Altered protein kinetics *in vivo* after single-limb burn injury

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Recovery from burn injury is associated with stimulated whole-body protein turnover. Since skeletal muscle and liver are the tissues most likely to influence whole-body measurements, we studied protein kinetics in soleus and plantaris muscles as well as liver 3 days after a 3 s burn on one hindlimb of the rat. Muscles from both the burned and unburned limbs of burned rats were compared with those of uninjured controls to distinguish between local and systemic factors involved. The following measurements were performed: (1) fractional growth rate of the tissue protein pool, determined from tissue protein content on days 2, 3 and 4; (2) fractional protein-synthetic rate, measured by [^{14}C]tyrosine constant infusion on day 3; (3) fractional protein-degradation rate, calculated from the difference between the rates of protein synthesis and growth. Protein growth by soleus and plantaris muscles of control rats and unburned limb of burned rats was not paralleled by those in the burned limb, which showed progressive atrophy between 2 and 4 days post-burn ($P < 0.005$). Protein synthesis by soleus but not plantaris muscle in the unburned limb of burned rats was enhanced by 62% ($P < 0.04$) above control. Protein synthesis by burned-limb soleus and plantaris muscles was elevated by 114% ($P < 0.001$) and 67% ($P < 0.02$) respectively above control. Protein degradation by both soleus and plantaris muscles in the unburned limb of burned rats did not differ from control. In contrast, that of soleus and plantaris muscles in the burned limb was stimulated by 230% ($P < 0.001$) and 164% ($P < 0.001$) respectively compared with controls. Protein turnover of soleus muscles in both control and burned rats was more rapid than in corresponding plantaris muscles. Liver protein mass exhibited steady growth in control rats, but remained unchanged in burned animals between 2 and 4 days post-burn. Liver protein synthesis in burned rats was elevated by 56% ($P < 0.01$) and protein breakdown was stimulated by 61% ($P < 0.002$) above those of controls. The data indicate that both local and systemic factors influence tissue protein turnover in animals recovering from a single-hindlimb scald.

One of the most severe consequences of burn injury is the slow but protracted erosion of body protein (Artz & Moncrief, 1969). Net proteolysis after burn injury could result from stimulated protein degradation, inhibited protein synthesis or any situation whereby degradation outstrips synthesis. Skeletal muscle is an important site in which to investigate the dynamics of protein metabolism post-burn because, by virtue of its large mass, it represents a major source of lost body protein. From experiments with incubated muscle preparations, different authors have explained the post-burn net protein breakdown on the basis of stimulated proteolysis with unchanged synthesis

(Odessey & Parr, 1982), impaired synthesis with unchanged breakdown (Frays & Maycock, 1979), or concurrently stimulated synthesis and breakdown rates with synthesis exceeded by degradation (Shangraw & Turinsky, 1982). Liver also represents an important determinant of post-burn protein metabolism, owing to its high basal protein-synthetic rate (McNurlan & Garlick, 1980) and its propensity to alter its protein synthesis markedly under a variety of physiological conditions, such as starvation (Garlick *et al.*, 1975; Stein *et al.*, 1976a) and trauma (Stein *et al.*, 1976b, 1977). Clinical studies of hypermetabolic burned children have revealed increased rates of whole-body

protein synthesis (Kien *et al.*, 1978a,b). Although their method could not localize the tissue site of increased activity, skeletal muscle and/or liver are the most probable site(s) of the enhanced protein turnover.

Previous studies from our laboratory (Shangraw & Turinsky, 1982) have shown that, after a 3 s scald on one hindlimb, rat soleus muscle from the burned region uniquely exhibits increased rates of protein synthesis and degradation *in vitro*. The present study was designed to test whether the enhanced protein turnover observed *in vitro* could be demonstrated *in vivo*. In addition, since soleus muscle is composed primarily of slow oxidative fibres (Ariano *et al.*, 1973), protein synthesis and degradation were also determined in plantaris muscles, which contain predominantly fast glycolytic and fast oxidative glycolytic fibres (Ariano *et al.*, 1973).

Finally, liver protein synthesis and breakdown were assessed to establish whether the single-limb injury produces systemic effects in non-muscle tissue at a time when the metabolic and endocrine environment has returned to normal, as judged by circulating concentrations of glucose and insulin (Turinsky & Shangraw, 1979).

