PROTEIN METABOLISM AND PROTEIN RESERVES DURING ACUTE STERILE INFLAMMATION

High Protein Intake Compensates for Increased Catabolism*

By S. C. MADDEN, M.D., and W. A. CLAY

(*From the Department of Pathology, The University of Rochester School of Medicine and Dentistry, Rochester, New York*)

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INTRODUCTION

Are protein anabolic mechanisms inhibited for a period immediately after body injury? The evidence presented herein does not support a theory of inhibition. Such inhibition for one or more days after injury has been postulated because of the marked rise in urinary nitrogen and the difficulty of obtaining nitrogen balance (2). This rise in nitrogen excretion is much out of proportion to the local injury and must represent a more general reaction in body protein metabolism (3, 7). The evidence of this paper and of earlier ones noted in the Discussion below suggests that *catabolism of protein reserves* is responsible for the *increased output of nitrogen.*

Methods

Mongrel adult dogs vaccinated against distemper were used in all experiments. They were kept in metabolism cages and continual nitrogen determinations were done of the total urinary output for 1-, 2-, or 3-day periods, depending upon the course of the experiment. Stool collection periods varied from 7 to 10 days. Hair and epidermis losses were not calculated into the balance, creating a small error.

The basal diet in grams per 100 gm. was dextrose 80, corn oil 17, and salt mixture (19) 3, fed to provide 80 calories per kilo. When casein or amino acids were added, sufficient dextrose was deducted to maintain the same caloric intake. The basal "normal" nitrogen intake was about 0.4 gm. per kilo in most experiments (2.5 gm. protein per kilo). In one experiment it was 0.3 gm. nitrogen per kilo. For "high" intakes this quantity was doubled. Vitamins were supplied by a synthetic emulsion, 5 cc. daily per dog. This emulsion (Lilly MH43-8111) contains per 5 cc. vitamin A 2500 U.S.P. units, vitamin D 250 U.S.P. units, and in milligrams thiamin chloride 3, riboflavin 3, pyridoxin hydrochloride 2.5, calcium pantothenate 2.5, nicotinamide 25, ascorbic acid 25, mixed natural tocopherols 25, choline chloride 150, rice polish concentrate 500, 2-methyl-1,4-naphthoquinone 0.5, inositol 25, *p*-aminobenzoic acid 25, linoleic acid esters 250. During the low protein intake periods of dog 43-346 the vitamin supplements were altered as recorded in the experimental history.

The amino acid mixture Vuj consists of, in grams per 100 gm.: *dl*-threonine 10.8, *dl*-valine 13.8, *l*(−)-leucine 15.4, *dl*-isoleucine 10.8, *l*(+)-lysine·HCl. H_2O 12.3, *dl*-tryptophane 1.8, *dl*-phenylalanine 6.9, *dl*-methionine 6.2, *l*(+)-histidine·HCl. H_2O 4.0, *l*(+)-arginine·HCl

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8.0, glycine 10.0. It was prepared in sterile solution in distilled water with the addition of sodium lactate, 1 M solution of sodium content chemically equivalent to the chloride (4.5 per cent) content of the Vuj mixture. The solution had as final concentrations, amino acids 10 per cent and sodium lactate 1.42 per cent. Its pH was 5.0.

All nitrogen determinations were by macro-kjeldahl analysis. The nitrogen content of the commercial casein used was 14.0 per cent; of the amino acid mixture Vuj, 13.6 per cent.

The amino acid solution was given intravenously at the rate of 2 mg. nitrogen/kilo/minute except when glucose was also given in experiments of Tables 3 and 4. The glucose of these experiments was given at the maximum rate (18) of 0.8 gm./kilo/hour and the amino acids were accordingly slowed to about 1 mg. nitrogen/kilo/minute.

