Nitrogen retention in rats fed on diets enriched with arginine and glycine

1. Improved N retention after trauma

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1. Nitrogen retention was measured in adult rats (250–350 g) subjected to the trauma of hind-leg fracture and given diets with or without arginine plus glycine supplementation. Observations were also recorded on excretion of creatine, creatinine, allantoin, and orotic acid. Liver and skeletal muscle transaminase activities were also determined.

2. When traumatized rats weighing approximately 250 g were given a diet with 200 g casein/kg, supplemented with 20 g arginine and 10 g glycine/kg (EC diet) or a casein diet made isonitrogenous with the EC diet by addition of aspartic acid (C diet), a 60–70 % increase in N retention was observed for the first 5 d post-injury for animals consuming the EC diet. A soya-bean (S) diet, isonitrogenous to the diet containing 20 % casein, supplemented with arginine and glycine was as effective as the EC diet in promoting significantly better N retention of traumatized rats (350 g) in comparison to rats given the C diet.

3. When the dietary casein content was reduced to 100 g/kg, supplements of 10 g arginine and 5 g glycine or 20 g arginine and 10 g glycine/kg did not improve N retention. It is suggested that both protein quality and protein quantity are important following injury.

4. An increased excretion of creatine was observed in traumatized rats given the high-protein diets supplemented with arginine and glycine. No consistent changes were noted for urine creatinine.

5. Urine allantoin levels remained stable after leg-fracture in rats consuming either the C or EC diets. Differences in the levels of urine orotic acid were found during both the pre- and post-injury periods in rats given the C, EC or S diets.

6. The mechanisms responsible for the improved N retention of traumatized rats consuming the high-protein diets with supplements of arginine and glycine may be related to the role of arginine both as a constituent of muscle tissue and as an intermediate in the urea cycle.

7. In traumatized rats fed the C or EC diets, liver transaminase activity increased whereas the transaminase activity in skeletal muscle decreased. These results support the recent concept that the increased excretion of N following injury arises from diminished reutilization of amino acids by muscle tissue without an acute increase in the rate of muscle catabolism.

Serious injury to man and rats is associated with increased excretion of urinary nitrogen. In man, the quantity of N lost may exceed the amount of N present in the liver (Cuthbertson, 1964). The severity and duration of the N response to injury are usually related to the type of trauma suffered, and have been shown to be modified by age (Cuthbertson, 1964), sex (Cuthbertson, 1970), previous protein nutrition (Cuthbertson 1964; Munro & Chalmers, 1945; Calloway, Grossman, Bowman & Calhoun, 1955; Browne, Schenker & Stevenson, 1944; Abbott & Albertsen, 1963), and environmental temperature (Cuthbertson, Fell, Rahimi & Tilstone, 1972; Davies, Liljedahl & Burke, 1970; Caldwell, 1962).

Conventional diet therapy programs following severe injury have been aimed at providing liberal amounts of protein and energy, parenterally, if necessary.

A few nutritional and pharmacological attempts directed at reducing N excretion in the post-traumatic period have been reported (Cuthbertson, Shaw & Young, 1941; Hinton, Allison, Littlejohn & Lloyd, 1971; Blackburn, Flatt, Clowes, O'Donnell & Hensle, 1971) with varying success.

Evidence to show that the extra urinary N is non-hepatic in origin led to the conclusion that, after injury, there occurs a generalized catabolism of muscle protein (Cuthbertson, 1964; Munro & Chalmers, 1945). This concept has recently been challenged by O'Keefe, Sender & James (1974), whose results suggest that the N response to injury involves a decrease in the normal rate of muscle protein synthesis without an acute increase in the rate of proteolysis.

It is conceivable that the conditions of traumatic shock which modify protein metabolism may result in alteration of the amino acid requirements of the body. The basis for this approach is the possibly changed pattern of amino acids needed for the synthesis of different tissue proteins and the possible inability of the animal to maintain synthesis of semi-dispensable or totally dispensable amino acids towards this end. To carry out repair of muscle tissue requires, among other nutrients, substantial amounts of the two amino acids arginine and glycine. According to Cuthbertson, Fell, Smith & Tilstone (1972), skin and connective tissue protein have 'a very considerable requirement for the biosynthesis of glycine and a considerable requirement for arginine'. For optimal growth the weanling rat apparently cannot produce sufficient endogenous glycine and benefits from a dietary source (Breuer, Pond, Warner & Loosli, 1964).

