose + insulin (100 munits/h)	9.6 $\pm$ 0.8	14.5 $\pm$ 0.9

rat, RNA activity was restored to the control value. Higher rates of insulin infusion did not increase synthesis further. The fact that the overall rate of protein synthesis was not restored to normal reflects the inability of insulin to restore fully the lower RNA content to normal in the 6 h of the infusion.

This suggested that elevated corticosterone concentrations were responsible for the acute resistance to insulin in the diabetic rat. Further evidence that this was the case was provided by measurement of plasma corticosterone. In the diabetic rats this was $36.7 \pm 10.1 \mu\text{g}/100\text{ml}$ ($n = 6$), a value 50% higher than in controls ($25 \pm 9 \mu\text{g}/100\text{ml}$). When this concentration of plasma corticosterone was achieved by a single subcutaneous injection of 5mg of corticosterone/100g body wt. 17h before the infusion (giving $44 \pm 15.9 \mu\text{g}/100\text{ml}$), the infusion of 100munits of insulin/h was less effective in increasing the RNA activity compared with adrenalectomized-diabetic rats. Since the RNA activity was not depressed as much as in the rats treated with corticosterone for 6 days, it appears that the full effects of corticosterone take longer than 17h to be achieved.

Rates of protein synthesis were also measured in the plantaris and soleus muscles (Table 2). For the plantaris muscle, which comprises mainly pale glycolytic fibres, the pattern and degree of response were very similar to that in the gastrocnemius, i.e. a lower rate of protein synthesis in the diabetic rat, which was restored by insulin pretreatment, but was only restored to normal by insulin infusion when the diabetic rats had been adrenalectomized. Also a single dose of corticosterone suppressed the response to insulin infusion. For the soleus muscle, comprising mainly red oxidative fibres, the muscle appeared to be much less sensitive to insulin lack or treatment,

but once again the pattern of response was similar to that of the other two muscles. Thus, although the synthesis rate was depressed in diabetes, the decrease was only 22%, compared with 66% in the plantaris and 60% in the gastrocnemius. Infusion of insulin did not restore the rate of protein synthesis to normal in the soleus, except in the adrenalectomized-diabetic rats.

Discussion

The depressed protein-synthetic rate observed in skeletal muscle of diabetic rats is accompanied by a loss of polyribosomes and accumulation of ribosomal subunits, and this is usually assumed to reflect an impairment in the process of peptide-chain initiation (Flaim *et al.*, 1980). Addition of insulin to the perfusion medium (25 munits/ml) could reverse the block in peptide-chain initiation and restore protein synthesis and RNA activity to normal values in mixed fast-twitch muscles from 2-day-alloxan-diabetic rats, but not in long-term diabetes of 7 days duration. Flaim *et al.* (1980) argued that, since the decreased RNA activity after 7 days was associated with a less marked accumulation of ribosomal subunits than after 2 days, the defect resulting from long-term diabetes may involve elongation or termination reactions which do not respond instantly to insulin administration. Although our results do not indicate the nature of the defect in long-term diabetic rats, they do suggest that the decreased acute response to insulin in long-term diabetes is not related to the insulin lack itself, since it is not observed in the adrenalectomized rat. A circulating factor could have been responsible for the insulin resistance in the 7-day-alloxan-diabetic rats studied by Flaim *et al.* (1980), since the effects of any such

factors may well persist for the relatively short period of the perfusion (50 min). The fact that rates of protein synthesis calculated over the entire 6 h period of our measurements in the adrenalectomized-diabetic rats were fully restored to normal by insulin infusion suggests that insulin restored the translational phase of protein synthesis to normal early in the infusion, most likely within the first hour.

