

## Thyroid hormones and muscle protein turnover

### The effect of thyroid-hormone deficiency and replacement in thyroidectomized and hypophysectomized rats

John G. BROWN,\* Peter C. BATES,\* Malcolm A. HOLLIDAY† and David J. MILLWARD\*

\*London School of Hygiene and Tropical Medicine, Clinical Nutrition and Metabolism Unit, Hospital for Tropical Diseases, 4 St. Pancras Way, London NW1 2PE, U.K. and †University of California (San Francisco) Childrens Renal Center, 400 Parnassus Avenue, San Francisco, CA 94143, U.S.A.

(Received 4 August 1980/Accepted 13 October 1980)

We have investigated the effects of thyroidectomy, hypophysectomy and 3,3',5-tri-iodothyronine replacement on protein synthesis and degradation in skeletal muscle *in vivo*. Thyroidectomy resulted in a decrease in the rate of protein synthesis as a result of a loss of RNA. However, RNA activity, the rate of protein synthesis per unit of RNA, was not decreased. This was the case in both young growing rats and mature non-growing rats. Tri-iodothyronine treatment of thyroidectomized rats increased protein synthesis by increasing RNA concentration without changes in RNA activity, and this occurred even when food intake was restricted to prevent any increase in growth. The rate of protein degradation was decreased by thyroidectomy and increased by tri-iodothyronine replacement in both animals fed *ad libitum* and food-restricted animals. Hypophysectomy decreased protein synthesis by decreasing both RNA concentration and activity. These changes were reversed by tri-iodothyronine treatment even in the presence of persistent marked hypoinsulinaemia. This indicates that tri-iodothyronine can activate the translational phase of protein synthesis in muscle in the absence of significant quantities of insulin. However, tri-iodothyronine does not seem to be obligatory for the maintenance of normal RNA activity in muscle, since in the thyroidectomized rat, in which plasma insulin concentrations are normal, RNA activity is maintained. From a consideration of the magnitude of changes in RNA activity observed in these experiments, it would appear that alterations in rates of elongation as well as initiation are involved in the changes in RNA activity.

Thyroid hormones appear to participate in an extensive and diverse range of metabolic processes encompassing most, if not all, cell types of the body (Ingbar & Woeber, 1974; Oppenheimer, 1979; Sterling, 1979a,b). One of these processes is protein turnover. It was reported some years ago that variations in thyroid status in man were associated with marked changes in the rate of protein turnover in the whole body (Crispell *et al.*, 1956). Such changes in whole-body protein turnover reflect, in part, changes in both protein degradation and synthesis in skeletal muscle, at least as judged by measurements on thyroidectomized and hypophysectomized rats in the perfused hemicorpus preparation (Flaim *et al.*, 1978a,b) and in muscles from hypophysectomized rats incubated *in vitro* (Goldberg *et al.*, 1977).

As far as changes in protein degradation are concerned, one difficulty in interpreting the reported

relationship between diminished thyroid activity in hypophysectomized or thyroidectomized rats and the decreased rate of protein degradation in muscle is that the degradation rate is generally higher during growth and falls when growth is suppressed, by whatever means (Millward *et al.*, 1975a, 1976; Waterlow *et al.*, 1978, chapter 18; Laurent *et al.*, 1978b; Millward, 1980a). Thus decreased protein degradation in muscle of young animals with depressed thyroid activity could be a consequence of the growth suppression rather than a primary effect of the hormonal deficiency. For this reason we have investigated the changes in muscle protein synthesis and degradation in rats after thyroidectomy and treatment with 3,3',5-tri-iodothyronine, attempting to separate the effects of the hormone from effects caused by an alteration in the growth rate.

As far as changes in the rate of protein synthesis are concerned, the mechanism of such changes is

difficult to interpret because after thyroidectomy plasma concentrations of somatotropin (growth hormone) also fall (Coiro *et al.*, 1979). Thus it is not clear whether a lack of tri-iodothyronine (the active thyroid hormone) or somatotropin is responsible for the fall in the rate of protein synthesis. In addition, experiments on thyroidectomized rats do not yield unequivocal information about the involvement of tri-iodothyronine in the translational phase of protein synthesis. This is because plasma insulin concentrations are maintained after thyroidectomy or even occasionally elevated (Lenzen *et al.*, 1976), and insulin itself will maintain the translational phase of protein synthesis in muscle (Jefferson *et al.*, 1977). In the present paper we report experiments in which the effects of the active thyroid hormone (tri-iodothyronine) on protein synthesis in muscle of both thyroidectomized and hypophysectomized rats are examined. In the latter case the effects of tri-iodothyronine can be judged in the absence of somatotropin. Furthermore, since plasma insulin concentrations are very low in hypophysectomized rats and are not restored after tri-iodothyronine treatment, the effects of tri-iodothyronine on protein synthesis in muscle can be, to some extent, assessed independently from any effects of insulin.

A preliminary report of some of these experiments has already been published (Brown & Millward, 1980).

## Materials and methods

### Chemicals

3,3',5-Tri-iodothyronine (sodium salt) was obtained from Sigma Chemical Co. (Poole, Dorset, U.K.). All other chemicals, unless otherwise stated, were obtained from BDH Chemicals (Poole, Dorset, U.K.).

### Animals and thyroidectomy

Two types of rat were used in the experiments reported: male Sprague-Dawley outbred albino and female Wistar outbred albino [Charles River (U.K.) Ltd., Margate, Kent, U.K.]. Rats were caged individually and fed on Oxoid cubed diet. Daily weighing was between 09:00h and 10:00h. Hypophysectomized rats (male Sprague-Dawley outbred albino) were also obtained from Charles River (U.K.) Ltd.

Thyroidectomy was performed as follows. Rats were given a 0.4% calcium lactate solution in place of drinking water for 24h before operation. Total thyroid excision, including parathyroid glands, was performed under ether anaesthesia. Fresh 0.4% calcium lactate was provided for the 24h period after the operation. Thereafter decreasing calcium lactate concentrations were provided in the drinking water (changed daily) for 1 week. At this time rats

were returned to drinking tap water. This procedure prevents serious hypocalcaemia in the initial post-operative days due to loss of hormonal calcium control.

### Constant infusion and measurement of muscle protein turnover

Tissue protein-synthesis rates were measured by using the constant-intravenous-infusion method (Waterlow & Stephen, 1968; Garlick *et al.*, 1973). A 0.25 mM solution of L-[U-<sup>14</sup>C]tyrosine (10 mCi/mmol; The Radiochemical Centre, Amersham, Bucks., U.K.) in saline (0.9% NaCl) was infused at a rate of 0.5 ml/h for 6h into a lateral tail vein as described previously (Millward *et al.*, 1975a). At the end of the infusion rats were killed by decapitation, with collection of blood and rapid removal of gastrocnemius and quadriceps muscles (from both hind-limbs) and heart. These tissues were immediately frozen on solid CO<sub>2</sub> and subsequently stored under liquid N<sub>2</sub>.

### Tissue analysis

Gastrocnemius and quadriceps muscles from one hind-limb and cardiac muscle were analysed for specific radioactivity of free and protein-bound tyrosine as described by Garlick & Marshall (1972). Gastrocnemius and quadriceps muscles from the other hind-limb of each rat were analysed for protein, RNA and DNA concentrations. Protein was measured by the method described by Lowry *et al.* (1951), with bovine serum albumin as standard, and the nucleic acids by the method of Munro & Fleck (1969), with the modification that DNA was extracted with 0.8 M-HClO<sub>4</sub> at 70°C and determined colorimetrically by the diphenylamine method as modified by Giles & Myers (1965).