Since trauma is associated with an increase in the catabolism of amino acids (either due to increased muscle protein breakdown or decreased muscle protein synthesis), the demands for arginine might also be increased because of its role in the urea cycle, where it is essential for the detoxification of ammonia (Greenstein, Winitz, Gullino, Birnbaum & Otey, 1956) arising from the catabolism of other amino acids.

This paper describes the results of experiments in which N retention was measured in adult rats subjected to the trauma of anaesthesia + hind-leg fracture and fed on diets supplemented with arginine plus glycine. A preliminary report of this work has appeared (Sitren, Fisher & Griminger, 1975). We also recorded observations on allantoin, orotic acid, creatine and creatinine excretion and tissue transaminase activity as influenced by traumatic shock.

EXPERIMENTAL

General

Four experiments were carried out with adult, male Sprague–Dawley rats weighing between 250 and 350 g. Prior to the start of experiments, all animals were provided with a stock diet (Purina Rat Chow, Ralston Purina Co., St Louis, Mo., USA) and tap-water *ad lib.*

Each experiment consisted of two sequential periods: a pre-injury period of 8–12 d duration and a post-injury period of 5 or 7 d. Semi-purified diets were given during these periods.

Table 1. *Composition (g/kg) of the diets given to rats*

Ingredient	High-protein casein		Low-protein casein		High-protein soya-bean (S)
	Control (20C)	Experimental (20EC)	Control (10C)	Experimental (10EC)	
Casein (800 g protein/kg)	200	200	100	100	—
Isolated soya-bean protein (900 g protein/kg)	—	—	—	—	178
DL-methionine	3	3	2	2	3
L-arginine HCl	—	24	—	12	15
Glycine	—	10	—	5	6
L-aspartic acid	78	—	39	—	64
Cellulose	20	20	20	20	20
Sucrose	500	500	500	500	500
Maize oil	50	50	50	50	50
Vitamin mix*	2	2	2	2	2
Mineral mix†	40	40	40	40	40
Choline chloride (700 mg/g)	1	1	1	1	1
Maize starch	106	150	246	268	121

* To provide (mg/kg diet): thiamin HCl 25, riboflavin 16, calcium pantothenate 20, pyridoxine HCl 6, biotin 0·6, folic acid 4, 2-methyl-1,4-naphthoquinone 5, cyanocobalamin 0·02, ascorbic acid 250, nicotinic acid 150, retinyl acetate (30 μg retinol equivalent) 100, cholecalciferol (6 μg/mg) 2·4, DL-α-tocopheryl acetate (0·5 mg α-tocopherol equivalent) 20.

† To provide (mg/kg diet): sodium chloride 5·58, potassium phosphate 15·56, magnesium sulphate 2·29, calcium carbonate 15·26, ferrous sulphate 1·08, potassium iodide 0·32, manganese sulphate 0·18, zinc chloride 0·010, copper sulphate 0·019, cobalt chloride 0·001.

At the start of each experiment, rats were assigned into groups of similar body-weight and housed in individual metabolism cages.

Traumatization of the rats was carried out under diethyl ether anaesthesia between 08.00 and 10.00 hours. The method of leg fracture has already been described (Sitren, Fisher & Ali, 1975). Following recovery from anaesthesia, the rat showed decreased mobility about the cage; by next morning the animal could stand on both hind-legs again and activity appeared normal. In this study the control animals were not anaesthetized (but see paper 2, Sitren & Fisher, 1977).

The results from each experiment were subjected to analysis of variance according to the principles outlined by Steele & Torrie (1960). A probability level of 5% ($P < 0.05$) was selected as the criterion for statistical significance between the means.

In experiments where N retention was measured, excreta from the entire post-injury period and from the last 3 d of the pre-injury period were collected daily and frozen at $-15°$ pending analysis.

Throughout the text the following code is used to describe the treatment groups: C-U, C diet pre- and post-stress, animals unstressed; C-F, C diet pre- and post-stress, animals with leg fracture; C-EC-F, C diet pre- and EC diet post-stress, animals with leg fracture; EC-F, EC diet pre- and post-stress, animals with leg fracture; S-F, S diet, animals with leg fracture.

Diets

The compositions of the diets are given in Table 1. The high (200 g/kg) and low-(100 g/kg) protein experimental casein (20EC and 10EC) diets were supplemented

with arginine and glycine to provide levels three times those normally found in diets with 200 and 100 g crude casein/kg, respectively (Block & Bolling, 1951). The control diets with 200 and 100 g casein/kg (20C and 10C) were made isonitrogenous to the experimental diets by the addition of L-aspartic acid. The soya-bean (S) diet was formulated to contain an amount of soya-bean protein isonitrogenous with 200 g casein/kg. Supplements of arginine and glycine were added so that the final concentration of these two amino acids was equal to that found in 20EC diets. Aspartic acid was also added to the S diet to make it isonitrogenous with the diet 20EC.

