METABOLIC ALTERATIONS FOLLOWING THERMAL BURNS. VII. EFFECT OF FORCE-FEEDING, METHIONINE, AND TESTOSTERONE PROPIONATE ON NITROGEN BALANCE IN EXPERIMENTAL BURNS

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Research Article

METABOLIC ALTERATIONS FOLLOWING THERMAL BURNS. VII. EFFECT OF FORCE-FEEDING, METHIONINE, AND TESTOSTERONE PROPIONATE ON NITROGEN BALANCE IN EXPERIMENTAL BURNS [1]

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Previous work (1) demonstrated that following an experimental burn animals were in negative nitrogen balance on a caloric and protein intake that had been sufficient to maintain nitrogen equilibrium during several control periods prior to burning. The total circulating plasma proteins in these animals remained the same, or in some instances increased slightly, while the concentration and total amount of albumin decreased (2). It has been well established (3 to 6) that patients suffering from burns show a nitrogen deficit and a decrease in the plasma protein concentration. Since it has been suggested that the prevention or alleviation of the nitrogen loss would improve the status of the patient it seemed important to attempt to do this by increasing the protein intake or by reducing the protein catabolism through the administration of testosterone propionate, or methionine. It has been proposed that if force-feeding of protein is employed that such feeding should be started at the earliest possible moment (6, 7). By means of increasing the nitrogen intake in a group of patients, the negative nitrogen balance was prevented or diminished, but in most instances when the very high diets were given early, they were poorly tolerated (8). In order to have a better understanding of the importance of securing a positive nitrogen balance at an early stage, and ways of attaining it, dogs were treated shortly after being burned, by force-feeding or by a normal diet plus methionine or intramuscular injections of testosterone propionate.

EXPERIMENTAL

Selection of the animals, diet, and methods of analyses as well as method of burning have been previously described (1). Four animals were used for the force-feeding experiments and 2 each for the testosterone and methionine studies. The first 2 animals in the force-fed groups (Nos. 10 and 11) were given a mixture of Nutramigen and Amigen [2] following the burn, in an amount sufficient to triple the nitrogen and double the caloric intake they had received previous to the burn. The other animals (Nos. 12 and 13) in the force-fed group following the burn were fed a mixture of Amigen and Aminoids [3] which provided a nitrogen intake approximately 3 times the amount they had taken during the control period. The caloric intake in this latter group was not materially altered. The food was mixed in a Waring Blendor with a measured amount of water and fed by stomach tube in 3 divided doses. The first feeding was administered a few hours following the burn. Two animals (Nos. 14 and 15) were maintained on the same diet they had received previous to the burn and were given intramuscular injections of 25 mgm. of testosterone propionate [4] daily for 15 days. Two other animals (Nos. 16 and 17) following the burn were given the regular diet plus methionine [5] in an amount equal to 1.5 per cent of the diet.

RESULTS

The data on 3 of the 4 force-fed animals (Nos. 11, 12 13) are shown in Table I. The nitrogen deficit was reduced over that of the previously reported normally fed dogs (1), but the institution of early force-feeding was poorly tolerated. No collections were obtained on dog No. 10 of the force-fed group because of marked vomiting and diarrhea, and the clinical condition of this animal was such that it was sacrificed on the sixth day following the burn. The food intake was reduced for dog No. 11 and a 3-day urine sample was obtained from the 4th to the 7th day. The results indicate that the animal was close to nitrogen balance dur-

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[2] Kindly supplied by Mead Johnson and Co., Evansville, Ind.

[3] Kindly supplied by the Arlington Chemical Co., Yonkers, N. Y.

[4] Perandren, kindly supplied by the Ciba Pharmaceutical Products, Inc.

[5] Kindly supplied by Merck & Co., Inc. Rahway, N. J.

TABLE I

The effect of force-feeding on the nitrogen balance following an experimental burn