To produce a controlled reaction of inflammation, turpentine, 1 cc., was injected subcutaneously over the lateral thorax at a point approaching the lower rib margin. This chemical irritant stimulates a local inflammatory reaction with the production of an abscess in about 3 days and a general reaction with fever, leucocytosis, and increased nitrogen excretion (3). The reaction terminates promptly on the 3rd to 5th day with the rupture or incision of the abscess, and in the dog with normal protein reserves healing is rapid.

EXPERIMENTAL OBSERVATIONS

The five tables record the nitrogen metabolism of dogs disturbed by sterile inflammation during periods of controlled protein intake. The data for 2 or more days are averaged when variations are minor or not illuminating. In assessing day-to-day variations in the urinary nitrogen one need remember that the 24-hour periods were not separated by catheterization of bladder urine.

Table 1, dog 43-346, shows first, the reaction of a *normal dog* to turpentine inflammation while receiving a "normal average" intake of protein nitrogen (lines 1 to 10); second, the reaction with the same intake but *after a period of very low protein intake* (lines 11 to 19); and third, the reaction during a *high protein intake* following a similar period of low intake (lines 20 to 30). Other details are given below in the experimental history.

In the first experiment, the *normal dog with normal intake* shows a *greatly increased urinary nitrogen* after turpentine. The doubled nitrogen output is in the range expected during fasting experiments (3, 9), but the negative balance is halved by the existence of the moderate nitrogen intake in this experiment. The development of the satellite abscess, drained (line 8) 5 days after the first one, was also associated with increased nitrogen excretion and negative balance. Weight was well maintained in contrast to the losses which occurred during inflammation with fasting.

In the second experiment the *depleted dog with normal intake* shows a very *slightly increased urinary nitrogen* after turpentine. This expected reaction (13, 9, 11) in the dog with reduced protein reserves permits a close approach to nitrogen balance during a level "normal" intake. Some weight loss occurred in this more strenuous experiment.

In the third experiment, the *depleted dog with high intake* shows a *greatly increased urinary nitrogen* after turpentine. The increase is of the same order as in the first experiment but with a more than doubled nitrogen intake the *nitrogen balance is strongly positive.* Weight actually increases during the course of this inflammation experiment.

TABLE 1

Protein Metabolism during Turpentine Inflammation

Normal Protein Intake in Normal Dog Yields Negative Nitrogen Balance
Normal Protein Intake in Depleted Dog Yields Approximate Nitrogen Balance
High Protein Intake in Depleted Dog Yields Positive Nitrogen Balance

Dog 43-346

Line	Days	Diet and procedure	Weight	Nitrogen, daily average			
				Intake	Urine	Feces	Balance
			kg.	*gm.*	*gm.*	*gm.*	*gm.*
1	4	Casein, oral	10.8	4.2	5.0	0.3	−1.1
2	1	Vuj, s.c.; turpentine, s.c.		4.1	6.8	0.3	−3.0
3	1	Vuj, s.c.		3.0	7.1	0.2	−4.3
4	1	Vuj, s.c.		3.0	7.1	0.2	−4.3
5	1	Vuj, s.c.; abscess drained		3.0	2.5	0.2	−0.6*
6	2	Casein, oral	10.7	4.2	2.1	0.2	+1.9
7	2	Casein, oral	10.7	4.2	5.8	0.2	−1.8
8	1	Casein, oral; abscess drained	10.4	4.2	4.0	0.2	−0.7*
9	1	Casein, oral		4.2	3.5	0.2	+0.5
10	5	Casein, oral	10.7	4.2	—	—	—
11	15	Low protein	10.1	0.4	1.4	—	—
12	14	Low protein	9.8	0.4	1.1	—	—
13	3	Casein, oral	9.9	3.0	2.1	0.2	+0.7
14	1	Vuj, i.v.; turpentine, s.c.		3.0	3.3	0.2	−0.5
15	1	Vuj, i.v.		3.0	3.3	0.1	−0.4
16	1	Vuj, i.v.	9.8	3.0	2.9	0.1	0.0
17	1	Vuj, i.v.; abscess drained		3.0	3.6	0.1	−1.0*
18	1	Vuj, i.v.; abscess drained		3.0	2.4	0.1	0.0*
19	4	Casein, oral	9.1	3.0	2.0	0.2	+0.8
20	16	Casein, oral	10.5	3.0	1.6	0.3	+1.1
21	14	Low protein	10.2	0.4	1.0	0.2	−0.8
22	15	Low protein	9.6	0.4	0.8	0.1	−0.5
23	2	Casein, oral		8.5	2.6	0.2	+5.7
24	1	Vuj, i.v.; turpentine, s.c.		8.5	7.5	0.2	+0.9
25	1	Vuj, i.v.	10.3	8.5	4.3	0.2	+4.0
26	1	Vuj, i.v.		8.5	3.5	0.2	+4.8
27	1	Vuj, i.v.		8.5	6.8	0.2	+1.5
28	1	Vuj, i.v.; abscess drained		8.5	4.7	0.2	+2.8
29	7	Casein, oral	10.7	8.5	3.3	0.2	+5.0
30	7	Casein, oral	11.1	8.5	6.1	—	—