Analytical procedures

Urinary and faecal N were estimated by a Kjeldahl technique. Urinary urea N was assayed by an automated procedure (Technicon Instruments Corp., Tarrytown, N.Y., USA, method N-1C), which was modified after the one described by Marsh, Fingerhut & Miller (1965) as were urinary creatinine (Technicon method N-11b, modified after Oser (1965)) and creatine (Griffiths, 1964). The method of Pentz (1969) was used to measure the concentration of allantoin in urine. Orotic acid was assayed by the combined methods of Stajner, Suva & Musil (1968) and of Rogers & Porter (1968). Transaminase activity, represented by L-aspartate aminotransferase (*EC* 2.6.1.1) (GOT), and L-alanine aminotransferase (*EC* 2.6.1.2) (GPT) was determined with the use of an automated kinetic analyser (Abbot Bichromatic Analyzer-100, Abbott Scientific Products Division, South Pasadena, Calif, USA). Total protein in tissue homogenates was estimated by an automated technique (Technicon method N-72-I/II), based on the method of Lowry, Rosebrough, Farr & Randall (1951).

Experimental

Groups of five or six rats of approximately equal body-weight were used for each treatment. In Expt 1, the rats weighed approximately 260 g and the high-protein 20C and 20EC diets were given and in Expt 2 body-weights were approximately 290 g and the low-protein 10C and 10EC diets were given. In Expt 3, body-weights were approximately 335 g and the S diet was compared with the high-protein 20C and 20EC diets. In Expt 4, rats given diets 20C or 20EC, and weighing 300–350 g were killed at 0, 1, 3 and 7 d post-injury. Gastrocnemius muscle from uninjured limbs and samples of liver tissue were quickly excised and were homogenized in cold, distilled-deionized water according to the procedure of Bird, Berg & Leathem (1968).

In Expt 1 and 2, faeces were not analysed for N content since it has been shown that faecal N does not change as a result of trauma (Cuthbertson, 1936; Upjohn & Levenson, 1958). In Expt 3, however, a complete N balance was carried out.

Paired feeding was used in all experiments.

RESULTS

The balance results for rats in Expt 1 are shown in Fig. 1. No significant differences were observed for the 3 d pre-injury period. On day 1 post-fracture there was a significant decrease in N retention for all treatment groups as well as the uninjured

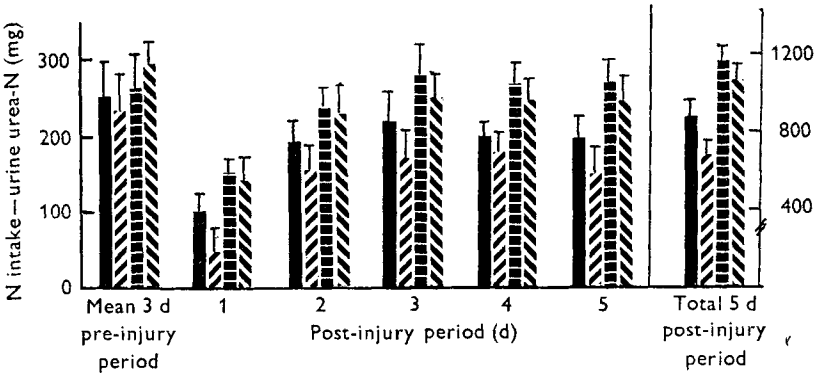

Fig. 1. Nitrogen retention of traumatized rats given a diet with 200 g casein/kg without (C) or with 20 g arginine and 10 g glycine supplementation (EC), ▬, C-U (C diet pre- and post-stress; unstressed); ▨, C-F (C diet pre- and post-stress; leg fracture); ▦, C-EC-F (C diet pre- and EC diet post-stress; leg fracture); ▩, EC-F (EC diet pre- and post-stress; leg fracture).

control group. Group C-F showed the least positive N balance on day 1. Diminished food intake (rats in groups C-U, EC-F and C-EC-F were pair-fed the amount consumed by rats in group C-F) was partially responsible for these results, since group C-U retained only 41 % as much N as in its mean 3 d pre-injury period.

Food intake of the fracture groups decreased by approximately one-third on the first day after injury but by day 3 it was back to 90 % of the pre-injury level.

The two fracture groups consuming the 20EC diet after injury retained significantly greater quantities of N than injured rats given the 20C diet for each of the first 5 d post-injury.