Dog	Period	No. of days in period	Ave. N intake per day	Ave. nitrogen output per day			Ave. N balance	Caloric intake	Body wt.*
				Total urinary N	Fecal N	Total N			
11	1	4	9.76	7.87			1.89†	850	15.2
	2	5	9.76	7.00			2.76†	850	15.3
	3	6	9.67	8.41			1.26†	850	15.4
	Burned 20 per cent								
	4	4	26.27¶	discarded‡				1,799	15.9
	5	3	21.40¶	19.21			1.49†	1,439	16.2
12	1	6	8.90	6.79	.86	7.65	1.25	750	13.7
	2	3	8.90	7.41	.23	7.64	1.26	750	13.7
	3	5	25.70**	discarded‡					
	4	4	8.90	8.61	.89	9.50	−0.60	750	14.2
	5	5	8.90	7.50	.79	8.29	0.61	750	14.2
	6	5	8.90	7.77	.65	8.42	0.48	750	14.2
	7	4	8.90	7.54	.65§	8.19	0.71	750	14.2
	8	5	8.90	7.03	.42	7.45	1.45	750	14.2
	9	4	8.87	7.07	.47	7.54	1.33	750	14.3
	Burned 20 per cent								
	10	5	25.70††			21.56‖	4.14	925	15.5
	11	5	25.70††			27.14‖	−1.44	925	13.8
	12	2	13.27	sample lost				1,535	14.0
	13	5	8.87	9.17	.74	9.91	−1.04	925	12.7
	14	5	21.28	14.01	2.11	16.12	5.16	1,818	14.2
	15	6	14.47	11.78	.85	12.63	1.84	1,041	14.4
13	1	4	8.90	7.14	.61	7.75	1.15	750	13.8
	2	5	8.90	6.78	.35	7.13	1.77	750	13.7
	3	5	25.70**			20.43‖	5.27	1,725	15.0
	4	4	8.90	9.99	.61	10.60	−1.70	750	14.4
	5	5	8.90	8.88	.37	9.25	−0.35	750	14.3
	6	5	8.90	7.56	.58	8.14	0.76	750	14.5
	7	4	8.90	7.49	.50	7.99	0.91	750	14.2
	8	5	8.89	7.30	.41	7.71	1.18	750	14.2
	9	4	8.87	6.93	.32	7.25	1.62	750	14.3
	Burned 20 per cent								
	10	5	25.70††			22.19‖	3.51	925	14.2
	11	5	25.70††			28.21‖	−2.51	925	13.8
	12	7	1.84	5.93	.46	6.39	−4.55	156	11.2
	13	5	7.10	7.59	.48	8.07	−0.97	605	11.0
	14	6	8.81	5.11	.47	5.58	3.23	750	11.5

* Weight at end of period.
† Intake minus urinary nitrogen output.
‡ Discarded because urine contaminated with vomitus and feces.
§ Average fecal nitrogen output.
‖ Includes urine, vomitus, and fecal nitrogen.
¶ 300 grams Nutramigen and 200 grams Amigen in 550 ml. of water 1st day; then reduced to 200 grams Nutramigen, 133 grams Amigen in 360 ml. of water.
** 240 grams Nutramigen and 160 grams Amigen in 440 ml. of water.
†† 140 grams Amigen, 100 grams Aminoids, 16 grams yeast, 5.4 grams salt and 1.5 grams cod liver oil in 300 ml. of water.

ing this time. However, the animal rapidly grew worse and was sacrificed on the 12th day.

Since the response to the early high food intake in these 2 animals was unsatisfactory, it seemed desirable to study the effect of such a regime on 2 normal animals (Nos. 12 and 13). It was found that this high intake (at least in the form given) was also poorly tolerated by the normal dog, al-

TABLE II

The effect of daily injections of 25 mgm. testosterone propionate on the nitrogen balance following experimental burns

Dog	Period	No. of days in period	Ave. N intake per day	Ave. nitrogen output per day			Ave. N balance	Body wt.*
				Total urinary N	Fecal N	Total N		
14	1	5	8.37	6.05	.66	6.71	1.66	14.3
	2	5	8.37	7.17	.57	7.74	0.67	13.8
	3	5	8.41	6.75	.63	7.38	1.03	13.7
	Burned 20 per cent							
	4	5	8.45‡	9.50	.55	10.05	−1.55	13.8
	5	6	8.59‡	9.70	.60	10.30	−1.71	12.8
	6	4	5.57‡	8.21	.60†	8.81	−3.24	—
15	1	5	10.71	8.46	.74	9.20	1.51	16.4
	2	5	10.77	9.46	1.01	10.47	0.30	16.8
	3	5	10.77	9.74	0.74	10.48	0.29	16.9
	Burned 20 per cent							
	4	5	10.82‡	12.17	0.66	12.83	−2.02	16.9
	5	6	11.00‡	11.22	0.64	11.86	−0.86	16.7
	6	4	11.00‡	9.63	1.00	10.63	0.37	16.2
	7	5	10.43	9.57	0.37	9.94	0.49	15.5
	8	5	10.43	7.94	1.00	8.94	1.49	15.0
	9	5	10.53	7.92	0.78	8.70	1.83	15.2
	10	7	10.56	7.59	0.62	8.21	2.35	15.0
	11	7	10.58‡	7.43	0.78	8.21	2.36	15.8

* Weight at end of period.
† Average fecal nitrogen output.
‡ 25 mgm. testosterone propionate intramuscular daily.