The present results strongly suggest that the insulin resistance in long-term diabetic rats results from the elevated circulating concentrations of corticosterone. Glucocorticoids exert a catabolic effect on muscle protein mass, an action opposite to that of insulin. Whether the major effect is an impairment of protein synthesis or an increase in protein degradation is still not clear (Millward *et al.*, 1976; Shoji & Pennington, 1977; Rannels *et al.*, 1978; Tomas *et al.*, 1979; Odedra *et al.*, 1980). However, all would agree that the suppression of protein synthesis by corticosterone is an important part of this response. The present results show that treatment for 6 days with corticosterone markedly suppresses protein synthesis. The effect is a result of a loss of RNA and decreased RNA activity, and occurs even in the presence of supra-physiological concentrations of insulin. The decreased RNA activity results from an inhibition of initiation (Rannels & Jefferson, 1980). The high concentration of corticosterone in the diabetic rats is comparable with those observed in rats treated with 5 mg of corticosterone/100 g body wt. (Tomas *et al.*, 1979), and this must play a large part in the catabolic response to diabetes. Similar elevated concentrations of cortisol are found in human diabetes (Hall *et al.*, 1979).

These arguments are complicated by two factors. The first is our observation that, although pre-treatment with insulin at 17 and 1 h before the infusion restored protein synthesis to normal, corticosterone concentrations were still elevated to the same extent as observed in the diabetic rats. We can only assume that the dose of insulin in these rats was sufficient to overcome any insulin resistance induced by the corticosterone or any other mechanism. The second difficulty is the fact that both starvation (Goodman & Ruderman, 1979; Le Marchand-Brustel & Freychet, 1979; Brady *et al.*, 1981) and streptozotocin-induced diabetes (Le Marchand-Brustel & Freychet, 1979) appear to increase insulin sensitivity of glucose uptake. However, regulation of glucose uptake in muscle is related to intracellular glycogen content (Rennie & Edwards, 1981), and in both starvation and diabetes glycogen content of muscle is low. Thus, teleologically, increased insulin sensitivity to glucose uptake might be described as an appropriate response in starvation or diabetes. There is no reason, however, to suppose that the

insulin sensitivity of protein synthesis changes in the same way as glucose uptake and metabolism. On the contrary, in starvation a catabolic response of muscle protein is a necessary adaptation, so that it is not surprising that, in addition to the low concentrations of insulin that occur in starvation, insulin insensitivity also develops. We can only assume that diabetes this is also the case.

Stress can induce a dramatic increase in the plasma concentrations of corticosterone (Henning, 1978). Maximum care was taken to ensure that the rats were comfortable during the 6 h of infusion. Hence the increased corticosterone in diabetic rats compared with controls was not due to the stress of infusion. In any case, it would appear that the effect of corticosterone does not occur immediately, since even after 17 h a single injection of the hormone did not induce such a marked effect as 6 days of treatment. This suggests that, whereas insulin is involved in the acute regulation of protein balance in muscle, corticosterone is more involved in chronic changes. If this is true, the acute changes in glucocorticoids in response to stress or food intake must be more related to the other physiological roles of the hormone.

The present results confirm previous reports that the response of muscle to hormonal factors varies with fibre type (Rannels *et al.*, 1978; Rannels & Jefferson, 1980; Flaim *et al.*, 1980). In particular, cardiac muscle is less sensitive to insulin lack than is skeletal muscle with mixed fibre populations, such as gastrocnemius (Jefferson *et al.*, 1974), an effect attributed to the protection afforded to cardiac muscle by the high concentrations of circulating fatty acids in diabetes (Rannels *et al.*, 1974). Flaim *et al.* (1980) showed that soleus muscle, containing high proportions of slow-twitch fibres, was much less sensitive to diabetes than muscles with fast-twitch fibres. Our results confirm these findings, showing that the fall in protein synthesis in our diabetic rats is 22%, compared with 66% in plantaris muscle. However, although the changes in soleus muscle were much less marked, the pattern of response was the same as in the other muscles, with significant differences between the acute response to insulin in the diabetic-adrenalectomized and non-adrenalectomized rats. Thus, whatever mechanisms are responsible for the protection of the slow-twitch muscles, they are not complete.