The 5 d totals showed that, compared with the C-F group, groups C-EC-F and EC-F had, respectively, 73 % and 58 % higher N-balance figures. There were no significant differences between groups C-EF-F and EC-F. Therefore, it made no difference whether the EC or C diet was given prior to injury, as long as the EC diet was consumed post-injury. Rather surprising were the results that the two fracture groups receiving the EC diet had significantly better N balances than the uninjured control group C-U, for the 5 d post-injury period.

In Expt 2, urinary urea N was used as the indicator of N excretion. In preliminary studies a highly significant ($P < 0{\cdot}001$, $r + 0{\cdot}83$) correlation between total urinary N and urea-N was found in both the pre- and post-injury periods. Urinary urea-N accounted for 75 % of the total N excreted. The difference between N intake and urinary urea-N for this experiment is shown in Fig. 2.

There were no significant differences found for the pre-injury period. On day 1 post-injury, all groups again showed a decrease in N retention. The unstressed control group (C-U) went from a mean pre-injury level of 178 to 147 mg N, a 17 % decrease. This amount equalled that due to the reduction in food consumption on day 1 post-injury. The 5 d N-retention totals showed that there were no significant differences among the traumatized rats. All three fracture groups had significantly lower N-retention values than the unstressed control group ($P < 0{\cdot}05$).

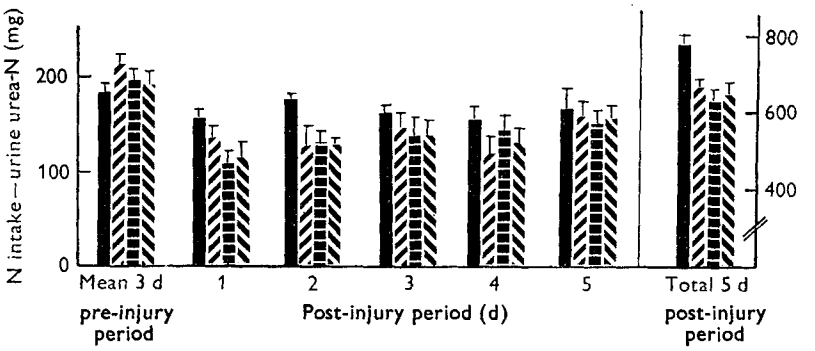

Fig. 2. Nitrogen retention of traumatized rats given a diet with 100 g casein/kg without (C) or with 10 g arginine and 5 g glycine supplementation (EC). ▬, C-U (C diet pre- and post-stress; unstressed); ▨, C-F (C diet pre- and post-stress; leg fracture); ▦, C-EC-F (C diet pre- and EC diet post-stress; leg fracture; ▩, EC-F (EC diet pre- and post-stress; leg fracture).

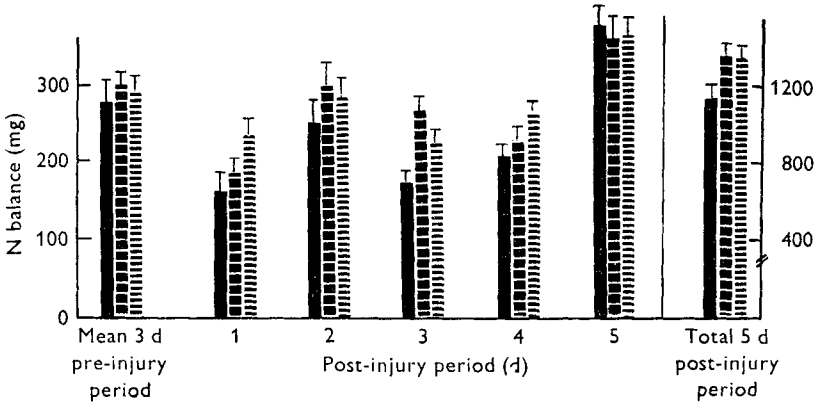

Fig. 3. Nitrogen balance of traumatized rats given a casein diet (C), a casein diet supplemented with arginine and glycine (EC), or a soya-bean diet supplemented with arginine and glycine (S). ▬, C-F (C diet; leg fracture); ▦, EC-F (EC diet; leg fracture); ▥, S-F (S diet; leg fracture).

The addition of arginine and glycine to a diet with 100 g casein/kg did not improve N retention of traumatized rats whereas rats on the high-protein (200 g/kg) diet derived considerable benefit from the amino acid supplementation.