### Calculation of fractional synthesis rate and fractional degradation rate of tissue protein

The rate of muscle protein synthesis was calculated from the ratio of protein-bound to free tyrosine specific radioactivity (Garlick *et al.*, 1973). The rate of protein degradation was calculated as the difference between the rate of synthesis and net change in protein mass in muscle (Millward *et al.*, 1975a).

### Serum measurements

3,3',5-Tri-iodothyronine concentrations were measured by radioimmunoassay (T<sub>3</sub> RIA kit IM.74; The Radiochemical Centre).

Insulin concentrations were measured by coated-charcoal immunoassay as described by Herbert *et al.* (1965).

Blood for these measurements was obtained from the tail vein, except when the animals were killed, when blood collected after decapitation was used.

### Experimental design

Three experiments were performed. In the first, the aim was to induce growth by the administration of tri-iodothyronine to young male thyroidectomized rats fed *ad libitum* and to compare muscle protein turnover with a similarly hormone-treated group that could not increase their growth because food intake was restricted to the amount taken by untreated thyroidectomized rats. In this experiment an attempt was also made to follow the time course of the effects of thyroidectomy on muscle growth and protein turnover.

In the second experiment the effect of thyroidectomy was examined in mature female rats that had virtually ceased to grow. In this way we could assess the importance of thyroid hormones in the control of the rate of muscle protein turnover in the non-growing adult.

In the third experiment the aim was to investigate the relationship between muscle protein synthesis and the concentrations of insulin, tri-iodothyronine and somatotropin in intact and hypophysectomized rats with and without tri-iodothyronine treatment.

#### Experiment 1

Fifty-four male Sprague–Dawley rats, obtained at approx. 50 g body wt., were divided into nine groups of six. When the rats had grown to approx. 100 g body wt. this experiment was commenced. One group was killed to obtain day-0 tissue composition values, five groups were thyroidectomized, one group was sham-operated and the remaining two groups were left unoperated. All rats were fed *ad libitum* after the operation, and serum tri-iodothyronine concentrations were measured on the first few days post-operative to check the efficacy of the operation and to follow the time course of the fall in circulating tri-iodothyronine concentration. On day 5 (post-operative) one thyroidectomized group and the sham-operated rats were infused. On day 16, by which time the growth of the operated rats had markedly slowed, one control (unoperated) and one thyroidectomized group were infused. One of the remaining three groups of thyroidectomized rats continued on the same regime (feeding *ad libitum* with no treatment), but tri-iodothyronine replacement was initiated in the other two, namely 5 µg of tri-iodothyronine/2 days in 0.2 ml of 10% (v/v) ethanol in saline. One of these treated groups continued to be fed *ad libitum*, whereas the other was restricted to approximately the same food intake as the untreated thyroidectomized group. Intraperitoneal tri-iodothyronine administration (or sham injection of carrier to the untreated thyroidectomized group) occurred on days 16, 18, 20 and 22 post-operative. On day 22 the untreated thyroidectomized and final unoperated group were infused

for comparison with the two treated groups, which were infused on day 23, i.e. after 7 days of tri-iodothyronine treatment.

#### Experiment 2

Ten female Wistar rats, obtained at approx. 230 g body wt., were divided into two groups of five. The rats were growing only at a very low rate, having practically reached maturity. At approx. 250 g body wt. one group was thyroidectomized and the other sham-operated. Both groups were fed *ad libitum*, and on day 16 post-operative both groups were infused.

#### Experiment 3

Groups of hypophysectomized rats at 90–100 g body wt. were treated with tri-iodothyronine for a total period of 7 days before the measurements were made. They were treated at two dosages, 5 µg/2 days and 50 µg/2 days by intraperitoneal injection. Measurements were made on two separate groups of untreated hypophysectomized rats, two separate groups of hypophysectomized rats treated with tri-iodothyronine at a dosage of 5 µg/2 days and one group of hypophysectomized rats treated with tri-iodothyronine at a dosage of 50 µg/2 days (five to seven rats per group). These measurements were compared with those for two groups of intact rats forming approximate weight- and age-matched controls and those made in the first experiment on control, thyroidectomized and tri-iodothyronine-treated thyroidectomized rats.

### Results and discussion

#### *Effects of thyroidectomy and tri-iodothyronine treatment on growth*

In comparison with sham-operated rats, the removal of the thyroid gland in young male rats had no effect on the growth rate during the first 5 days after the operation (Expt. 1). Furthermore, on the basis of our experience with normal rats of this sex and strain, the growth rate of the two groups of rats over this time, shown in Table 1, indicates that the surgery itself had little effect on growth. After 5 days the growth of the thyroidectomized rats began to slow, difference being apparent by day 8. However, over the time course of this experiment growth did not stop altogether, since even after 22 days growth was still occurring, although at a very much lower rate. These findings are broadly similar to those reported by Coiro *et al.* (1979), except that in their experiment growth did cease entirely by day 10 after the operation. However, the present results show that the continuation of growth after the removal of the thyroid involves increases in muscle mass, muscle protein and DNA (as shown in Table 2), as

Table 1. *Effects of thyroidectomy and tri-iodothyronine replacement on growth (Expt. 1)*  
Muscle used was combined gastrocnemius and quadriceps. Experimental details are given in the text. Results are given as means  $\pm$  s.d. for six animals

Time after operation (days)	Group	Initial body wt. (day 0) (g)	Day-16 body wt. (g)	Final body wt. (at death) (g)	Muscle wt. (at death) (g)	Total muscle protein (mg)	Fractional growth rate of muscle protein mass (%/day)
5	Sham-operated	93 $\pm$ 3	—	141 $\pm$ 5	1.36 $\pm$ 0.03	202 $\pm$ 13	4.65
	Thyroidectomized	102 $\pm$ 4	—	142 $\pm$ 9	1.40 $\pm$ 0.05	205 $\pm$ 12	4.86
16	Non-operated	99 $\pm$ 5	248 $\pm$ 14	248 $\pm$ 14	2.75 $\pm$ 0.15	412 $\pm$ 36	4.56
	Thyroidectomized	99 $\pm$ 4	175 $\pm$ 14	175 $\pm$ 14	1.91 $\pm$ 0.11	277 $\pm$ 22	1.86
22-23	Thyroidectomized	103 $\pm$ 5	174 $\pm$ 12	189 $\pm$ 19	2.13 $\pm$ 0.08	313 $\pm$ 26	1.65
	Thyroidectomized + tri-iodothyronine; fed <i>ad libitum</i>	98 $\pm$ 4	163 $\pm$ 15	207 $\pm$ 17	2.39 $\pm$ 0.07	331 $\pm$ 14	2.24
	Thyroidectomized + tri-iodothyronine; food-restricted	100 $\pm$ 5	161 $\pm$ 8	185 $\pm$ 7	2.16 $\pm$ 0.06	314 $\pm$ 8	1.53
	Non-operated	98 $\pm$ 7	224 $\pm$ 24	302 $\pm$ 26	3.58 $\pm$ 0.18	543 $\pm$ 12	3.46

well as the increases in body weight reported by Coiro *et al.* (1979).