* Abscess nitrogen deducted

Experimental History—Table 1.

Dog 43-346. An adult female mongrel was transferred from kennel ration to casein plus the basal diet for 4 days (line 1, Table 1). The plasma protein level was 6.44 per cent.

Turpentine was injected and amino acids Vuj given subcutaneously replaced the casein. Temperature 38.3° C. Plasma protein level 6.44 per cent. The basal diet was spoon-fed and some was vomited—consumption 70 per cent.

The *next day* the temperature was 39.9° C. The abscess swelling was 6 cm. in diameter.

The *3rd day* the temperature was 40.5° C.

The *4th day* the temperature was 39.5° C. The dog vomited after spoon-feeding. Consumption of the basal diet averaged about 60 per cent during these 4 days. At the close of the day the abscess, now 7 × 12 cm., was incised and drained of 65 cc. pus, nitrogen content 0.88 gm. Bacteriological culture of the pus gave no growth.

Casein by mouth replaced the amino acid injections on the 5th day (line 6). The abscess sealed and healed but 4 days later in a more dependent site a small abscess appeared and was drained of 45 cc. pus, nitrogen content 0.68 gm.

After 11 days of casein feeding the plasma protein level was 5.91 per cent. Casein was omitted for the next 29 days (lines 11 and 12) and vitamins were supplied by 2 gm. liver powder (Eli Lilly and Company, liver fraction rich in B_2 complex), 2 gm. yeast powder (Standard Brands Inc., No. 200-B), and 400 mg. choline chloride daily.

After 7 days of this *very low nitrogen intake* the plasma protein level was 5.38 per cent; after 28 days, 5.01 per cent. Casein was returned to the diet for 3 days at the end of which the total plasma protein concentration was 4.99 per cent (line 13).

Turpentine was injected for a second time (line 14, Table 1) and amino acids this time by vein replaced the oral casein. Temperature 38.7° C. Vomiting occurred twice apparently related to the spoon-feeding of the basal diet.

The *next day* the temperature was 40.9° C. Vomiting occurred once again.

The *3rd day* the temperature was 40.2° C. Food consumption was only fair.

The *4th day* the temperature was 39.1° C. The abscess was incised and drained of 45 cc. pus, nitrogen content determined the next day.

The *5th day* (line 18) further drainage occurred of 36 cc. pus, which added to that of the day before had a nitrogen content of 0.45 gm. Temperature 39.8° C. at the beginning of the day dropped to 38.7° C. near the end. Vomiting once again reduced the food intake so that for the 5 days following turpentine injection only about half of the basal diet was eaten. By the end of the day another 60 cc. of pus was collected, nitrogen content 0.33 gm.

The *6th day* casein replaced intravenous amino acids and the dog vomited once more after spoon-feeding. Temperature 38.5° C.

Casein was continued a total of 20 days. Near the beginning of this period the plasma protein level was 5.20 per cent; near the end, 5.96 per cent.