In comparing the 10EC diet given in Expt 2 with the 20EC diet used in Expt 1, although the ratios (arginine + glycine):(total protein) were the same, the absolute amounts differed by a factor of 2. Therefore another trial was carried out in which the low-protein casein diet was supplemented with 20 g arginine and 10 g glycine/kg. The results (not shown here) demonstrated no significant improvement in N retention of traumatized rats consuming this diet when compared with injured rats given the casein control diet without arginine plus glycine.

Thus N retention of traumatized rats was not improved by supplements of arginine plus glycine to a diet with 100 g casein/kg whereas these same supplements added to

Table 2. *Urinary metabolites of traumatized rats given a casein diet (C), a casein diet supplemented with arginine and glycine (EC), or a soya-bean diet supplemented with arginine and glycine (S)*

	Mean 3 d pre-injury period		Post-injury period									
			Day 1		Day 2		Day 3		Day 4		Day 5	
Group†	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
C-F												
Creatinine (mg/kg body-wt)	36·1	0·6	33·4	0·4	34·7	1·0	30·3	1·2*	28·3	2·2*	32·2	1·0
Creatine (mg/kg body-wt)	3·7	0·1	3·5	0·0	4·1	0·1	3·8	0·2	3·8	0·1	4·3	0·1
Orotic acid (μg)	23·8	1·9	14·7	2·0*	11·5	1·1*	23·4	1·7	30·5	2·2*	28·0	1·1
EC-F												
Creatinine (mg/kg body-wt)	38·7	0·9	33·0	2·5*	37·0	0·7	31·0	2·6*	38·6	0·9	32·2	2·4*
Creatine (mg/kg body-wt)	9·0	1·6	8·2	0·8	23·5	5·5*	17·9	3·6*	56·4	4·7*	40·9	2·6*
Orotic acid (μg)	15·9	1·0	19·6	1·6	20·8	2·1*	22·0	1·5*	24·4	1·4*	20·2	1·9
S-F												
Creatinine (mg/kg body-wt)	37·7	1·3	34·9	2·8	38·5	1·1	34·7	2·2	39·7	0·8	34·1	1·5
Creatine (mg/kg body-wt)	7·5	0·7	16·9	4·2*	45·4	4·1	46·0	3·9*	77·5	3·4*	57·1	3·5*
Orotic acid (μg)	16·1	1·0	16·4	1·8	19·0	1·7	20·9	1·7*	22·4	1·7*	18·6	2·1

* Value significantly different from that for mean 3 d pre-injury period: $P < 0.05$.

† C-F = C diet, day 5 post-fracture; EC-F = EC diet, day 5 post-fracture; S-F = S diet, day 5 post-fracture.

a diet with 200 g casein/kg led to significant improvement in N retention of injured rats.

The N balance results from Expt 3 are shown in Fig. 3. Faecal N for all rats ranged from 22 to 29 mg/d. The average coefficient of variation for the three groups of rats was only 14%. Differences in N retention between the treatment groups were, therefore, due primarily to variations in urinary N excretion. There were no significant differences in N balance during the pre-injury period. Following leg fracture, rats given the C diet showed lower N balance values than animals in groups EC-F or S-F for the first 4 d post-injury. The 5 d post-injury totals demonstrated that rats in groups EC-F and S-F retained 14% more N than animals in group C-F. This amount, although significant ($P < 0.05$), was less than the differences recorded previously for injured rats given diets 20C or 20EC. This discrepancy will be discussed later.

In Expt 1 the average pre-injury urinary allantoin level for all five groups was 10·7 mg/100 g body-weight per d. This amount is similar to values reported by Pentz (1969) and Pak, Donoso & Tagle (1973) for normal rats. In the post-injury period, no significant changes were found.

Urinary metabolites from rats in Expt 3 are given in Table 2. Groups C-F and EC-F excreted significantly lower quantities of creatinine during several days of the post-injury period. However, there was no consistent pattern to these changes. Group S-F did not show any significant differences in creatinine excretion after injury. Some of these results contrast with early work by Cuthbertson, McGirr & Robertson (1939) in which no changes in creatinine excretion were found following hind-leg fracture in the rat.

Changes in urinary creatine excretion were striking for groups EC-F and S-F.

Table 3. *Specific activity (m-units†/mg protein) of liver and muscle* L-*aspartate aminotransferase (EC* 2.6.1.1) *(GOT) of traumatized rats given a diet with* 200 *g casein/kg without (20C) or with* 20 *g arginine and* 10 *g glycine/kg supplementation (20EC)*

Stress	Day post-stress	Liver Mean	Liver SE	Muscle Mean	Muscle SE
		Diet 20C			
None	—	615	45	1704	75
Leg fracture	1	933	71*	1496	144
Leg fracture	3	1177	26*	1923	202
Leg fracture	7	823	86*	1127	59*
		Diet 20EC			
None	—	537	88	1736	54
Leg fracture	1	1015	114*	1295	100*
Leg fracture	3	1175	37*	1184	71*
Leg fracture	7	982	57*	1215	50*

* Values significantly different from the unstressed group given the same diet: $P < 0.05$.
† One unit of enzyme activity is that amount that will catalyse the transformation of 1 μmole of substrate/min under the conditions of the assay (see text for references).