Experimental

Treatment of animals and removal of tissues

Adult male Sprague-Dawley rats were cannulated in the right external jugular vein (Steiger *et al.*, 1972). Paired rats were infused with 48 ml of 0.9% NaCl/day with a Holter 903 infusion pump (Criticon, Tampa, FL, U.S.A.) as described by Stein *et al.* (1976a) and were allowed free access to laboratory food and water. We have verified that this infusion did not alter the protein/weight ratio of tissues. At 3–4 days after cannulation, one of the animals was anaesthetized with diethyl ether and burned on one hindlimb by immersing the limb in 90–91°C water for 3 s. The remaining animal, which served as a control, was only anaesthetized. Burned rats exhibited no sign of discomfort and, similarly to burn patients not requiring wound débridement or dressing changes, did not receive pain medication.

At 3 days after the burn injury, the infusate of the overnight-starved animals was supplemented with 1.4 μ Ci of [U-¹⁴C]tyrosine (Amersham, Arlington Heights, IL, U.S.A.)/ml for a 6 h period. Rats were killed by stunning and exsanguination immediately thereafter, and weighed 171–201 g at the time of death. Soleus and plantaris muscles from both limbs of burned rats and from controls as well as samples of liver tissue from both groups of animals were quickly excised, blotted, weighed and frozen with solid CO₂ in 1 ml of 5% (w/v)

trichloroacetic acid. Blood was also collected, allowed to coagulate at 26°C, and centrifuged at 1800g for 30 min at 4°C. Serum and tissue samples were stored at –30°C until analysis. Tissues were later homogenized in 5% (w/v) trichloroacetic acid, and the homogenates were centrifuged at 1250g for 30 min at 4°C. The precipitate was washed with 2.5 ml of 5% (w/v) trichloroacetic acid, and the supernatant and wash for each tissue sample were combined. Also, 4 vol. of serum was deproteinized by mixing with 1 vol. of 25% (w/v) trichloroacetic acid, adjusted to 4 ml volume with 5% trichloroacetic acid and subsequently treated identically with the tissue supernatants.

Preparation of supernatant fraction

Tissue supernatant/wash and deproteinized serum/wash samples were mixed with 5 vol. of water-saturated diethyl ether to extract the trichloroacetic acid, then frozen and freeze-dried. Freeze-dried samples were treated in accordance with the technique of Garlick & Marshall (1972) to separate L-tyrosine from racemized D-tyrosine, which does not represent the precursor pool for protein synthesis. Of the resulting H₂SO₄ extract, 1 ml was taken for counting of ¹⁴C radioactivity in 10 ml of Aquassure (New England Nuclear, Boston, MA, U.S.A.), and 1 ml was assayed for tyramine (i.e. L-tyrosine) by the fluorimetric method of Waalkes & Udenfriend (1957).

Preparation of protein fraction

The washed trichloroacetic acid precipitate was decontaminated by using the procedure of Wool & Krahll (1959). Proteins were hydrolysed in 6M-HCl at 110°C for 12–15 h, after which 1 ml was taken for counting of ¹⁴C radioactivity in 10 ml of Aquassure, and 0.2 ml was diluted with distilled water (1:50) for tyrosine assay (Waalkes & Udenfriend, 1957).

Calculation of muscle protein-synthetic rate

The fractional rate of protein synthesis, k_s , was calculated by using the equation derived by Garlick *et al.* (1973):

$$\frac{S_B}{S_T} = \frac{R}{(R-1)} \times \frac{(1-e^{-k_s t})}{(1-e^{-Rk_s t})} - \frac{1}{(R-1)}$$

where S_B and S_T are the specific radioactivities of the protein-bound and free tissue L-tyrosine pools respectively in d.p.m./nmol of tyrosine, R is the ratio of bound/free tyrosine in the muscle, and t is the duration of [¹⁴C]tyrosine infusion in days. The equation was solved for k_s by the Newton-Raphson iterative procedure with numerical differentiation, with the use of predetermined values of S_B/S_T , R and t .