The *diet without casein* as given after the first abscess was again supplied for 29 days (lines 21 and 22). The plasma protein level fell to 5.07 per cent at the end of this period, but after 2 days of high casein feeding is recorded as 5.43 per cent.

Turpentine a third time was injected (line 24, Table 1) and casein was replaced by amino acid solution Vuj by vein. Temperature 39.9° C. 8 hours after the turpentine. The dog vomited once after eating.

The *next day* the temperature was 40.1° C. Vomiting again occurred once.

The *3rd and 4th days* the temperature ranged from 39.8° C. in the morning before injection of amino acids to 38.6° C. in the afternoon after injection.

The *5th day* the abscess was incised and drained of 68 cc. fluid and pus, nitrogen content 0.80 gm. Temperature 38.6° C.

Casein replaced the intravenous amino acids for the next 2 weeks (lines 29 and 30). Two days after the amino acids were discontinued the plasma protein level was 6.02 per cent.

Table 2, dog 40-122, *normal dog with high protein intake* shows approximate *nitrogen balance* after turpentine despite a greatly increased urinary nitrogen.

The urinary nitrogen is more than doubled during the height of the reaction and on the 3rd day (line 5) balance is negative 1.1 gm., at least partly if not entirely related to the reduction in intake. Some of the increase is due to the large injection of amino acids but increase would occur on any regime and negative balance would be great without the high intake. There was no weight loss.

Experimental History—Table 2.

Dog 40-122. An adult female beagle hound was transferred from kennel ration to casein and the synthetic basal diet for 11 days. The plasma protein level was 6.11 per cent.

Turpentine was then injected and the basal diet was continued with casein replaced by amino acids Vuj, 868 cc., partly subcutaneously and partly by vein. Temperature 38.1° C.

TABLE 2

Protein Metabolism during Turpentine Inflammation
High Protein Intake in Normal Dog Yields Approximate Nitrogen Balance

Dog 40-122

Line	Days	Diet and procedure	Weight	Nitrogen, daily average		
				Intake	Urine	Balance*
			kg.	*gm.*	*gm.*	*gm.*
1	4	Casein, oral	14.8	5.9	4.5	+1.1
2	7	Casein, oral	14.8	5.9	4.3	+1.3
3	1	Vuj, s.c. and i.v.; turpentine, s.c.		11.8	11.2	+0.3
4	1	Vuj, i.v.	16.0	11.8	11.6	−0.1
5	1	Vuj, i.v.	15.4	8.9	9.7	−1.1
6	2	Casein, oral; abscess ruptured		8.9	3.3	+5.3
7	2	Casein, oral		8.9	6.4	+2.2
8	6	Casein, oral	15.3	8.9	6.0	+2.6

* Fecal nitrogen estimated 0.3 gm. daily

The *next day* some of the subcutaneous fluid had not been absorbed, contrary to custom and the remaining injections were all given intravenously. This dog had had a great many subcutaneous infusions in previous experiments. Temperature 40.3° C.

The *3rd day* the amino acid solution given was reduced to 651 cc. Temperature 38.9° C. Basal diet consumption averaged 60 per cent during these 3 days.

The *4th* day the abscess ruptured during the night and its contents were not collected. Amino acids were replaced by casein. The plasma protein level was 6.10 per cent.

Measured intake with casein was continued for 16 days. Fecal nitrogen was not analyzed in this experiment.

Table 3, dog 43-345, ***normal dog with high protein intake*** shows approximate ***nitrogen balance*** after turpentine. This experiment is parallel to that of Table 2 and demonstrates again that in the normal dog with normal protein reserves the increased catabolism following injury, as illustrated in the first experiment of Table 1, can be compensated for by doubling the nitrogen intake. A little loss in weight occurred despite apparently good utilization of the amino acids.