After thyroidectomy, serum thyroxine concentrations have been reported to fall to barely detectable values after 4 days (Coiro *et al.*, 1979). In the present experiment total serum tri-iodothyronine concentrations fell from  $1.20 \pm 0.2$  ng/ml in unoperated rats to half this value after 24 h, to below 0.25 ng/ml after 72 h and to undetectable values after 4 days. Thus operated rats continued to grow at normal rates for several days after circulating tri-iodothyronine had disappeared. Measurements of food intakes in these experiments indicate that the growth failure, when it occurred, was not associated with a decrease in the absolute food intake, but rather a failure to increase it. Thus food intakes were 15.2 g/day pre-operative and 16.4 g/day on days 11-14 post-operative, whereas in the unoperated rats intakes at this time had increased to 28 g/day. This means that, whereas intake per unit body weight had stayed more or less constant in the unoperated rats (at approx. 15 g/day per 100 g body wt.), intakes by thyroidectomized animals fell to about 10 g/day per 100 g body wt.

Although the rate of increase of muscle protein and DNA post-operatively was not significantly decreased until after 5 days, a marked fall in RNA concentration was apparent at 5 days. Thus this effect of thyroidectomy on RNA concentration, which has been reported by Flaim *et al.* (1978b) in rats several weeks after surgery, is a very early event according to the present results. Indeed the fall in RNA concentration at this early stage is so marked that RNA must have been lost from the muscle rather than just failing to accumulate.

As stated in the introduction, one objective of the present study was to treat the thyroidectomized rats with tri-iodothyronine and manipulate subsequent growth by controlling food intakes. The tri-iodothyronine treatment with feeding *ad libitum* did not in fact restore growth to control rates. Thus the growth rate of the muscle protein mass was increased from 1.65%/day in the untreated rats to 2.24%/day after tri-iodothyronine treatment compared with 4-5%/day in control rats of the same weight or 3-4%/day in control rats of the same age. This small increase in growth was accompanied by a small increase in food intake from 17.0 to 20.3 g/day over the 7 days of tri-iodothyronine treatment.

This failure to restore growth to normal with tri-iodothyronine administration reflects at least two major difficulties in attaining tri-iodothyronine replacement therapy. Firstly, the regime for hormone administration in these experiments involved a single 5  $\mu$ g intraperitoneal injection of tri-iodothyronine every other day. Subsequent work we have done on tri-iodothyronine administration methods and dosage indicates that, although this

Table 2. *Effects of thyroidectomy and tri-iodothyronine replacement on skeletal-muscle composition (Expt. 1)*  
Muscle used was combined gastrocnemius and quadriceps. Experimental details are given in the text. Results are given as means  $\pm$  S.D. for six animals.

Time after operation (days)	Group	Total muscle DNA ( $\mu$ g)	Protein/DNA ratio	$10^3 \times$ RNA/protein ratio	RNA/DNA ratio
5	Sham-operated	588 $\pm$ 109	349 $\pm$ 49	7.72 $\pm$ 1.01	2.71 $\pm$ 0.59
	Thyroidectomized	507 $\pm$ 103	411 $\pm$ 73	4.88 $\pm$ 0.48	2.02 $\pm$ 0.26
16	Non-operated	1245 $\pm$ 40	331 $\pm$ 36	4.96 $\pm$ 0.94	1.64 $\pm$ 0.23
	Thyroidectomized	817 $\pm$ 81	345 $\pm$ 44	3.56 $\pm$ 1.21	1.18 $\pm$ 0.27
22–23	Thyroidectomized	748 $\pm$ 39	390 $\pm$ 29	3.21 $\pm$ 0.53	1.22 $\pm$ 0.18
	Thyroidectomized + tri-iodothyronine; fed <i>ad libitum</i>	695 $\pm$ 97	467 $\pm$ 95	5.23 $\pm$ 1.05	2.29 $\pm$ 0.88
	Thyroidectomized + tri-iodothyronine; food-restricted	695 $\pm$ 121	464 $\pm$ 80	5.33 $\pm$ 1.91	2.45 $\pm$ 0.88
	Non-operated	1349 $\pm$ 86	393 $\pm$ 13	4.17 $\pm$ 0.59	1.69 $\pm$ 0.30

dose would produce normal serum tri-iodothyronine concentrations during the second day (the non-injection day), on the day of injection the animals were most probably in a hyperthyroid state. Certainly growth was consistently more on the days after injection than on injection days in the tri-iodothyronine-treated group fed *ad libitum*.

Nevertheless their growth was increased so that comparisons could be made of muscle protein turnover in these rats and in similarly tri-iodothyronine-treated thyroidectomized rats in which food intake was restricted to that consumed by untreated thyroidectomized rats. This group behaved in an almost exactly similar way to the untreated rats in terms of their muscle growth (i.e. 1.53%/day compared with 1.65%/day). However, as far as muscle composition was concerned, in both groups of tri-iodothyronine-treated rats muscle RNA concentrations were increased by over 60% (Table 2) to values higher than either age- or weight-matched control unoperated rats. These results then are in marked contrast with those obtained by Flaim *et al.* (1978b), since in their studies, although treatment with thyroxine (the precursor of tri-iodothyronine) increased muscle RNA concentration over a range of dosages, in none of their groups were they able to restore concentrations to that in weight-matched controls.

In the mature female rats (Expt. 2) a very low growth rate of <0.5%/day was observed in the sham-operated controls. In the thyroidectomized group growth ceased altogether by day 8 post-operative, and these rats maintained stable body weights from day 8 to day 16 post-operative when they were infused.

#### *Effects of thyroidectomy and tri-iodothyronine treatment on muscle protein turnover*

The rate of protein synthesis in these experiments

was calculated from the relative specific radioactivities of tyrosine isolated from tissue supernatant and protein at the end of a 6 h infusion, and two potential sources of error must be considered (Waterlow & Stephen, 1968; Garlick *et al.*, 1973; Laurent *et al.*, 1978a; Waterlow *et al.*, 1978, chapter 10). The first involves the assumption that the measured tyrosine specific radioactivity ( $S_1$ ) is similar to that of the precursor. Although this is a significant problem in tissues where  $S_1$  is very different from that of the plasma tyrosine labelling ( $S_p$ ) (e.g. liver or intestine), in muscle the range for error is small. In these experiments  $S_1/S_p$  was on average 0.82, with a range of 0.76–0.90. Since the precursor labelling for tyrosine, an essential amino acid, is likely to be less than in plasma and higher than the true intracellular value (as discussed in Laurent *et al.*, 1978a), it is highly unlikely that the measured value of  $S_1$ , which includes tyrosine from intracellular and extracellular spaces; will differ by more than 10% from the true value for the precursor. Furthermore, because the concentrations of tyrosine in the intracellular and extracellular spaces are similar, any differences in the distribution of tissue water in these groups will not have significant effects on the difference between real and assumed precursor labelling. Since the difference in calculated rates of protein synthesis observed in these experiments ranges from 22% to 90%, then it is not possible for such differences to result from errors in assumptions about precursor labelling.