Calculation of liver protein-synthetic rate

The fractional rate of protein synthesis, k_s , was calculated by using the equation derived by Garlick *et al.* (1973):

$$\frac{S_B}{S_T} = \frac{\lambda_p}{(\lambda_p - k_s)} \times \frac{(1 - e^{-k_s t})}{(1 - e^{-\lambda_p t})} - \frac{k_s}{(\lambda_p - k_s)}$$

where λ_p is the rate constant for the rise to plateau for the tyrosine specific radioactivity. Preliminary experiments were conducted to determine λ_p in burned and control rats by examining the radioactivity of deproteinized serum at 20, 40, 60, 80 and 120 min of infusion. Blood samples (0.5 ml) were taken from the cut tail, allowed to coagulate, and serum was deproteinized as described above. The calculated rate for burned rats was $\lambda_p = 34 \text{ days}^{-1}$ and for controls was $\lambda_p = 51 \text{ days}^{-1}$ (Fig. 1). All other values were determined in the manner described above for muscle protein synthesis.

Determination of tissue protein growth rate

A second group of rats was used to determine the rate of growth for liver as well as soleus and plantaris muscles. Three rats with a weight range of 2 g were combined per cage, and one overnight-starved animal was killed per day at 2, 3 and 4 days after the day of the burn injury. Protein contents of 100 mg samples of liver and entire skeletal muscles were determined in 1M-NaOH digests by a modified isothermic Lowry procedure (Lowry *et al.*, 1951; Hartree, 1972). Fractional growth rate of liver and skeletal muscles was calculated as the

daily change in protein content in the entire tissue between 2 and 4 days post-burn, expressed in %/day.

Calculation of tissue protein-breakdown rate

Protein degradation in muscle and liver was calculated as the difference between the protein-synthesis and protein growth rates, in accordance with Garlick *et al.* (1975).

Statistical analysis of data

Data on muscle protein degradation are expressed as means $\pm$ S.D. Statistical differences between groups were determined by two-way analysis of variance at the 95% confidence level. All other data are expressed as means $\pm$ S.E.M., and statistical differences between groups were determined by one-way analysis of variance coupled with Student's *t* test at the 95% confidence level.

Results

Effect of burn injury on body growth

Rats recovering from a single-hindlimb scald consistently weighed less than corresponding controls ($P < 0.002$). On days 2, 3 and 4 post-burn, the burned rats weighed 183 ± 2 (mean $\pm$ S.E.M.), 186 ± 2 and 189 ± 2 g as compared with respective control weights of 193 ± 1 , 199 ± 2 and 203 ± 2 g. The lower body weight can be explained, in part, by the food-consumption pattern between the day of injury and 4 days post-burn. Food intake by burned rats was diminished markedly during the first day post-burn, and then began a slow and incomplete return towards control consumption rate during the subsequent 3 days.

Effect of burn injury on tissue protein mass and growth

Both soleus and plantaris muscles from the burned limb exhibited a decreased protein content per mg of tissue (Table 1). At 2 days post-burn, soleus-muscle protein/weight ratio was decreased by 14% ($P < 0.001$) compared with control and by 20% ($P < 0.001$) compared with the contralateral unburned-limb muscle. The depression in protein content per muscle weight continued through the fourth day post-burn. For burned-limb plantaris muscle, the protein/weight ratio did not differ from controls, but was lower than that of the contralateral unburned-limb muscle for the major portion of the experimental period. More importantly, at 4 days post-burn, the protein mass of soleus and plantaris muscles in the burned limb was only 88% ($P < 0.002$) and 86% ($P < 0.001$) respectively of the mass of uninjured muscles.

No difference was found in protein content per mg of liver between control and burned rats during

Fig. 1. Specific radioactivity of free tyrosine in serum of rats during infusion of U-[^{14}C]tyrosine

Control (—) and burned (---) animals were infused with $2.7 \mu\text{Ci/h}$ via the infusion apparatus starting at time zero. Blood samples of ether-anaesthetized rats were taken from the tail vein, and serum was separated for assay of L-tyrosine and counting of radioactivity. During studies on protein synthesis, duration of the infusion was 6 h. Each value is an average for two rats.