though the symptoms were not so severe as those exhibited by the burned animals. The total nitrogen output (urine, feces, and vomitus) was analyzed for dog No. 13 for the 5-day period and in spite of loss through vomiting, the positive nitrogen balance (period 3) was markedly increased over the control periods. A gain in weight occurred, which was thought to be partly due to water retention. After allowing several weeks for these animals to return to normal, they were burned and then force-fed a diet containing 3 times the nitrogen content of the control diet, but with little change in caloric content. Clinically the animals seemed better than the first 2 dogs (Nos. 10 and 11) but their course was far from satisfactory. The total nitrogen output in these dogs was analyzed during two 5-day periods of force-feeding. Both animals were in marked positive nitrogen balance during the first 5 days following the burn, but in negative balance the second 5 days. Following the 10 days of force-feeding, the animals were given the regular diet *ad libitum.* Dog No. 12 consumed large amounts of food, and nitrogen retention was fairly marked. A gain in weight without evidence of edema also occurred. The other animal ate poorly and did not regain his control weight.

The results on the 2 testosterone-treated dogs (Nos. 14 and 15) are shown in Table II. Dog No. 14 showed a slight skin disorder during the control period which did not seem significant. However, 10 days following the burn, it became worse and there was every indication that the results obtained during the last 5 to 7 days were complicated by this factor. The dog started to refuse food during the sixth period and the experiment was discontinued on the fifteenth day post burn.

The other animal (No. 15) showed only a slight loss of nitrogen after the fifth day and was in positive balance after the tenth day. This was an improvement over the 4 control dogs which were in negative balance for 15 days following the burn.

The nitrogen balance studies on the methionine-treated dogs (Nos. 16 and 17) are shown in Table III. The animals refused the food with the methionine supplement; so it was given by stomach

TABLE III

The effect of methionine supplement on the nitrogen deficit following experimental burns

Dog	Period	No. of days in period	Ave. N intake per day	Total urinary N	Ave. N balance*	Body wt.†
16	1	4	13.70	9.80	3.90	20.2
	2	5	13.92	10.42	3.21	20.6
	3	7	13.92	11.63	2.00	20.3
	Burned 20 per cent					
	4	5	14.23‡	16.33	− 2.19	20.0
	5	2	0	15.45	−15.45	—
17	1	4	14.27	11.78	2.49	21.8
	2	5	14.50	11.40	3.10	21.8
	3	7	14.50	11.07	3.43	22.0
	Burned 20 per cent					
	4	5	14.83‡	17.65	−2.95	21.8
	5	4	2.50	10.82	−8.32	20.0

* Intake minus urinary N output.
† Weight at end of period.
‡ Supplemented with 1.5 per cent methionine daily (dog 16, 3.60 grams; dog 17, 3.75 grams).

tube. One of the animals (No. 16) started to vomit on the third day and the course of both animals was poor. Since the supplement in no way seemed to improve the condition over the untreated animals, the methionine was discontinued after the fifth day and the regular diet resumed. Dog No. 16 refused all food and died on the seventh day post burn. The other animal died on the tenth day.

DISCUSSION

No conclusions can be drawn concerning the nitrogen balance studies in the first 2 force-fed dogs (Nos. 10 and 11) which were given the high protein and high caloric diet, but from the 1 collection period for dog No. 11, it would appear that the nitrogen loss was reduced. The nitrogen balance of the other force-fed dogs (Nos. 12 and 13), which were given triple the nitrogen intake but almost the same caloric intake, was negative during the second 5-day period in spite of the high nitrogen intake. The course of the animals was certainly in no way improved by the positive balance during the first 5 days. Cuthbertson (9) noted in his work on fracture patients that high caloric and protein diets (as much as 231 grams protein and 4,100 calories) failed to eliminate the negative nitrogen balance during the height of the catabolic phase. He first observed a period of retention, then a period of loss which was followed later by a period of retention. This pattern is similar to that noted in our animals. Howard, Winternitz, Parson, Bigham and Eisenberg (10) found that fracture patients on a low protein, low caloric diet lost no more nitrogen than those on a higher nitrogen and caloric intake. However, their high diets contained only 15 grams of nitrogen and 24 calories per 1.73 sq. m. area and therefore cannot be considered similar to those of Cuthbertson (9).

No attempt was made to force-feed animals with large amounts of their regular diet. It seemed of particular interest to use amino acid preparations since their use has been advocated for increasing the protein intake. It is quite possible that large amounts of native protein may be somewhat better tolerated than the hydrolysates or that the amino acid mixtures if started after the shock phase had subsided and gradually increased might have been beneficial. Free and Leonards (11) found that when large equivalent amounts of nitrogen were taken in the form of meat, blood, and amino acids, the latter form caused gastrointestinal upsets in both subjects. While the form of nitrogen and the fact that it was given in such large amounts may have aggravated the diarrhea and vomiting noted in our animals, the gastrointestinal upset was not the only undesirable effect noted in these experiments.