The second assumption involves the rate of rise to plateau of the precursor. As discussed by Waterlow *et al.* (1978, chapter 10), the rate of protein synthesis ( $K_s$ ) is calculated from the equation:

$$\frac{S_B}{S_1} = \frac{\lambda_1}{\lambda_1 - K_s} \cdot \frac{(1 - e^{-K_s t})}{(1 - e^{-\lambda_1 t})} - \frac{K_s}{(\lambda_1 - K_s)} \quad (1)$$

where  $S_b$  and  $S_i$  are the tissue protein-bound and free tyrosine labelling,  $\lambda_1$  is the rate constant describing the rise to plateau of the free tyrosine labelling and  $t$  is the time in days. Because tyrosine quickly achieves a plateau during the infusion, the actual magnitude of  $\lambda_1$  need not be defined with great accuracy, and for muscle an approximation is used (i.e.  $RK_s$ , where  $R$  is the ratio of pool sizes of free and protein-bound tyrosine). In fact our measurements of the rise to plateau of intracellular tyrosine in muscle during a constant infusion indicate a value for  $\lambda_1$  of 46 day<sup>-1</sup> compared with the value of 49 day<sup>-1</sup> calculated from  $RK_s$  (P. C. Bates, G. K. Grimble & D. J. Millward, unpublished work). Thus the assumptions involved in the choice of  $RK_s$  as an approximation for  $\lambda_1$  discussed in Garlick *et al.* (1973) and Waterlow *et al.* (1978, chapter 10) would appear to be valid.

However, in the present experiments, given the possibility that the alteration in thyroid status may change amino acid transport rates (Sterling, 1979a), and hence alter the turnover rates of the intracellular tyrosine pool, consideration must be given to possible differences in  $\lambda_1$  between the groups.

The overall turnover of the intracellular pool is described by the expression  $PRK_s$ , where  $P$  is the overall flux expressed as a multiple of the flux between intracellular and protein-bound tyrosine ( $RK_s$ ). Thus the flux between extracellular and intracellular tyrosine is  $(P-1)RK_s$ . The magnitude of  $P$  is equal to  $1/[1-(S_i/S_p)]$ . In the present experiments, because  $S_i/S_p$  was not significantly different, the magnitude of  $P$  was not different among any of the groups (Table 3). Thus there is no reason to believe that the changes in thyroid status have altered amino acid transport and turnover of the intracellular tyrosine pool sufficiently to affect either the rise to plateau of the precursor pool or the validity of the calculated rate of protein synthesis. At 5 days after the operation the average rate of protein synthesis in muscle of the thyroidectomized rats was 20% less than in the sham-operated animals. This difference was not significant, however, because of the variability in the rate among individual sham-operated rats. In our experience the variability in rates of protein synthesis between individual animals is such that to assess the significance of such small differences very large numbers of individual measurements would have to be made. However, as discussed above, the capacity for protein synthesis was markedly diminished ( $P < 0.001$ ), as judged by the RNA/protein ratio (Table 2). Because the fall in the RNA/protein ratio was greater than the fall in the fractional rate of protein synthesis, the RNA activity (i.e. the rate of protein synthesis per unit RNA) was elevated, although once again the significance of this difference was low ( $P < 0.20$ ). However, at 16 and 22 days after the operation the

Table 3. Radioactive labelling of free and protein-bound tyrosine and calculated rates of protein synthesis and degradation in skeletal muscle (Expt. 1)  
Experimental details are given in the text. Results are given as means  $\pm$  s.d. for six animals.

Time after operation (days)	Group	$S_i$ (d.p.m./ $\mu$ mol)	$S_i/S_p$ ratio	$S_b/S_i$ ratio	Fractional synthesis rate (%/day)	RNA activity (g of protein synthesized/day per g of RNA)	Fractional degradation rate (%/day)
5	Sham-operated	50 500 $\pm$ 9800	0.76 $\pm$ 0.13	0.0232 $\pm$ 0.0064	10.48 $\pm$ 2.59	13.9 $\pm$ 4.6	5.83
	Thyroidectomized	55 900 $\pm$ 7200	0.82 $\pm$ 0.13	0.0189 $\pm$ 0.0023	8.56 $\pm$ 0.95	17.7 $\pm$ 3.1	3.70
16	Non-operated	29 700 $\pm$ 5300	0.76 $\pm$ 0.08	0.0183 $\pm$ 0.0013	8.54 $\pm$ 0.29	18.5 $\pm$ 2.9	3.98
	Thyroidectomized	53 700 $\pm$ 12 800	0.77 $\pm$ 0.06	0.0105 $\pm$ 0.0022	5.11 $\pm$ 0.89	15.0 $\pm$ 4.9	3.25
22-23	Thyroidectomized	67 100 $\pm$ 16 200	0.89 $\pm$ 0.08	0.0104 $\pm$ 0.0035	5.04 $\pm$ 1.47	17.2 $\pm$ 5.5	3.39
	Thyroidectomized + tri-iodothyronine; fed <i>ad libitum</i>	53 400 $\pm$ 9600	0.90 $\pm$ 0.05	0.0212 $\pm$ 0.0030	9.60 $\pm$ 1.13	19.6 $\pm$ 2.0	7.36
	Thyroidectomized + tri-iodothyronine; food-restricted	52 500 $\pm$ 7800	0.84 $\pm$ 0.09	0.0179 $\pm$ 0.0030	8.09 $\pm$ 1.35	16.2 $\pm$ 3.5	6.56

rate of protein synthesis was significantly decreased in comparison with any of the control groups, because, even though RNA activities were still in the normal range, there was a diminished capacity for protein synthesis as judged by the RNA/protein ratio.

After treatment with tri-iodothyronine, rates of protein synthesis were significantly increased in both rats fed *ad libitum* and food-restricted rats. Once again the mechanism of these changes in protein synthesis involved alterations in the capacity for protein synthesis (Table 2) rather than changes in the translational phase of protein synthesis.

In the mature female rats (Expt. 2) the changes in protein synthesis after thyroidectomy were qualitatively similar to those observed in the younger male rats. Thus the rate of protein synthesis fell by 40%, and this was due to a significant fall in muscle RNA concentration, diminishing the capacity for protein synthesis. The RNA activities of the two groups were not significantly different. The values were markedly lower than those observed in the younger rats, however, and are lower than those observed in well-nourished male rats throughout development (Millward *et al.*, 1975a). This may reflect a sex difference, since measurements in young 150g female rats, more comparable with the young males in the current study, indicate RNA activities of  $9.5 \pm 1.2$ g of protein synthesized/day per g of RNA (J. G. Brown & D. J. Millward, unpublished work). This is clearly lower than any of the values for the males in the current study and closer to the values given in Table 4.

These changes in protein synthesis in muscle are in one important respect quite different from most of the changes we have previously reported in response to altered nutritional or hormonal states (Millward *et al.*, 1976; Millward & Waterlow, 1978). In response to protein-energy malnutrition, diabetes or hypophysectomy the loss of muscle RNA is accompanied by decreases in RNA activity (see Millward & Waterlow, 1978). This has led us to believe that the loss of muscle ribosomes was a consequence of increased RNA degradation after decreased initiation and polyribosome disaggregation in muscle, as postulated some years ago by Munro (see Waterlow *et al.*, 1978, chapter 18), although such a

mechanism has not been confirmed. In the present experiment the changes in muscle RNA content after changes in thyroid status occur with no changes in RNA activity. This suggests a mechanism involving the regulation of ribosome synthesis, possibly at the level of transcription of the ribosomal-RNA gene rather than through a cytoplasmic mechanism involving altered RNA degradation. Such a mechanism is consistent with the proposal that part of the response to thyroid hormones is due to an interaction of tri-iodothyronine with nuclear chromatin followed by changes in gene expression (Andrea *et al.*, 1979; Limas, 1979; Oppenheimer, 1979; Sterling, 1979b). What we do not know is the extent to which the pattern of gene expression is changed with these alterations in thyroid status. Although alterations in thyroid status result in changes in the activity and structure of mitochondria in muscle (Gustafsson *et al.*, 1965), this does not necessarily involve changes in gene expression, as postulated by Winder *et al.* (1975), but could occur as a direct effect of the hormone on mitochondria (Sterling, 1979b).