Table 1. *Tissue protein mass and protein/weight ratio at 2, 3 and 4 days post-burn*

Tissues from control rats are compared with tissues from rats recovering from a single-hindlimb scald. Muscles from both the burned and contralateral unburned hindlimbs of burned rats were studied. Tissue samples were digested in 1M-NaOH at 100°C. Duplicate portions of each sample were assayed for protein content by using the Lowry procedure as modified by Hartree (1972) except that no incubation at 50°C was performed. Each value is a mean $\pm$ S.E.M. for 12–14 rats. Differences from controls ($P < 0.02$) are denoted by an asterisk (*), and those from the unburned limb of burned rats ($P < 0.02$) are indicated by a dagger (†).

Tissue	Group	Parameter	Time post-burn		
			2 days	3 days	4 days
Soleus muscle	Control rats	Mass (mg)	16.4 $\pm$ 0.3	16.1 $\pm$ 0.4	16.8 $\pm$ 0.3
	Unburned limb		16.2 $\pm$ 0.3	16.5 $\pm$ 0.4	17.2 $\pm$ 0.5
	Burned limb		16.7 $\pm$ 0.4	15.5 $\pm$ 0.4	14.9 $\pm$ 0.4*†
	Control rats	Protein/weight	0.197 $\pm$ 0.001	0.199 $\pm$ 0.002	0.197 $\pm$ 0.002
	Unburned limb		0.212 $\pm$ 0.003*	0.208 $\pm$ 0.004*	0.211 $\pm$ 0.003*
	Burned limb		0.169 $\pm$ 0.002*†	0.162 $\pm$ 0.003*†	0.166 $\pm$ 0.003*†
Plantaris muscle	Control rats	Mass (mg)	34.4 $\pm$ 0.3	35.4 $\pm$ 0.3	35.1 $\pm$ 0.3
	Unburned limb		32.5 $\pm$ 0.4*	34.7 $\pm$ 0.5	35.2 $\pm$ 0.4
	Burned limb		31.7 $\pm$ 0.4*	30.3 $\pm$ 1.2*†	30.4 $\pm$ 0.5*†
	Control rats	Protein/weight	0.198 $\pm$ 0.002	0.191 $\pm$ 0.002	0.193 $\pm$ 0.002
	Unburned limb		0.219 $\pm$ 0.003*	0.205 $\pm$ 0.002*	0.200 $\pm$ 0.002
	Burned limb		0.193 $\pm$ 0.002†	0.192 $\pm$ 0.006†	0.199 $\pm$ 0.003
Liver	Control rats	Mass (mg)	1264 $\pm$ 14	1294 $\pm$ 15	1338 $\pm$ 30
	Burned rats		1278 $\pm$ 22	1278 $\pm$ 21	1262 $\pm$ 27
	Control rats	Protein/weight	0.206 $\pm$ 0.003	0.205 $\pm$ 0.002	0.207 $\pm$ 0.003
	Burned rats		0.206 $\pm$ 0.003	0.204 $\pm$ 0.003	0.207 $\pm$ 0.003

2–4 days post-burn. However, by the fourth day post-burn, the liver protein mass in burned rats was only 95% ($P = 0.068$) of the control value.

Table 1 suggests that control and unburned-hindlimb muscles exhibit a trend towards growth between 2 and 4 days post-burn, whereas this trend is absent from burned-limb muscles. Similarly, liver of controls exhibits a growth trend, whereas the protein mass of liver of burned rats remains unchanged. To assess growth of the tissue protein pool more accurately, groups of three rats were formed whose body weights differed by no more than 2g on the day of the burn injury. One animal from each group was killed per day at 2, 3 and 4 days later. Each group of three was then considered as one 'n' value when growth was calculated in %/day (Table 2). Between 2 and 4 days, protein growth in soleus muscle of the unburned limb of burned rats did not differ from control. In contrast, the burned-limb soleus not only failed to grow, but actually atrophied at almost 6%/day ($P < 0.005$). In plantaris muscle, similar growth trends were observed, except that the growth of the unburned-limb muscle protein pool exceeded that of controls ($P < 0.003$). Growth of the liver protein mass in burned and control animals is also compared in Table 2. In contrast with liver from control animals, that of rats burned on one hindlimb failed to show any growth trend during 2–4 days post-burn ($P < 0.05$). To determine whether these alterations in tissue growth reflected synthetic or

degradative changes, the fractional synthetic rates of muscle and liver were assessed.