Table 4. *Specific activity (m-units†/mg protein) of liver and muscle* L-*alanine aminotransferase (EC* 2.6.1.2) *(GPT) of traumatized rats given a diet with* 200 *g casein/kg without (20C) or with* 20 *g arginine and* 10 *g glycine/kg supplementation (20EC)*

Stress	Day post-stress	Liver Mean	Liver SE	Muscle Mean	Muscle SE
		Diet 20C			
None	—	367	43	235	8
Leg fracture	1	385	21	162	17*
Leg fracture	3	533	19*	190	18
Leg fracture	7	302	25	170	6*
		Diet 20EC			
None	—	341	38	222	14
Leg fracture	1	383	23	134	18*
Leg fracture	3	458	13*	197	33
Leg fracture	7	132	11*	160	5*

* Values significantly different from the unstressed group given the same diet: $P < 0.05$.
† One unit of enzyme activity is that amount that will catalyse the transformation of 1 μmole of substrate/min under the conditions of the assay (see text for references).

Following injury, rats consuming the S diet doubled their creatine excretion on the first day. Creatine excretion then increased dramatically through the fourth day, at which time the level was ten times higher than normal. On day 5 the excretion of creatine decreased but still remained considerably higher than normal. Animals given the EC diet showed no change in urinary creatine level on day 1 post-injury. Creatine level then rose significantly on days 2 and 3 and reached a maximum on day 4, when it was six times higher than normal. On day 5 creatine excretion dropped but the concentration remained 4·5 times higher than normal. Contrary to this extensive

creatinuria seen for groups EC-F and S-F, rats in group C-F, by comparison, showed only minimal changes. There was an 11% increase on day 2 and a 16% increase on day 5 for group C-F. However, these differences from the level found in the pre-injury period did not attain statistical significance. The common variance used to test differences between the means was probably magnified by the supernormal values recorded for groups EC-F and S-F.

If arginine becomes a limiting amino acid following severe injury then a deficiency might be expected to reveal itself as an orotic aciduria. In the pre-injury period, orotic acid excretion was one-third less in groups EC-F and S-F than it was in group C-F, indicating that rats consuming the C diet were less efficient in disposing of their normal load of ammonia via the urea cycle. Following leg fracture, group C-F exhibited significantly depressed orotic acid excretion on days 1 and 2. The level returned to normal on day 3, increased significantly to a higher level on day 4, and finally decreased slightly on the fifth day but remained higher than normal.

In the post-injury period of groups EC-F and S-F urinary orotate showed no decrease. Rather, excretion in both groups increased steadily through the fourth day post-injury.

In Expt 4, specific activities of liver and muscle transaminase were measured in rats given the high-protein C and EC diets (used in Expt 1). L-aspartate aminotransferase (GOT) and L-alanine aminotransferase (GPT) are two important enzymes that aid in making amino groups available for entry into the urea cycle. Measurement of their activities provides an indication of amino acid catabolism. In livers of traumatized rats given diet C, GOT increased significantly (Table 3) the first day after stressing and remained elevated on days 3 and 7. Animals given diet C and subjected to leg fracture showed a decrease in muscle GOT, then an increase on day 3 to a level greater than that of unstressed rats, and finally a significant decline on day 7.

Muscle GOT activity of rats given diet EC changed differently; leg fracture resulted in a significant decrease in activity on day 1 which lasted till day 7. The activity remained at a significantly lower level.

Changes in GPT activity (Table 4) were not as great as those found for GOT activity. In rats given diets C and EC, liver GPT increased on day 3 only. On day 7 rats consuming diet EC had significantly decreased liver GPT concentrations, only 39% of normal.

Muscle GPT decreased a significant 31% on the first day in rats consuming diet C. This level increased slightly on day 3 and then decreased again on day 7. In rats fed on diet EC, injury resulted in a 40% decrease in activity on day 1. GPT then increased on day 3 to 89% of normal before it decreased again on day 7.

In general, liver GOT activity increased after injury in rats given either diet. Liver GPT increased significantly on the third day after leg fracture in rats given diet C and in those given diet EC. Muscle transaminase activity showed a general decrease following trauma in rats given diet C or diet EC. One notable exception occurred on the third day for GOT levels. In traumatized rats consuming diet EC, enzyme activity decreased 32%, whereas the activity in injured animals given diet C increased 13%.