As far as cardiac muscle is concerned, alterations in thyroid status have marked effects on protein turnover and cardiac size (Hjalmarson *et al.*, 1975; Sanford *et al.*, 1978), as well as on cardiac myofibrillar ATPase activity (see Morkin, 1979). Our own measurements of protein synthesis in cardiac ventricular protein (results not shown) in the present experiments showed parallel changes to those reported in the present paper for skeletal muscle. However, protein turnover and organ size in heart are very sensitive to alterations in metabolic rate and accompanying cardiac work rates (see, e.g., Sanford *et al.*, 1978), so that it is more difficult to determine whether thyroid hormones exert a direct effect on the heart.

The rate of protein degradation in muscle falls with development (Millward *et al.*, 1975a; Millward, 1980a), and in the present experiments the rate was higher in the sham-operated rats 5 days after the operation than in the older (16-day) control rats. Any effect of thyroidectomy on protein degradation must be judged against this developmental fall. As far as the initial response to thyroidectomy is concerned, results suggest a more

Table 4. Radioactive labelling of free and protein-bound tyrosine and calculated rates of protein synthesis in skeletal muscle of thyroidectomized and sham-operated mature female rats (Expt. 2)

Muscle used was gastrocnemius. Experimental details are given in the text. Results are given as means  $\pm$  s.d. for five animals.

Group	$S_1$ (d.p.m./ $\mu$ mol)	$S_2/S_1$ ratio	Fractional synthesis rate (%/day)	RNA activity (g of protein synthesized/day per g of RNA)	$10^3 \times$ RNA/protein ratio
Sham-operated	$47\ 800 \pm 10\ 400$	$0.00734 \pm 0.00204$	$4.18 \pm 0.81$	$8.1 \pm 1.6$	$5.18 \pm 0.09$
Thyroidectomized	$44\ 300 \pm 20\ 100$	$0.00493 \pm 0.00131$	$2.67 \pm 0.57$	$6.9 \pm 1.8$	$3.88 \pm 0.17$

rapid fall in the rate of degradation than in the control rats, but the difference in the rate of degradation 5 days after the operation was not in fact statistically significant. At 16 and 22 days after thyroidectomy the rate of degradation had fallen, but not to a significantly lower rate than in the age-matched control rats. These rats were, however, heavier, and therefore in that sense may not be appropriate controls. Proof of the fact that thyroidectomy does diminish protein degradation in muscle is provided by the measurement of the changes in the mature female rats after the operation. In this case, when development changes had ceased, thyroidectomy lowered the degradation rate from 3.7%/day to 2.7%/day, a rate that we have previously observed only in chronically malnourished rats (Millward *et al.*, 1975a) or in senile rats (Millward & Bates, 1978).

In fasting human adults the falls in metabolic rate and circulating tri-iodothyronine concentration (Portnay *et al.*, 1974; Spaulding *et al.*, 1976) are accompanied by a decrease in the urinary excretion of  $N^{\alpha}$ -methylhistidine (Young *et al.*, 1973). Furthermore, treatment with tri-iodothyronine during a fast increases urinary  $N^{\alpha}$ -methylhistidine excretion (Burman *et al.*, 1979). These results indicate that in man changes in thyroid status are accompanied by changes in muscle protein degradation if  $N^{\alpha}$ -methylhistidine in the urine originates from the degradation of actin in skeletal muscle rather than from non-muscle sources (Millward *et al.*, 1980a).

In the perfused hemicorpus preparation from thyroidectomized rats degradation rates were decreased by 30% compared with normal rats, but in these studies neither the age nor the weight of the control and thyroidectomized rats was stated (Flaim *et al.*, 1978b).

After treatment with tri-iodothyronine, degradation rates were increased in animals fed *ad libitum* or in food-restricted animals to rates that were 65–85% higher than in the somewhat heavier 16-day controls and also higher than in the smaller, younger, sham-operated control rats. More important is the fact that the increase in degradation occurred, not only in the animals that were allowed food *ad libitum* and could therefore increase their growth, but also in those rats that were restricted to the food intake of untreated thyroidectomized rats. Thus the increase in degradation must be different from what we have previously called an anabolic increase (Millward *et al.*, 1978; Millward, 1980b) observed in muscle when growth is increased during nutritional rehabilitation (Millward *et al.*, 1975a) or during stretch-induced hypertrophy (Laurent *et al.*, 1978b; Laurent & Millward, 1980; Millward, 1980a). In this case the rates of muscle protein degradation and resynthesis are increased without a change in muscle growth rate.

Flaim *et al.* (1978b) reported that treatment of thyroidectomized rats with thyroxine at doses that either partly restored growth (10  $\mu$ g/day per 100 g body wt.), or at higher doses that did not allow growth (100 or 200  $\mu$ g/day per 100 g body wt.), increased protein degradation in the perfused hemicorpus to control values, but not to an elevated value as observed in the present work. However, whether the rate is elevated above normal values depends on the choice of control, and, from our own previous measurements, degradation rates in younger rats than those examined in the present work are higher than in the tri-iodothyronine-treated rats (see, e.g., Millward *et al.*, 1975a).

Little is known about the factors that determine the overall rate of degradation in muscle. Rates are generally high in immature muscle, and fall during development to a value that is specific for each muscle type (Millward *et al.*, 1975a; Laurent *et al.*, 1978a; Millward, 1980a). Degradation rates are highest in cardiac and oxidative slow-contracting muscles. All these changes and differences in degradation in muscle are accompanied by parallel changes and differences in the activities of acid proteinases in muscle (see Cambell, 1979; Millward, 1980b). Similarly changes in the activities of cathepsin D and other lysosomal acid proteinases in muscle also accompany changes in thyroid status (DeMartino & Goldberg, 1978; Flaim *et al.*, 1978b). Our own measurements (S. J. Rosochacki, J. G. Brown & D. J. Millward, unpublished work) indicate a fall in the rate of pepstatin-sensitive haemoglobin degradation (cathepsin D activity) after thyroidectomy but no change in pepstatin-insensitive acid autolysis (i.e. activities of thiol and other acid proteinases). After tri-iodothyronine treatment cathepsin D activity is restored to control values, but pepstatin-insensitive acid autolysis is elevated above control values (see Millward *et al.*, 1980b). However, it is not known whether the rate of degradation in muscle is determined primarily by the concentration of the degrading system or by the nature of the substrate in terms of the structure of the contractile apparatus. Thus a particular specific activity of the degrading system could occur in muscle in response to a need for a change in the degradation rate. Although there are marked changes in mitochondrial structure and function in muscle with alterations in thyroid status (Gustafsson *et al.*, 1965), it is not known to what extent changes in the structure and function of the myofibrils occur that might affect their properties as substrates for the degradation system in the muscle fibres.