Effect of burn injury on muscle protein dynamics

Table 2 illustrates the fractional rate of protein synthesis, k_s , in soleus and plantaris muscles from controls and the burned and unburned limbs of burned rats. In soleus muscles, the rates in both thermally injured and uninjured muscles of burned rats were stimulated above control by 114% ($P < 0.001$) and 62% ($P < 0.04$) respectively. Furthermore, protein synthesis in the burned-limb muscle was increased by 32% ($P < 0.05$) when compared with the contralateral unburned-limb muscle. Plantaris muscle from the unburned limb of burned rats exhibited a protein-synthetic rate that did not differ from controls. In contrast, protein synthesis in burned-limb plantaris muscle was elevated by 67% above that in controls ($P < 0.02$). Although the burned-limb plantaris muscle did not differ from the contralateral unburned-limb muscle when the standard *t* test was used, its protein synthesis was elevated by 20% ($P < 0.05$) over that of the corresponding unburned-limb muscle when the paired *t* test was employed.

When control soleus and plantaris muscles are compared with one another, the rate of protein synthesis by soleus muscle was 66% higher ($P < 0.02$). A similar pattern was also evident in muscles from burned rats, with the rate in soleus

Table 2. *Muscle and liver protein turnover 3 days after a single-hindlimb scald in the rat*

Muscles from control rats are compared with muscles from both the unburned limb and the contralateral burned limb of burned rats. To measure the fractional growth rate of the tissue protein pool, groups of three rats were formed whose body weights ranged no more than 2 g on the day of the burn injury. One animal from each group was killed per day at 2, 3 and 4 days later after overnight deprivation of food. Each group of three animals was then considered as one 'n' value when growth was calculated in %/day. Tissue samples were digested in 1M-NaOH at 100°C. Duplicate portions of each sample were assayed for protein content by using the Lowry procedure as modified by Hartree (1972) except that no incubation at 50°C was performed. Each value of fractional growth rate is a mean with $n = 12-14$. Fractional protein synthetic rate was measured by [^{14}C]tyrosine constant infusion technique (Garlick *et al.*, 1973). Fractional protein-degradation rate was calculated from the difference between the fractional synthetic and growth rates for each group of tissues. Each value of fractional protein synthetic or degradation rate is an average for five to 14 rats. Significant difference from controls ($P < 0.05$) is indicated by an asterisk (*). Difference from the unburned limb of burned rats ($P < 0.03$) is denoted by a dagger (†).

Tissue	Group	Fractional growth rate (%/day, $\pm$ S.E.M.)	Fractional synthesis rate (%/day, $\pm$ S.E.M.)	Fractional breakdown rate (%/day, $\pm$ S.D.)
Soleus muscle	Control rats	1.28 ± 1.25	8.78 ± 0.79	7.50 ± 4.67
	Unburned limb	3.01 ± 1.83	$14.23 \pm 1.11^*$	11.22 ± 7.38
	Burned limb	$-5.92 \pm 1.71^{*\dagger}$	$18.81 \pm 1.65^{*\dagger}$	$24.73 \pm 7.57^{*\dagger}$
Plantaris muscle	Control rats	1.02 ± 0.59	5.00 ± 0.68	3.98 ± 2.52
	Unburned limb	$3.94 \pm 0.50^*$	6.96 ± 1.02	3.03 ± 3.11
	Burned limb	$-2.17 \pm 0.76^{*\dagger}$	$8.36 \pm 0.90^*$	$10.52 \pm 3.60^{*\dagger}$
Liver	Control rats	2.77 ± 1.11	108 ± 6	105 ± 13
	Burned rats	$-0.59 \pm 1.16^*$	$168 \pm 30^*$	$169 \pm 66^*$

muscle being 104% higher in the unburned limb ($P < 0.001$) and 125% higher in the burned limb ($P < 0.001$).

Protein breakdown in skeletal muscle can be calculated as the difference between the rates of protein synthesis and growth, because no appreciable protein is exported. As also shown in Table 2, the rate of protein degradation in soleus muscle from the unburned limb of burned rats did not differ from control. However, protein breakdown by soleus muscle of the burned limb was accelerated by 230% ($P < 0.001$) compared with controls and by 120% ($P < 0.003$) compared with the contralateral unburned limb. Similar results were obtained with plantaris muscle, where proteolysis by burned-limb muscle was increased by 164% above controls and by 247% over that of the unburned limb ($P < 0.001$). Thus thermal injury leads to local stimulation of both protein synthesis and breakdown by skeletal muscle. Systemic effect of burn injury is also observed, but is restricted to stimulation of protein synthesis in soleus muscle.