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References

- Brady, L. J., Goodman, M. N., Kalish, F. N. & Ruderman, N. B. (1981) *Am. J. Physiol.* **240**, E184–E190

- Feldhoff, R. C. Taylor, J. M. & Jefferson, L. S. (1977) *J. Biol. Chem.* **252**, 3111–3116
- Few, J. D. & Cashmore, G. C. (1971) *Ann. Clin. Biochem.* **8**, 205–209
- Flaim, K. E., Copenhaver, M. E. & Jefferson, L. S. (1980) *Am. J. Physiol.* **239**, E88–E95
- Frayn, K. N. & Maycock, P. F. (1979) *Biochem. J.* **184**, 323–330
- Garlick, P. J. & Marshall, I. (1972) *J. Neurochem.* **19**, 577–583
- Garlick, P. J., Millward, D. J. & James, W. P. T. (1973) *Biochem. J.* **136**, 935–945
- Goodman, M. N. & Ruderman, N. B. (1979) *Am. J. Physiol.* **236**, E519–E527
- Hall, S. E. H., Saunders, J. & Sönksen, P. H. (1979) *Diabetologia* **16**, 297–306
- Hay, A. M. & Waterlow, J. C. (1967) *J. Physiol. (London)* **191**, 111P
- Henning, S. J. (1978) *Am. J. Physiol.* **235**, E451–E456
- Herbert, V., Lau, K.-S., Gottlieb, C. W. & Bleicher, S. J. (1965) *J. Clin. Endocrinol. Metab.* **25**, 1375–1384
- Jefferson, L. S. (1975) *Methods Enzymol.* **39**, 73–82
- Jefferson, L. S. (1980) *Diabetes* **29**, 487–496
- Jefferson, L. S., Koechler, J. O. & Morgan, H. E. (1972) *Proc. Natl. Acad. Sci. U.S.A.* **69**, 816–820
- Jefferson, L. A., Rannels, D. E., Munger, B. L. & Morgan, H. E. (1974) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **33**, 1098–1104
- Le Marchand-Brustel, Y. & Freychet, P. (1979) *J. Clin. Invest.* **64**, 1505–1515
- Millward, D. J., Garlick, P. J., James, W. P. T., Nnanyelugo, D. O. & Ryatt, J. S. (1973) *Nature (London)* **241**, 204–205
- Millward, D. J., Garlick, P. J., James, W. P. T. & Nnanyelugo, D. O. (1974) *Br. J. Nutr.* **32**, 127–142
- Millward, D. J., Garlick, P. J., Nnanyelugo, D. O. & Waterlow, J. C. (1976) *Biochem. J.* **156**, 185–188
- Odedra, B. R., Bates, P. C., Nathan, M., Rennie, M. & Millward, D. J. (1980) *Proc. Nutr. Soc.* **39**(3), A82
- Pain, V. M. & Garlick, P. J. (1974) *J. Biol. Chem.* **249**, 4510–4514
- Rannels, D. E., Hjalmarson, A. C. & Morgan, H. E. (1974) *Am. J. Physiol.* **226**, 528–539
- Rannels, D. E., Pegg, A. E., Rannels, S. R. & Jefferson, L. S. (1978) *Am. J. Physiol.* **235**, E126–E133
- Rannels, S. R. & Jefferson, L. C. (1980) *Am. J. Physiol.* **238**, E564–E572
- Rennie, M. J. & Edwards, R. H. T. (1981) in *Carbohydrate Metabolism and its Disorders* (Dickens, F., Randle, P. J. & Whelan, W., eds.), vol. 3, pp. 1–119, Academic Press, London
- Shoji, S. & Pennington, R. J. T. (1977) *Mol. Cell. Endocrinol.* **6**, 159–169
- Tomas, F. M., Munro, H. N. & Young, V. R. (1979) *Biochem. J.* **178**, 139–146
- Waterlow, J. C. & Stephen, J. M. L. (1968) *Clin. Sci.* **35**, 287–305
- Wool, I. G., Stirewalt, W. S., Kurihara, K., Low, R. B., Bailey, P. & Oyer, D. (1968) *Recent Prog. Horm. Res.* **24**, 139–213