#### *Relationship between tri-iodothyronine, insulin and somatotropin in the regulation of muscle protein synthesis*

The hypophysectomized rats (Expt. 3) showed no



growth during these experiments. The rates of protein synthesis were very much diminished in both groups of untreated hypophysectomized rats (Table 5), as we have previously reported (Millward *et al.*, 1975*b*). This decreased rate of protein synthesis involved both a diminished capacity, indicated by the lowered RNA/protein ratio, and a decreased RNA activity (i.e. rate of protein synthesis per unit RNA). Essentially similar results were reported for measurements of RNA concentration and protein synthesis in the perfused hemicorpus preparation from hypophysectomized rats (Flaim *et al.*, 1978*a*).

In the hypophysectomized rats there are multiple hormone deficiencies, so that the cause of the decreased rate of protein synthesis is not immediately apparent. In addition, we have previously reported that food intake is depressed in the hypophysectomized rat to the extent that pair-feeding of intact rats resulted in an equal depression of protein synthesis (Millward *et al.*, 1975*b*, 1976). In the present experiments food intake of the hypophysectomized rats was 8 g/day per 100 g body wt., less than half the intake of the control rats fed *ad libitum*. This diminished food intake is likely to be a major factor in the depressed plasma insulin concentrations in these rats. As shown in Table 5, insulin was undetectable. Thus lack of insulin must be added to the lack of thyroid hormones and somatotropin as possible explanations for the diminished protein synthesis.

The treatment of the hypophysectomized rat with tri-iodothyronine at either 5 or 50 µg/2 days mar-

kedly increased protein synthesis (Table 5); this involved increases in both RNA/protein ratios and RNA activity. Indeed, in one group of hypophysectomized rats treated with tri-iodothyronine RNA activities were increased to values as high as we have ever observed previously (Millward *et al.*, 1975*a*; Millward & Waterlow, 1978). Surprisingly, these changes appeared to be independent from changes in the plasma insulin concentrations. As shown in Table 5, plasma insulin concentrations were still very low after tri-iodothyronine treatment, being less than 2 µunits/ml on average, and including some cases where insulin was undetectable. This itself was not surprising, since after tri-iodothyronine treatment of these hypophysectomized rats food intake was not markedly increased (and no growth occurred). This suggests therefore that the increased protein synthesis, and particularly the increased RNA activity, in response to the tri-iodothyronine treatment resulted from a mechanism that did not involve insulin but was a direct effect of tri-iodothyronine. If this is correct, then it means that tri-iodothyronine can activate protein synthesis in muscle in the absence of somatotropin and with only very low plasma concentrations of insulin. However, the fact that at 5, 16 and 22 days after thyroidectomy in the complete absence of tri-iodothyronine RNA activity is maintained (Table 3) demonstrates that there is no obligatory requirement for tri-iodothyronine for normal translation in the presence of normal plasma concentrations of insulin (Table 5). Thus it would

Table 5. Rates of protein synthesis and RNA concentration in skeletal muscle and plasma immunoreactive-insulin concentrations of rats in vivo

Muscle used was combined gastrocnemius and quadriceps. Experimental details are given in the text. Results are given as means ± S.D. for five to seven animals.

Group	Body wt. (g)	$10^3 \times$ RNA/protein ratio	Fractional synthesis rate (%/day)	RNA activity (g of protein synthesized/day per g of RNA)	Plasma concn. of insulin (µunits/ml)
Expt. 3					
Hypophysectomized	89 ± 6	3.36 ± 1.3	3.56 ± 0.01	10.1 ± 1.7	<2.0
Hypophysectomized	96 ± 3	4.04 ± 0.2	3.01 ± 0.55	7.5 ± 1.1	<2.0
Hypophysectomized + tri-iodothyronine (5 µg/2 days)	89 ± 5	5.43 ± 0.5	12.9 ± 0.12	23.4 ± 4.0	<2.0
Hypophysectomized + tri-iodothyronine (5 µg/2 days)	102 ± 6	6.19 ± 1.0	8.4 ± 2.10	13.4 ± 1.2	<2.0
Hypophysectomized + tri-iodothyronine (50 µg/2 days)	95 ± 9	6.00 ± 0.2	8.2 ± 0.90	13.6 ± 1.2	<2.0
Control	124 ± 9	7.7 ± 1.0	16.5 ± 1.7	18.2 ± 3.7	22.2 ± 5.0
Control	156 ± 2	8.4 ± 1.4	12.4 ± 0.5	12.2 ± 0.6	24.4 ± 1.8
Expt. 1					
Control	248 ± 14	5.0 ± 0.9	8.5 ± 0.30	18.5 ± 2.9	33.8 ± 3.4
Thyroidectomized	175 ± 14	3.56 ± 1.2	5.11 ± 0.89	15.0 ± 4.9	18.4 ± 5.0
Thyroidectomized	189 ± 19	3.21 ± 0.5	5.04 ± 1.47	17.2 ± 5.5	26.5 ± 5.5
Thyroidectomized + tri-iodothyronine (5 µg/2 days)	207 ± 17	5.23 ± 1.0	9.60 ± 1.13	19.6 ± 2.0	14.2 ± 8.5

appear that insulin and tri-iodothyronine are independently able to stimulate the translational phase of protein synthesis in muscle. However, the decreased RNA concentrations in the thyroidectomized rats indicate that insulin is not able to maintain the ribosome concentration in muscle in the absence of tri-iodothyronine. In this respect, then, tri-iodothyronine appears to have a wider range of influence on protein synthesis in muscle than insulin, being able to increase both ribosome concentration and activity.

Although RNA activity was raised to control values in the hypophysectomized rats treated with tri-iodothyronine, and muscle RNA concentrations were increased, the hypophysectomized rats did not grow. There were two reasons for this. Firstly, tri-iodothyronine treatment increased the degradation rate as well as the rate of protein synthesis, as discussed above. However, the second reason is the failure of tri-iodothyronine to induce DNA synthesis in muscle. This was indicated by the observation that there was no increase in DNA in the tri-iodothyronine-treated hypophysectomized rats (results not shown), and the production of new nuclei is a key part of the growth of muscle (see Millward & Waterlow, 1978; Millward, 1980a). Furthermore, in thyroidectomized rats DNA accumulation continues in muscle for several weeks after tri-iodothyronine has disappeared. Thus it would appear that tri-iodothyronine does not play a direct role in regulating DNA synthesis and mitosis in satellite cells in muscle. According to the experiments performed by Cheek & Hill (1970), DNA synthesis in muscle is a major site of action of somatotropin. Thus the failure of DNA synthesis in the hypophysectomized rats and the eventual slowing of DNA synthesis in thyroidectomized rats probably reflects somatotropin deficiency.

The present results suggest that somatotropin is not obligatory for the maintenance of protein synthesis in muscle. Thus in the tri-iodothyronine-treated hypophysectomized rats the normal rates of translation and the increased RNA concentrations occurred in the absence of somatotropin. Also, in the thyroidectomized rats pituitary somatotropin content and secretion are decreased (Herlant, 1964; Peake *et al.*, 1973), and plasma somatotropin concentrations become very much depressed, at least after 10 days (Coiro *et al.*, 1979), so that the maintenance of normal RNA activity at 16 and 22 days post-operative in Expt. 1 is a further indication that somatotropin is not essential for normal translation. It is noteworthy that, according to Flaim *et al.* (1978a), although the treatment of hypophysectomized rats with somatotropin did increase ribosomal aggregation, suggesting an increase in initiation, neither RNA concentrations nor RNA activities in muscle were restored to normal values.