Effect of burn injury on liver protein dynamics

The fractional synthetic rate by liver of burned rats was elevated by 56% ($P < 0.01$) over that of controls (Table 2).

Liver proteolysis can be calculated as the difference between the rates of protein synthesis and growth because the 6h-constant-infusion method does not measure plasma protein synthesis (McNurlan & Garlick, 1980). Since the stimulated protein synthesis in liver of burned rats occurred

concomitantly with arrested protein growth (Table 2), the rate of hepatic proteolysis, calculated from the two above values, was increased by 61% ($P < 0.002$) above controls (Table 2). Thus the data indicate that both hepatic protein synthesis and breakdown are stimulated by the single-hindlimb burn injury.

Discussion

A 3 s single-hindlimb scald produces an injury of moderate severity with only modest systemic responses (Turinsky & Shangraw, 1979; Turinsky, 1983). It enables comparison of metabolic changes in tissues located near the site of injury with those more remote within the same animal, and tissues from both burned and unburned regions of burned animals can be compared with those from uninjured controls. In this way, both local and systemic responses to burn injury can be separately evaluated. Previously studies in our laboratory (Shangraw & Turinsky, 1982) have shown that protein synthesis by soleus muscle *in vitro* is elevated by 128% in the burned limb, whereas that from the contralateral unburned limb was not different from controls. The present study confirms our finding of stimulated protein synthesis by soleus muscle in the burned limb *in vivo*, where it is enhanced by 114% above control and by 62% above that by unburned soleus muscle from the same animal. In addition, plantaris-muscle protein synthesis in the burned limb is also increased, by

67% above control and by 20% above that by the contralateral plantaris.

Soleus muscle from the unburned limb of burned rats exhibited an increased rate of protein synthesis *in vivo* in relation to control, which was not apparent *in vitro* (Shangraw & Turinsky, 1982). However, neither the plantaris nor the gastrocnemius muscle (R. E. Shangraw & J. Turinsky, unpublished work) from the unburned limb synthesized protein at a rate different from control. Soleus muscle thus appears more responsive to systemic effects of the burn injury involving a small body surface area than are the plantaris and gastrocnemius muscles.

Two potential reasons must be considered for the systemic effects of the single-hindlimb scald on soleus muscle. One is the moderate hyperinsulinaemia that is sometimes seen in rats 3 days post-burn (Turinsky, 1983). Jefferson *et al.* (1977) have demonstrated that insulin exerts a powerful effect on protein synthesis by skeletal muscle. Further study is warranted into the relationship between insulin and muscle protein synthesis in rats burned on one hindlimb. The second possibility for enhanced protein synthesis in the unburned-limb soleus muscle in burned rats is increased activity as a consequence of the animal's tendency to disuse the injured limb (Shangraw & Turinsky, 1982). However, this appears unlikely, because immobilization of the unburned limb did not decrease soleus protein synthesis *in vitro* (Shangraw & Turinsky, 1982) and because similar alterations were not present in the unburned limb plantaris muscles *in vivo*.

We observed that during post-burn recovery the uninjured soleus muscle is more sensitive to systemic factors that affect protein synthesis than is plantaris muscle. This contrasts with the findings of other investigators that slow oxidative muscle (e.g. soleus) is more resistant to nutritional and endocrine influences than is muscle consisting predominantly of fast glycolytic and fast oxidative glycolytic fibres (e.g. plantaris, gastrocnemius, extensor digitorum longus). These investigators reported that protein synthesis in fast muscles is more readily inhibited than in the soleus as a result of starvation or alloxan-induced diabetes (Li & Goldberg, 1976; Flaim *et al.*, 1980). Furthermore, Goldberg (1969) has shown that cortisone treatment causes marked atrophy of plantaris muscle, but has only a small effect on the soleus muscle. However, our data are consistent with the findings by Buse (1981) that *in vivo* protein synthesis in soleus muscle is increased by infusion of branched-chain amino acids, whereas the gastrocnemius muscle is unaffected. Her work on gastrocnemius protein synthesis has been confirmed (McNurlan *et al.*, 1982). Thus it appears that fast muscles are

more responsive to stimuli which inhibit protein synthesis, whereas slow muscle is more responsive to factors which stimulate protein synthesis above the normal state.