Appetite lags during these abscess episodes as it does during most acute injury. The basal diet was only partially consumed during the acute phase of the preceding experiments, averaging 60 to 70 per cent of the 80-calorie-per-kilo diet. Intravenous glucose solution was substituted during the next two experiments at a caloric level similar to that actually obtained orally (Table 3, lines 11 to 18 and Table 4). It will be noted in the experimental histories that dog

TABLE 3

Protein Metabolism during Turpentine Inflammation

High Protein Intake in Normal Dog Yields Approximate Nitrogen Balance

Dog 43-345

Line	Days	Diet and procedure	Weight	Nitrogen daily average			
				Intake	Urine	Feces	Balance
			kg.	*gm.*	*gm.*	*gm.*	*gm.*
1	14	Casein, oral	8.3	3.4	2.7	0.2	+0.5
2	4	Casein, oral	8.4	3.4	—	0.2	—
3	3	Casein, oral		3.4	3.7	0.2	−0.5
4	1	Vuj, i.v.; turpentine, s.c.		6.7	5.9	0.2	+0.6
5	1	Vuj, i.v.		6.7	3.1*	0.2	—
6	1	Vuj, i.v.		6.7	5.1	0.2	+1.4
7	1	Vuj, i.v.; abscess drained		6.7	4.0	0.2	+2.3‡
8	2	Vuj, i.v.	7.8	6.7	4.8	0.2	+1.7
9	2	Casein, oral	8.0	3.4	3.5	0.2	−0.3
10	14	Casein, oral	7.9	3.4	2.3	0.2	+0.9
11	3	Casein, oral	8.1	3.4	2.8	0.2	+0.4
12	1	Vuj, glucose, i.v.; turpentine, s.c.		6.7	6.2	0.1	+0.4
13	1	Vuj, glucose, i.v.		6.1	4.7	0.1	+1.3
14	1	Vuj, glucose, i.v.		6.7	5.9	0.1	+0.7
15	1	Vuj, glucose, i.v.	7.9	6.7	7.7	0.1	−1.1
16	1	Vuj, i.v.; casein, oral		6.7	3.5	0.2	+3.0
17	7	Casein, oral	8.0	6.9	5.4	0.2	+1.3
18	6	Casein, oral	8.2	6.9	4.5	0.2	+2.2

* Urine collection incomplete

‡ Abscess nitrogen deducted

43-345 vomited on 3 of the 6 injection days in the first experiment of Table 3 but on none of the 5 amino-acid-glucose injection days of the second experiment; and that there was vomiting once in dog 43-391 as compared with its more frequent occurrence when the oral diet was given dog 43-346. Good tolerance for such amino acid mixtures has been shown (15).

The second experiment of Table 3 shows approximate ***nitrogen balance*** in a ***normal dog with high protein intake*** during turpentine inflammation. The urinary nitrogen increased promptly and on the 4th day (line 15) was larger by 1 gm. than the intake. The general maintenance of weight and nitrogen balance in the three experiments of Tables 2 and 3 should be compared with the observed losses of fasting dogs (3, 9).

Experimental History—Table 3.

Dog 43-345. An adult female mongrel terrier previously on kennel ration was given the basal diet with casein and synthetic vitamins for 21 days. The plasma protein level was 5.96 per cent at the end of this time.

Turpentine was then injected subcutaneously (line 4) and the amino acid solution Vuj was started intravenously. A total of 493 cc. of the 10 per cent solution was given during 6 hours and 40 minutes (6.7 gm. nitrogen at the rate of 2 mg. nitrogen/kilo/minute). The basal diet was 90 per cent consumed.

The *next day* the dog vomited once during injection and ate only 70 per cent of the basal diet and on the *3rd day* only 40 per cent.

The *4th day* the incised abscess drained 35 cc. pus and fluid containing 0.2 gm. nitrogen. Temperature 39.7° C. The dog vomited once, consumed 40 per cent of the basal diet.

The *6th day* amino acid injections were completed. The dog vomited once. Temperature 38.9° C. The plasma protein level was 5.77 per cent. The abscess wound healed promptly.