Plasma protein, albumin, and plasma volume alterations were followed in these animals and will be discussed in another report. However, it can be stated here that the volume changes were more marked and no doubt contributed to the undesirable effects of force-feeding. Since it has been shown (12) that increased intakes of nitrogen are accompanied by an increased intake of water, the imbalance following a burn was, no doubt, accentuated by feeding large amounts of protein. That the force-feeding in itself has an effect on water metabolism is indicated by the studies on the 2 normal animals. During the 5-day period of force-feeding (period 3) previous to the burn, dog No. 13 consumed more than 4 times the amount of water than that drunk on the regular diet and the apparent water retention (intake minus urine output) was doubled over the control periods. Following the burn, all the force-fed animals showed a marked edema and several had generalized muscular twitchings which had been noted by other workers (13, 14) in cases of "water intoxication."

Once the anabolic phase begins (from 15 to 20 days post burn in our animals) large amounts of food can be taken to advantage. During period 14, dog No. 12 voluntarily consumed 21 grams of nitrogen and retained 5 grams per day with a gain in weight of 1.5 kgm. Another animal (not shown in the table) was also given food *ad libitum* from the nineteenth to twenty-fifth day post burn and consumed an average of 33 grams of nitrogen per day with a urinary nitrogen output of 26 grams, and during this time gained 2.3 kgm. Neither of these dogs showed edema or the other undesirable effects seen in the early force-fed dogs.

Since testosterone propionate has been shown to increase nitrogen retention (15), there has been an interest in the use of this hormone to decrease the loss of nitrogen following injury. Its use has been reported by Howard (16) in a fracture patient in whom it seemed to have some effect in reducing the nitrogen loss. Testosterone propionate was therefore administered to 2 female dogs following a standardized burn. It is difficult to draw conclusions from this work, since only 2 animals were studied, and the results on the one animal were complicated by the skin disorder previously described. Because of comparable results obtained with testosterone propionate in patients (17), it seems that the improvement in the nitrogen balance noted in dog No. 15 might be of some significance. The use and cautions to be exercised in employing testosterone propionate have been brought out in another report (17).

Croft and Peters (18) reported that the addition of methionine reduced the nitrogen loss following burns in rats. They presented the hypothesis that there is a need for methionine following injury and that to cover this requirement, tissue is broken down resulting in a large loss of nitrogen. The methionine supplement administered to the 2 dogs in the present experiment was without effect on the nitrogen balance. While no conclusions can be drawn from this small group, the results are in keeping with the negative findings in a patient treated with this amino acid and with the report of Schenker (19) and Chanutin and Ludewig (20) who noted no improvement in the nitrogen balance with the administration of methionine.

Howard and his associates (10) have pointed out that the nitrogen loss following injury does not occur in malnourished patients and apparently feel that the nitrogen catabolism in healthy injured subjects may be a desirable reaction. There seems to be some question, therefore, whether it is advantageous to attempt to prevent or alleviate the nitrogen loss during the catabolic phase. Cuthbertson, Shaw and Young (21) reported that a crude anterior pituitary extract prevented weight loss and reduced the nitrogen loss in injured rats, but did not increase the rate of restoration of the muscles which atrophied as the result of injury.

These observations force one to question the advisability of attempting to prevent the loss of nitrogen by force-feeding during the first week or 2 following a burn or other severe trauma. It is obvious, however, that if a negative nitrogen balance persists for more than 2 or 3 weeks the patient will suffer from a serious protein depletion. It is important, therefore, to institute adequate feedings as soon as the patient can tolerate them. It is our impression (17) and that of others (22) that during the first 2 days the caloric and protein intake should not be forced. In several very severely burned adult patients we have recently given between 800 and 1,200 calories (mostly carbohydrate) during the first 2 days following the burn. The caloric and protein intake was then increased to 1.6 times the patients' basal caloric requirement and subsequently gradually increased to an intake of 200 grams protein and about 3,000 calories. These patients exhibited a minimum nitrogen loss and seemingly have been in as good a condition as can be expected.

SUMMARY

Four groups of 2 female dogs were fed a standardized diet and, after a burn, given the following treatment:

1. Force-fed a diet containing triple the protein (in the form of amino acids) and twice the caloric intake received during the control periods.
2. Force-fed a diet containing triple the protein, but little change in caloric intake.
3. The regular diet and injections of 25 mgm. testosterone propionate daily.
4. The regular diet plus 1.5 per cent methionine.

The results indicate that force-feeding improved the nitrogen balance, but the treatment was poorly tolerated.

There is some indication that testosterone propionate reduces the nitrogen loss following a burn. Methionine seemed to have no beneficial effect on the nitrogen deficit which occurs after a burn.

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