DISCUSSION

In assessing the effects of trauma in this study, attention is drawn to the possible separate contribution of the leg fracture and the anaesthesia. The subsequent paper describes the response of the rat to ether exposure equivalent to that necessary to impose the fracture. The effect of leg fracture without anaesthesia was obviously inadmissible. In our estimation the animal's metabolic behaviour to ether exposure represents a considerable portion of the total fracture + anaesthesia response. Nevertheless, some of the observations in the two studies differ as between ether alone and ether + leg fracture.

The improved N retention of traumatized rats consuming the high-protein diets supplemented with arginine and glycine may be related to the dual metabolic role of arginine in protein metabolism. This amino acid is required for normal functioning of the urea cycle as well as for protein synthesis. Although in the adult rat, arginine is considered a dispensable amino acid ((US) National Research Council, 1972), there may be a limited capacity for synthesis. The deletion of arginine from the diet has resulted in the excretion of large quantities of orotic and citric acids and also increased urinary urea (Milner & Visek, 1973; Milner, Wakeling & Visek, 1974; Milner, Visek & Hague, 1975).

Since traumatic shock results in increased amino acid degradation, there may be increased need for arginine for the urea cycle. The amount of arginine present in a 200 g/kg casein or soya-bean diet may not be adequate to operate the urea cycle while at the same time promoting normal tissue maintenance and repair after injury. In addition to the arginine requirement for normal maintenance of body protein and normal functioning of the urea cycle, the demand for this amino acid might be increased in two ways: (1) for repair of injured tissue, and (2) to cope with an accelerated urea cycle, resulting from the need to detoxify additional ammonia produced by increased amino acid catabolism. Because of the serious metabolic consequences of hyperammonaemia, it is possible that the urea cycle needs for arginine might take precendence over the arginine needs for muscle maintenance and repair, thereby leading to a positive feedback condition. Without sufficient arginine, normal protein synthesis would diminish, thereby providing more amino acids for deamination, with subsequent detoxification of the N moiety. Urea cycle activity would, therefore, increase and consequently, so would the demand for arginine. These additional requirements for arginine might tax the limited reserves of this amino acid in the rat. The amount of arginine present in the high-protein 20C diet (6·7 g/kg) may not be adequate to cover an increased requirement after injury, whereas a supplement of arginine (20EC diet) apparently could meet these increased demands.

In Expt 2, traumatized rats derived no benefit from supplements of arginine and glycine. There are two possible explanations for this difference, both of which are related to the previously proposed regulatory role of arginine in reducing amino acid degradation.

It has been established that the magnitude of the N response to traumatic shock is directly related to the level of previous protein intake (Munro & Chalmers, 1945).

Injured rats given a diet with 100 g protein/kg would, therefore, be expected to excrete less N than animals consuming one with 200 g protein/kg. Rats on the low-protein diet would catabolize comparatively less amino acids and also have less of a demand for arginine as an intermediate of the urea cycle. The total requirement for arginine would not be as great as it would be for animals consuming a high-protein diet, so that additional dietary arginine would be of no benefit to animals consuming the low-protein diet.

The second point to be considered is the difference in total N content of the diets. The amount of N supplied by a diet with 200 g protein/kg is substantially higher than the N requirement of the adult rat (US National Research Council, 1972). The excess of amino acids is degraded by the liver in similar fashion to an endogenous supply. Therefore, urea cycle activity and arginine requirements would be higher than those in animals given the diet with 100 g protein/kg. Supplementary arginine would benefit only those rats given a high-protein diet. This suggests that both protein quality and protein quantity are important after injury.

Injured rats given diets supplemented with arginine and glycine in Expt 3 did not exhibit as large a difference in N retention as did those in Expt 1. One possible explanation may be the difference in body-weight at the time of injury. Animals in Expt 1 averaged 260 g whereas those in Expt 3 weighed 335 g.

In relation to the arginine requirement of the rat, it is conceivable that the ability to synthesize arginine is related to growth rate or body size or both. The growth rate was relatively slower for the larger rats. Consequently, the arginine requirement may have been less.

In Expt 3 the response to the arginine plus glycine supplementation was similar with both casein and soya-bean as the dietary protein source. This strengthens the evidence for a specific role played by the added amino acids, rather than attributing the response to a peculiarity associated with casein as the dietary protein.