Turpentine again was injected (line 12, Table 3) after 19 days on casein plus the basal diet (lines 9 to 11) and this time amino acids and glucose intravenously replaced all oral feeding. Glucose, 10 per cent, in 0.9 per cent sodium chloride solution, volume 950 cc., was mixed with the amino acid solution, 493 cc., and given during 15 hours.

The *next day* 10 per cent of the amino acid solution was not given. Skin over the turpentine injection site appeared necrotic indicating some turpentine had been placed intracutaneously. Before the day was over necrotic skin sloughed and the wound was licked clean by the dog. Temperature 39.6°C.

The *3rd day* the wound presented as a clean ulcer about 3 cm. in diameter with fluctuation on the margins.

The *4th day* the skin defect had decreased greatly but some pus oozed and the temperature rose to 41.5° C. The plasma protein level was 5.37 per cent.

The *5th day* amino acids were discontinued as no longer indicated after 120 cc. had been given, and casein 36.3 gm. was fed with the basal diet. The ulcer-abscess margins were almost closed.

During the following 13 days casein, 49 gm. daily, was fed with the basal diet (lines 17 and 18). The ulcer-abscess healed promptly.

Table 4, dog 43-391, *normal dog with high protein intake* shows approximate *nitrogen balance* after turpentine. This dog was intended to be one with maximum protein reserves accomplished by preliminary feeding of a high protein diet. Because of lack of time the turpentine injection was given before nitrogen equilibrium had been reached on the high intake of 0.8 gm. nitrogen/kilo/day. Balance became slightly negative on the day before and the day of the drainage of the abscess (lines 7 and 8). The peak of nitrogen excretion has been noted at similar times in previous experiments and in fasting dogs (3). There is slight weight loss despite the high nitrogen intake.

Experimental History—Table 4.

Dog 43-391. An adult female short-haired mongrel was transferred from kennel ration to a high intake of casein plus the basal diet for 11 days. The plasma protein level was 6.31 per cent.

Turpentine was injected and the entire oral diet was replaced by intravenous amino acid solution, 669 cc., and dextrose, 10 per cent, 1360 cc. Temperature 38.6° C. The injection continued for 15 hours each day.

The *next day* the dog vomited mucus and fluid once midway in the course of the injection. Temperature 39.9° C.

The *3rd day* temperature 39.9° C. A trace of sugar was found in the urine of the first 2 days, none the 3rd day.

The *4th day* temperature 39.3° C.

The *5th day* temperature 39.5° C. At the end of the day the fluctuant abscess was drained of 38 cc. of pus, containing 0.5 gm. nitrogen.

TABLE 4

Protein Metabolism during Turpentine Inflammation

High Protein Intake in Normal Dog Yields Approximate Nitrogen Balance

Dog 43-391

Line	Days	Diet and procedure	Weight	Nitrogen, daily average			
				Intake	Urine	Feces	Balance
			kg.	*gm.*	*gm.*	*gm.*	*gm.*
1	2	Casein, oral		9.1	8.8	0.3	0.0
2	6	Casein, oral	11.4	9.1	6.5	0.3	+2.3
3	3	Casein, oral		9.1	5.4	0.3	+3.4
4	1	Vuj, glucose, i.v.; turpentine		9.1	6.4	0.1	+2.6
5	1	Vuj, glucose		9.1	8.3	0.1	+0.7
6	1	Vuj, glucose		9.1	8.1	0.1	+0.9
7	1	Vuj, glucose		9.1	9.8	0.1	−0.8
8	1	Vuj, glucose; abscess drained	10.9	9.1	9.7	0.1	−1.2*
9	2	Casein, oral		9.1	5.5	0.3	+3.3
10	4	Casein, oral	10.6	9.1	4.0	0.3	+4.8

* Abscess nitrogen deducted

Casein and the synthetic diet replaced the infusions during the next 6 days. The wound healed promptly.