However, the interpretation of such experiments is complicated by the possibility that all of the somatotropin action is mediated by the somatomedins (Phillips & Vassilopoulou-Sellin, 1980). Indeed, we cannot rule out the possibility that in the present experiments tri-iodothyronine may stimulate the production of somatomedins, although somatomedin production by the perfused liver of hypophysectomized rats is very much diminished (Phillips *et al.*, 1976).

Although these experiments do not reveal how the translational phase of protein synthesis is activated by tri-iodothyronine and insulin, there are implications resulting from the magnitude of the changes in RNA activity observed in the present experiments. It is usually considered that initiation (rather than elongation) is the main site of regulation in muscle and most eukaryotic cells (Jefferson, 1980; Henshaw *et al.*, 1980; Kay, 1980; Hunt, 1980). The evidence for this is the observation that, when changes in RNA activity occur, there are often changes in the number of ribosomal subunits or the degree of aggregation of ribosomes (see, e.g., Morgan *et al.*, 1971; Jefferson *et al.*, 1977; Jefferson, 1980). If changes in initiation are the only factor involved in alterations in RNA activity, then changes in ribosomal aggregation and activity should be proportional. Maximum ribosomal aggregation probably involves 80% of all ribosomes translating (Hirsch *et al.*, 1973; Kay, 1980). A fall in RNA activity from the maximum value to only one-third of this, as we observe in the hypophysectomized rats (or in diabetic rats with a similarly low RNA activity; Millward *et al.*, 1976), should involve a change to less than 30% ribosomes aggregated, with the rest as monomers or subunits. If changes in elongation rates also occurred, then the changes in aggregation need not be so marked. However, measurements of ribosomal subunits in conditions where the RNA activity is very low, such as in untreated hypophysectomized rats, indicate that the ribosomal subunits account for only 12% of total tissue RNA, compared with 6% in normal animals (Flaim *et al.*, 1978a). Although most workers usually stress the fact that the quantitative recovery of subunits is difficult from muscle (see, e.g., Morgan *et al.*, 1971), it would be surprising if measurements of subunits so markedly underestimated the actual changes. This means that changes in the elongation rate may occur more than is commonly supposed.

The reason that changes in elongation rate are not usually considered to occur probably reflects the fact that elongation rates are difficult to measure, particularly in muscle. In fact, thyroid hormones have been implicated in the regulation of elongation rates, at least in liver. Mathews *et al.* (1973) reported that, after thyroidectomy, transit times in rat liver were increased by 65%, and this slowing of

elongation was prevented with tri-iodothyronine treatment. Carter *et al.* (1975) have also implicated thyroid hormones in peptide-chain elongation in liver with their observation of a direct stimulation of incorporation of labelled amino acid into poly-ribosomes and the formation of polyphenylalanine after the addition of poly(U). This latter study involved a mitochondria-free cell-free system, and indicated that the effect of thyroxine was independent of changes induced in the cell after interactions of the hormone with mitochondria, which Sokoloff and co-workers had claimed as an essential part of the mechanism (Sokoloff & Kaufman, 1961; Sokoloff & Roberts, 1974).

Measurements of rates of elongation in rat and mouse liver and in HeLa cells indicate rates of 6–7 molecules of amino acids/s per ribosome (Haschemeyer, 1976). As we have previously reported, our values for RNA activity can be converted into minimum elongation rates by assuming that 80% of total RNA is ribosomal and that a maximum of 80% of ribosomes can be translating at any one time (Millward *et al.*, 1976; Laurent *et al.*, 1978b). Assuming molecular weights of 110 and  $2.46 \times 10^6$  for amino acids and ribosomal RNA respectively, the RNA activities reported in the present paper for control rats (12–18 g of protein synthesized/g of RNA per day) are equivalent to elongation rates of 4.7–7.2 molecules of amino acids/s per ribosome. The highest values for RNA activity observed in the tri-iodothyronine-treated thyroidectomized rats and in one group of tri-iodothyronine-treated hypophysectomized rats are 8–9.4 molecules of amino acids/s per ribosome. This latter value is higher than any previous value reported for eukaryotic cells, and may therefore indicate an accelerated rate of elongation. On the other hand, if initiation is rate-limiting in muscle at all values of RNA activity, so that any value less than the maximum observed value involves a decrease in initiation with fewer ribosomes translating, then it must be accepted that actual elongation rates at these values are higher than the calculated minimum values, i.e. more like 8–9 molecules of amino acids/s per ribosome. If this is so, then we must conclude that elongation rates in muscle are higher than in other cell types. There is no obvious reason why this should be so. We therefore propose that the variation in RNA activities from low values in the hypophysectomized rats to the lower end of the control range involves increases in initiation rates, whereas the further increases in the RNA-activity values reflect increased elongation rates with maximal ribosomal aggregation.

We therefore conclude from these experiments that in skeletal muscle tri-iodothyronine can activate maximally protein synthesis at the level of transcription and translation by increasing rates of

initiation and probably elongation, and that these effects can be achieved in the absence of measurable quantities of circulating insulin or somatotropin. Since the translational phase of protein synthesis is unimpaired in the absence of thyroid hormones when plasma insulin concentrations are normal, we conclude that insulin is a second factor that can independently stimulate translation. In other studies with diabetic rats we have observed that the RNA activity *in vivo* in muscle of diabetic rats can be increased from the low values of 5 g to the normal value of 15 g of protein synthesized/day per g of RNA by the infusion of insulin for 6 h (B. Odedra & D. J. Millward, unpublished work). However, insulin's role seems to be restricted to translation, since, unlike tri-iodothyronine, insulin appears to be unable to maintain ribosome concentration on its own. How these effects are achieved is not known. Furthermore, the fact that tri-iodothyronine concentrations in the blood tend to change much less rapidly than those of insulin implies that tri-iodothyronine may not be as involved in the acute regulation of translation *in vivo* as is insulin. However, this aspect of the role of tri-iodothyronine in the regulation of muscle protein synthesis can only be evaluated with more information about correlations between the free hormone concentration and protein synthesis in muscle *in vivo* and with better techniques for the replacement of the hormone in deficient animals.

We are grateful to Miss S. Dalal for assays of plasma insulin. These studies were generously supported by the Muscular Dystrophy Group of Great Britain and the Medical Research Council.