Because muscle protein is largely retained in the tissue after synthesis, the breakdown rate *in vivo* is easily calculated as the difference between fractional growth and synthesis rates (Garlick *et al.*, 1975). The present study showed that the stimulated rate of protein synthesis in thermally injured skeletal muscle is more than matched by proteolysis, resulting in a net muscle breakdown *in vivo* at 3 days post-burn. Neither soleus nor plantaris muscle from the unburned limb of burned rats showed a statistically significant alteration of the fractional breakdown rate compared with muscle from uninjured controls.

With regard to the mechanism(s) of the altered protein metabolism in thermally injured muscles, studies of soleus muscles *in vitro* (Shangraw & Turinsky, 1982) have shown that disuse of the burned limb may account for a fraction of the excessive proteolysis by muscle from the burned region. Subsequent experiments on the status of electrolytes in muscle from the burned limb have revealed that normal or near-normal gradients for Na^+ , K^+ , Mg^{2+} and P_i are re-established by 3 days post-burn (Turinsky & Gonnerman, 1982). Cellular Ca^{2+} , however, is increased 18-fold (Turinsky & Gonnerman, 1982). Conclusive statements about the true significance of the elevated cellular Ca^{2+} content are precluded by our uncertainty of the exact location of the Ca^{2+} within the cells, but elevated cellular Ca^{2+} content is associated with increased protein breakdown in skeletal muscle (Kameyama & Etlinger, 1979; Lewis *et al.*, 1982). Thus Ca^{2+} may be implicated as a possible stimulator of proteolysis in thermally injured muscle. Enhanced protein synthesis, which may be a direct component of the repair process in the thermally injured muscle, may also be related to elevated cellular Ca^{2+} content (Kameyama & Etlinger, 1979), although contradictory observations have been reported (Lewis *et al.*, 1982). It is noteworthy that Clowes *et al.* (1983) have characterized a circulating factor produced after trauma that stimulates muscle proteolysis. If this factor were to be responsible for stimulated proteolysis, which in our model is restricted to muscles in the burned region, our data would be consistent with a high concentration of this factor in the region of the burn, but with its absence (or insignificant concentration) in the systemic circulation.

Liver protein synthesis in burned rats is accelerated at 3 days post-burn, which is consistent with findings after femur fracture (Stein *et al.*, 1976b, 1977), surgical trauma and pancreatitis (Sakamoto *et al.*, 1983). Since it is believed that the 6h

constant infusion of tyrosine does not measure plasma protein synthesis by the liver (McNurlan & Garlick, 1980), and because the hepatic protein pool of burned rats failed to grow simultaneously, it is concluded that the rate of hepatic protein synthesis is matched by augmented cellular proteolysis. We attempted to evaluate hepatic protein degradation by measuring the ratio of L-tyrosine specific radioactivities of liver cells and plasma (Gan & Jeffay, 1967) (R. E. Shangraw & J. Turinsky, unpublished work). It could be assumed that accelerated proteolysis would increase the quantity of intracellular tyrosine, which in turn would decrease the ratio of liver cell/plasma tyrosine specific radioactivities. However, there was no significant difference in the cell/plasma tyrosine specific radioactivities between control [37.52 ± 2.66 (mean $\pm$ S.E.M.)] and burned-rat liver (30.34 ± 6.36). Further investigation is warranted into relationship between the rate of hepatic proteolysis measured by the difference between protein synthesis and growth rates, and dilution of the ^{14}C label with unlabelled tyrosine in burned rats.

In conclusion, both protein synthesis and breakdown are stimulated in the soleus and plantaris muscles of thermally injured rats. Concurrently, protein turnover by liver and protein synthesis by soleus muscle from the uninjured region are also augmented. It therefore appears that both local and systemic responses to injury converge in the animals recovering from a single-hindlimb scald. The enhanced muscle and liver protein synthesis is energy-expensive (Pain, 1978), and thus may underly hypermetabolism observed in the post-burn state (Kien *et al.*, 1978a,b).

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