Table 5, dog 42-1380, *normal dog with high protein feeding* shows approximate *nitrogen balance* after turpentine. The dog chosen for this experiment was known to eat most food very readily, and it voluntarily consumed the diet throughout the experiment. The urinary nitrogen tripled in the second 24 hours and then declined sharply to a level indicating considerable conservation after the injury. The oral nitrogen appears to be a little better utilized than the parenteral amino acids although the difference is not much when compared with the data of Table 3. The data also indicate that the preliminary 7 days were not sufficient to attain equilibrium at the intake of 0.4 gm. nitrogen/kilo upon raising from 0.3 gm./kilo. The plasma protein level of 5.60 per cent further suggests protein reserves to be not entirely filled.

Experimental History—Table 5.

Dog 42-1380. An adult male mongrel, studied for about 5 months during limited intakes of amino acids, was rested on a diet of casein 30 gm., dextrose 143 gm., corn oil 36 gm., salt mixture 6 gm., vitamin emulsion 10 cc., choline chloride 300 mg. Weight increased during 9 weeks from 11.1 kilos to 14.5 kilos. The data of Table 5 begin with the following 4 days during which the basal diet of these experiments was fed with casein, 43 gm. Casein was reduced during the succeeding 3 days to 41.4 gm. At the end of this period the plasma protein level was 5.60 per cent.

Turpentine was injected and the casein intake was doubled. The usual reaction with inflammation and abscess formation disturbed the dog generally but with minimal assistance all food was consumed throughout the experiment. Vomiting of mucus occurred once on the 2nd day.

TABLE 5

Protein Metabolism during Turpentine Inflammation
High Protein Feeding in Normal Dog Yields Approximate Nitrogen Balance

Dog 42-1380

Line	Days	Diet and Procedure	Weight	Nitrogen, daily average			
				Intake	Urine	Feces	Balance
			kg.	*gm.*	*gm.*	*gm.*	*gm.*
1	4	Casein, oral	14.5	6.0	4.1	0.3	+1.6
2	3	Casein, oral	14.5	5.8	3.8	0.3	+1.7
3	1	Casein, oral; turpentine, s.c.		11.6	4.4	0.3	+6.9
4	1	Casein, oral	14.7	11.6	12.1	0.2	−0.7
5	1	Casein, oral		11.6	6.7	0.2	+4.7
6	1	Casein, oral—abscess drained		11.6	6.7	0.2	+2.2*
7	1	Casein, oral		11.6	6.0	0.2	+5.4
8	2	Casein, oral		5.8	4.8	0.2	+0.8
9	2	Casein, oral		5.8	3.7	0.2	+1.9
10	2	Casein, oral		5.8	3.4	0.2	+2.2
11	2	Casein, oral		5.8	3.0	0.2	+2.6

* Abscess nitrogen deducted

The *abscess* was incised and drained on the 4th day after turpentine injection. The pus and fluid contained 2.54 gm. nitrogen. The abscess wound healed promptly.

DISCUSSION

The above data may be considered in the light of two observations of general importance in protein metabolism. In the first place, a *protein reserve* appears to be a normal part of the total body protein. It may be defined as that protein of which the body or an organ can be deprived and still live, albeit more precariously. The remaining protein of an animal thus depleted of reserve is certainly *essential protein*, but we do not believe that reserve protein is in any sense nonessential or inactive in normal existence. We would not call it "storage protein"

(17). It is not an inert fraction of the body protein but is continuously involved in the internal metabolism of protein. It may vary in amount. Normal limits are not well defined, but definite depletion is easily recognized by accompanying reduced tolerances to injurious agents. References to its nature (12) and value (14) have been previously cited. The second pertinent observation is the *increased nitrogen excretion which occurs after injury*, an increase larger than can be accounted for by the local injury (3, 5, 7). Of further interest it has been shown that *little or no increase occurs in protein-depleted animals* (9, 13, 11, 17, and Table 1 above).