## References

- Andrea, T. A., Dietrich, S. W., Murray, W. H., Kollman, P. A. & Jorgensen, E. C. (1979) *J. Med. Chem.* **22**, 221–232
- Brown, J. G. & Millward, D. J. (1980) *Biochem. Soc. Trans.* **8**, 366–367
- Burman, K. D., Wartofsky, L., Dinterman, R. E., Kesler, P. & Wannemacher, R. W. (1979) *Metab. Clin. Exp.* **28**, 805–813
- Cambell, J. B. (1979) M.Med.Sci. Thesis, University of Dundee
- Carter, W. J., Fass, F. H. & Wynn, J. (1975) *J. Biol. Chem.* **250**, 3588–3594
- Cheek, D. B. & Hill, D. E. (1970) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **29**, 1503–1509
- Coiro, V., Braverman, L. E., Christianson, D., Fang, S. L. & Goodman, H. M. (1979) *Endocrinology* **105**, 641–646
- Crispell, K. R., Parson, W. & Hollifield, G. (1956) *J. Clin. Invest.* **35**, 164–169
- DeMartino, G. N. & Goldberg, A. L. (1978) *Proc. Natl. Acad. Sci. U.S.A.* **75**, 1369–1373
- Flaim, K. E., Li, J. B. & Jefferson, L. S. (1978a) *Am. J. Physiol.* **234**, E38–E43

- Flaim, K. E., Li, J. B. & Jefferson, L. S. (1978b) *Am. J. Physiol.* **235**, E231–E237
- 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
- Giles, K. W. & Myers, A. (1965) *Nature (London)* **206**, 93
- Goldberg, A. L., Griffin, G. E. & Dice, J. F. (1977) in *Pathogenesis of Human Muscular Dystrophies* (Rowland, I. P., ed.), pp. 376–385, *Experpta Medica*, Amsterdam
- Gustafsson, R., Tata, J. R., Lindberg, O. & Ernster, L. (1965) *J. Cell Biol.* **26**, 555–578
- Haschemeyer, A. E. U. (1976) *Trends Biochem. Sci.* **1**, 133–136
- Henshaw, E. C., Centrella, M., Mastropaulo, W., Smith, K. E. & Wong, S. T. (1980) *Biochem. Soc. Trans.* **8**, 286–287
- Herbert, V., Lau, K. S., Gottlieb, C. W. & Bleicher, S. J. (1965) *J. Clin. Endocrinol. Metab.* **25**, 1375–1384
- Herlant, M. (1964) *Int. Rev. Cytol.* **17**, 299–276
- Hirsch, C. A., Cox, M. A., Van Venroo, W. J. W. & Henshaw, E. C. (1973) *J. Biol. Chem.* **248**, 4377–4385
- Hjalmarson, A. C., Rannels, D. E., Kao, R. & Morgan, H. E. (1975) *J. Biol. Chem.* **250**, 4556–4561
- Hunt, T. (1980) *Trends Biochem. Sci.* **5**, 178–181
- Ingbar, S. H. & Woeber, K. A. (1974) in *Textbook of Endocrinology* (Williams, R. H., ed.), pp. 95–232, W. B. Saunders Co., Philadelphia, London and Toronto
- Jefferson, L. S. (1980) *Diabetes* **29**, 487–496
- Jefferson, L. S., Li, J. B. & Rannels, S. R. (1977) *J. Biol. Chem.* **252**, 1470–1483
- Kay, J. E. (1980) *Biochem. Soc. Trans.* **8**, 288
- Laurent, G. J. & Millward, D. J. (1980) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **39**, 42–47
- Laurent, G. J., Sparrow, M. P., Bates, P. C. & Millward, D. J. (1978a) *Biochem. J.* **176**, 393–405
- Laurent, G. J., Sparrow, M. P. & Millward, D. J. (1978b) *Biochem. J.* **176**, 407–417
- Lenzen, S., Joost, H. G., Hasselblatt, A. (1976) *Endocrinology* **99**, 125–129
- Limas, C. J. (1979) *Am. J. Physiol.* **236**, H451–H456
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Mathews, R. W., Oronsky, A. & Haschemeyer, A. E. V. (1973) *J. Biol. Chem.* **248**, 1329–1333
- Millward, D. J. (1980a) in *Degradative Processes in Heart and Skeletal Muscle* (Wildenthal, K., ed.), pp. 161–197, Elsevier/North-Holland, Amsterdam
- Millward, D. J. (1980b) *Compr. Biochem.* **19B**, 153–232
- Millward, D. J. & Bates, P. C. (1978) *Proc. Nutr. Soc.* **37**, 30A
- Millward, D. J. & Waterlow, J. C. (1978) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **37**, 2283–2290
- Millward, D. J., Garlick, P. J., Stewart, J. C., Nnanyelugo, D. O. & Waterlow, J. C. (1975a) *Biochem. J.* **150**, 235–243
- Millward, D. J., Nnanyelugo, D. O. & Chatterjee, A. K. (1975b) *Proc. Nutr. Soc.* **34**, 94A–95A
- Millward, D. J., Garlick, P. J., Nnanyelugo, D. O. & Waterlow, J. C. (1976) *Biochem. J.* **156**, 185–188
- Millward, D. J., Bates, P. C., Laurent, G. J. & Lo, C. C. (1978) in *Protein Turnover and Lysosomal Function* (Segal, H. L. & Doyle, D. T., eds.), pp. 619–644, Academic Press, New York
- Millward, D. J., Bates, P. C., Grimble, G. K., Nathan, M. & Rennie, M. J. (1980a) *Biochem. J.* **190**, 225–228
- Millward, D. J., Bates, P. C., Brown, J. G., Rosochacki, S. J. & Rennie, M. J. (1980b) *Ciba Symp.* **75**, 307–329
- Morgan, H. E., Jefferson, L. S., Wolpert, E. B. & Rannels, D. E. (1971) *J. Biol. Chem.* **246**, 2163–2170
- Morkin, E. (1979) *Circ. Res.* **44**, 1–7
- Munro, H. N. & Fleck, A. (1969) *Mammalian Protein Metabolism*, vol. 3, pp. 423–460, Academic Press, New York
- Nutting, D. F. (1976) *Endocrinology* **99**, 1423–1431
- Oppenheimer, J. H. (1979) *Science* **203**, 971–979
- Peake, G. T., Birge, C. A. & Doughaday, W. H. (1973) *Endocrinology* **92**, 487–493
- Phillips, L. S. & Vassilopoulou-Sellin, R. (1980) *N. Engl. J. Med.* **302**, 371–380
- Phillips, L. S., Herington, A. C., Karl, I. E. & Doughaday, W. H. (1976) *Endocrinology* **98**, 606–614
- Portnay, G. I., O'Brian, J. T., Rush, J., Vagenakis, A. G., Aziz, F., Arky, R. A., Ingbar, S. H. & Braverman, L. E. (1974) *J. Clin. Endocrinol. Metab.* **39**, 191–194
- Sanford, E. C., Griffin, E. E. & Wildenthal, K. (1978) *Circ. Res.* **43**, 688–694
- Sokoloff, L. & Kaufman, S. (1961) *J. Biol. Chem.* **236**, 795–803
- Sokoloff, L. & Roberts, P. A. (1974) *J. Biol. Chem.* **249**, 5520–5526
- Spaulding, S. W., Chopra, I. J., Sherwin, R. S. & Lyall, S. S. (1976) *J. Clin. Endocrinol. Metab.* **42**, 197–200
- Sterling, K. (1979a) *N. Engl. J. Med.* **300**, 117–123
- Sterling, K. (1979b) *N. Engl. J. Med.* **300**, 173–177
- Waterlow, J. C. & Stephen, J. M. L. (1968) *Clin. Sci.* **35**, 287–305
- Waterlow, J. C., Garlick, P. J. & Millward, D. J. (1978) *Protein Turnover in Mammalian Tissues and in the Whole Body*, Elsevier/North-Holland, Amsterdam
- Winder, W. W., Baldwin, K. M., Terjung, R. L. & Holloszy, J. O. (1975) *Am. J. Physiol.* **228**, 1341–1345
- Young, V. R., Haverberg, L. N., Bilmazes, C. & Munro, H. N. (1973) *Metab. Clin. Exp.* **22**, 1429–1436