The present studies do not permit a differentiation in response between glycine and arginine or both supplements. A role for glycine is conjectured first, because in the face of an increased need for skin and connective tissue repair endogenous synthesis may well be inadequate (Breuer *et al.* 1964); secondly, because the inclusion of supplemental arginine increases the glycine requirement for obligatory creatine synthesis (Fisher, Salander & Taylor, 1956*a*, *b*).

Orotic acid is an intermediate in the synthesis of pyrimidines and is normally found in the urine in very small amounts. Pyrimidine biosynthesis is interrelated with the biosynthesis of arginine since both pathways share a common substrate, carbamyl phosphate (Jones, 1970). It has been shown that urea cycle activity in the rat is depressed when a deficiency of arginine is present (Prior & Visek, 1973; Milner *et al.* 1974). A portion of the ammonia arising from the normal degradation of amino acids is shunted into pyrimidine biosynthesis. The result is an accumulation of orotic acid with consequent spill-over into the urine.

The decrease in urinary orotic acid (Table 3) on days 1 and 2 post-injury for group C-F rats could have indicated that the urea cycle was operating more efficiently. Since, after injury, increased quantities of amino acids passed to the liver for degrad ation

the amount of arginine present in this excess may have been sufficient to operate the urea cycle adequately. However, the quantity of arginine required for tissue protein synthesis and maintenance may still have been limiting. On days 4 and 5 post-injury, the increase in orotic acid would indicate a return to a deficient state in the urea cycle.

Interpretation of these results must take into consideration the possible effect of other factors arising from conditions of traumatic shock, which may influence the excretion of orotic acid in a manner unrelated to arginine metabolism. The course of protein metabolism is certainly affected under shock conditions and normal pyrimidine synthesis may also be altered.

Allantoin excretion in the rat and of uric acid in man are useful as an indication of the state of purine metabolism. The excretion of urinary allantoin by rats was not affected after leg fracture. More serious injury, such as that produced by neutron irradiation, has been reported to result in the hyper-excretion of allantoin (Pentz, 1969). Very early work by Cuthbertson (1931) established the presence of a uric aciduria in injured patients. It appears, however, that the effects of traumatic shock on purine metabolism in the rat do not parallel the changes seen in man. If the state of purine metabolism is altered in the rat, it does not manifest itself in terms of allantoin excretion.

Creatine metabolism in traumatized rats was greatly affected when high-protein diets supplemented with arginine and glycine were given (Table 3). It should be mentioned that the method of leg fracture involved the disruption of skeletal muscle and therefore caused some degree of soft tissue damage. Muscle is the major storage site for body creatine (Mitchell, 1962). The precursors of creatine, glycine, arginine and methionine (Bloch & Schoenheimer, 1941) were present in liberal amounts in diets EC and S.

The adult animal can store fairly large quantities of creatine (Allison & Bird, 1964) and the urinary output of creatine can be doubled by the addition of arginine, glycine and methionine to a normal diet (Brown & Allison, 1948).

Before injury, animals consuming diets EC or S excreted more than twice the amount of creatine as did rats given the C diet. It is quite probable that the rats given the EC or S diets stored considerable quantities of creatine in muscle tissue during the pre-injury feeding period. After fracture, the disturbance of muscle tissue probably released creatine in quantities which exceeded the renal threshold, resulting in creatine hyper-excretion. In general, the disturbance of muscle tissue leads to an increase in urinary creatine along with a corresponding fall in creatinine excretion (Baron, 1973). However, the profound increase in urinary creatine of traumatized rats fed diets supplemented with arginine and glycine was not associated with any consistent change in the excretion of creatinine. This suggests that a part of the metabolism or excretion of creatinine was independent of creatine metabolism after injury.

In relation to the protein metabolic response to trauma, the results of liver and muscle transaminase activity do not support the concept of a generalized breakdown of skeletal muscle following injury. It would be expected that liver transaminase activity would increase after trauma as a result of increased amino acid degradation. This is supported by the results of Expt 4 (Tables 4 and 5). If the protein metabolic

response to trauma results from an increase in the rate of muscle catabolism, then it might be expected that muscle transaminase activity would also increase. However, just the opposite occurred: a decrease in muscle GOT and GPT was observed. This would suggest that skeletal muscle did not undergo increased degradation consequent on injury, thus supporting the results of O'Keefe *et al.* (1974) who showed that the protein metabolic response to injury involved a decrease in muscle protein synthesis without an acute increase in the rate of catabolism.

Further work is needed in order to elucidate the exact source of the extra urinary N excreted following injury. Also needed are experiments designed to clarify the mechanisms responsible for the better N retention of stressed rats given diets with elevated levels of arginine and glycine.

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