A relationship appears to be established, therefore, between the increased nitrogen excretion after injury and the body protein reserves. The nature of this relationship is not clear. It might appear that the increased nitrogen excretion is the result of fundamentally beneficial reactions involving use of reserve protein. Is it a breakdown of reserve protein to provide specific fractions for combatting the injury? It may be. If so, one might hope to be able to substitute the needed material from outside and spare the excess general catabolism. An extract of anterior pituitary gland (ox) has prevented urinary increases of nitrogen and creatine after bone fracture in rats (8). Even more recently in a brief note it is reported that the amino acid methionine may largely prevent urinary nitrogen increases after burns in rats (4).

If anabolism were seriously inhibited by injury one should not be able to reduce the negative balance by exogenous intake. Some have claimed this true—that nitrogen intake during acute injury merely increased output (discussed in 3). Others have questioned (10); and still others have demonstrated a great reduction in negative balance by high intake (6, 16). It is suggested in the experiments of this report that not only is the doubled intake generally adequate for balance *for this type and degree of injury* but that without doubling the intake (Table 1 above), that is, by maintaining the existing intake, the negative balance may be only half as great as during injury with no intake (3). The increased urinary nitrogen appears thereby *more* related to the amount of the protein reserve than to the quantity of the current intake, although it is obvious that this intake and its quality will have influence.

Evaluation of the state of the total body protein prior to an injury is very difficult clinically, and only less difficult experimentally to the degree that dietary and other conditions can be controlled and standardized over long periods. There are no "spot tests" to aid this evaluation. Yet without knowledge of the state of protein reserves we do not believe one can properly interpret reactions in the metabolism of protein after injury. And when one considers what may be the *influence of variable states in the protein total of individual organs* the problem becomes more complex. Addis, Poo, and Lew (1) have shown how the rates for the loss and the recovery of protein by individual organs and tissues may vary.

When balance has been achieved during injury (16) proponents of the antianabolic theory have pointed out, often rightly so, that it was accomplished in patients who were protein-depleted before the injury. Nitrogen balance is easier of attainment in depleted individuals, but that "inhibition of anabolism" should be the factor preventing its attainment in the well nourished appears unlikely. If inhibition were the important factor, one should expect it in the depleted as well as in the normal. More likely it is the difficulty and labor of the required high intake which prevents nitrogen balance in the well nourished.

Parenteral feeding is an aid but not a simple expedient. Many patients will continue in the future as in the past to recover by drawing upon their reserves. Skillful physicians will provide a high intake of nitrogen as early as possible after injury to restore protein reserves to combat subsequent injuries, or may provide specifics for protecting reserves if such are discovered.

SUMMARY

Adult dogs were given a proteinless diet plus casein, 80 calories/kilo, 0.4 gm. nitrogen/kilo/day. Sterile controlled inflammation was produced by subcutaneous injection of turpentine. The reaction is characterized by local swelling, induration, and abscess formation, terminated by rupture or incision after 3 to 5 days and by general reactions of malaise, fever, leucocytosis, and increased urinary nitrogen. For 3 to 6 days after turpentine the nitrogen intake was provided in seven experiments by amino acids given parenterally (a solution of the ten essential amino acids (Rose) plus glycine).

A normal dog with a normal protein intake showed a negative nitrogen balance after turpentine—urinary nitrogen doubled even as in inflammation during fasting.

A protein-depleted dog (low protein reserves produced by very low protein intake) given a normal protein intake after turpentine maintained nitrogen balance—urinary nitrogen rose only slightly. With a high (doubled) protein intake the depleted dog showed strongly positive balance.

Normal dogs with high (doubled) protein intakes react to turpentine with doubled urinary nitrogen outputs on individual days and therefore are maintained in approximate nitrogen balance and weight balance. This end may be achieved equally well or better by oral feeding, when such is possible and absorption unimpaired.

The increased nitrogen excretion after injury is again shown directly related to the state of body protein reserves. Increased catabolism not inhibition of anabolism best explains the excess urinary nitrogen. Protection during injury of valuable protein reserves appears possible through an adequate intake of protein nitrogen.

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