METABOLIC ALTERATIONS FOLLOWING THERMAL BURNS.[1] I. NITROGEN BALANCE IN EXPERIMENTAL BURNS

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Several investigators (1 to 4) have shown that a negative nitrogen balance exists following a burn. It was suggested that the prevention or alleviation of this loss of nitrogen might promote healing and improve the prognosis of the severely burned patient. Since, however, it is difficult to evaluate the significance of nitrogen balance studies in patients because of the marked variation in their age, their nutritional status previous to burning, and the depth and severity of their injury, it seemed advisable to carry out nitrogen balance studies in animals under controlled conditions.

METHODS AND MATERIALS

Adult female mongrel dogs were selected for the study. They were fed 10 or 11 grams per kilogram of body weight of the following mixture in parts per hundred: casein 37.4, dextrose 26.4, Crisco 22.2, salt mixture (U.S.P. Salt Mixture No. 1) 3.5, dried yeast 6.8, bone ash 2.7, and cod liver oil 1.0. The amount of food consumed daily by each animal was weighed, and samples of each batch of food were analyzed for nitrogen content. After the dogs had consumed this diet for a preliminary period of ten days to two weeks, they were placed in metabolism cages. Urine was collected under toluene and a daily aliquot removed and pooled for a 5-day period. At the beginning of the study, and at the end of each 5-day period, the bladder was catheterized to insure full collection. Feces, marked with carmine, were preserved with an acid-alcohol solution, dried on the steam bath and ground to a powder.

The nitrogen balance and body weight were followed for three 5-day periods to insure nitrogen equilibrium or a slightly positive balance and the maintenance of relatively constant weight. The animals were then burned under anesthesia (intravenous nembutal). Hot irons with a uniform surface area (12.57 cm.) were employed so that an estimate of the surface area burned could be calculated. A deep second or third degree burn was produced, involving 20 or 30 per cent of the body surface (calculated from surface area (5)). Nitrogen balance studies were continued during the postburn period with the same food intake as that during the control period.

Total nitrogen was determined by the macro-Kjeldahl method. The ammonia was distilled into 75 ml. of saturated boric acid, and the mixture of bromcresol green and methyl red suggested by Ma and Zuazaga (6) was used as an indicator. Urea and ammonia nitrogen were determined by the method of Van Slyke and Cullen (7).

RESULTS

The results are summarized in Table I. Following the burn, all the animals showed a marked negative nitrogen balance on an intake of nitrogen that had been sufficient to maintain a positive balance in the periods previous to the burn. The nitrogen loss continued for approximately the same length of time (15 days) in all 4 dogs. A mild diarrhea was exhibited in all the animals after the burn. Dog 3 developed a severe diarrhea and showed a marked loss of appetite a week after the burn and died 11 days postburn. An increased nitrogen loss did not occur in the feces during the period of loose stools; only dog 7 showed a slightly higher fecal nitrogen output and diarrhea was not so marked in this dog as in animals 3 and 4.

Although an actual increase in all of the nitrogenous products in the urine occurred after burning, the percentage of nitrogen excreted as urea plus ammonia nitrogen was not markedly altered. In the 5-day period following the burn, dogs 7 and 8 showed no change, but in dogs 3 and 4, the percentage of total nitrogen excreted as urea and ammonia was decreased approximately 10 per cent.

Fifteen days from the time of burning, the animals began to retain nitrogen, but they did not regain their weight. Dogs 7 and 8 were kept on the experimental diet for several weeks after col-

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TABLE I

The effect of experimental burns on nitrogen balance

Dog	Period †	Av. N intake per day	Av. Nitrogen Output per day				Av. N balance	Body weight
			Total urinary N	Urea + ammonia N	Fecal N	Total N		
		grams	*grams*	*per cent*	*grams*	*grams*	*grams*	*kgm.*
3	1	6.64	5.39	91	0.47	5.86	0.78	10.80
	2	6.41	4.80	90	0.48	5.28	1.13	10.91
	3	6.41	5.13	90	0.45	5.58	0.83	10.80
	Burned (25 per cent)							
	4	6 41	8.14	80	0.64	8.78	−2.37	9.77
	5	2.56*	7.42		0.57	7.99	−5.43	8.41
	6	0*				2.60**	−2.60	died
4	1	6.61	4.90	91	0.40	5.30	1.31	10.91
	2	6.38	5.16	89	0.54	5.70	0.68	10.91
	3	6.38	4.89	95	0.48	5.37	1.01	11.13
	Burned (25 per cent)							
	4	6.38	8.88	79	0.45	9.33	−2.95	10.11
	5	6.38	8.34	89	0.61	8.95	−2.57	9.77
	6	6.33	7.15	89	0.59	7.74	−1.41	9.09
	7	sample lost						
	8	6.33	4.43	90	0.41	4.84	1.49	9.55
	9	6.33	4.27	89	0.39	4.66	1.67	9.43
7	1	11.72	9.50	89	0.66	10.16	1.56	18.40
	2	11.80	9.85	89	0.79	10.64	1.16	18.51
	3	11.85	10.06	90	0.85	10.91	0.94	19.05
	Burned (20 per cent)							
	4	11.78	13.99	89	1.10	15.09	−3.31	18.18
	5	11.63	13.34	85	1.22	14.56	−2.93	17.28
	6	11.19	12.55	87	1.08	13.63	−2.44	16.35
	7	11.19	8.49	89	9.95	9.44	1.75	16.35
	8	11.72	8.17	86	0.77	8.94	2.78	16.23
	9	11.62	8.81	88	0.78	9.59	2.03	16.47
	10	11.64	8.97	89	0.86	9.83	1.81	16.69
	11	11.19	9.44	90	0.73	10.17	1.02	17.16
8	1	6.89	6.07	82	0.75	6.82	0.07	12.72
	2	7.07	5.78	87	0.52	6.30	0.77	13.18
	3	7.11	6.14	88	0.80	6.94	0.17	12.95
	Burned (20 per cent)							
	4	6.77	8.39	87	0.92	9.31	−2.54	12.14
	5	6.21	7.66	83	0.72	8.38	−2.17	10.91
	6	5.84	6.84	85	0.98	7.82	−1.98	10.56
	7	6.27	5.24	86	0.60	5.84	0.43	10.32
	8	8.39	6.20	89	0.73	6.93	1.46	10.00
	9	7.83	6.63	88	0.59	7.22	0.61	10.45
	10	6.89	5.02	89	0.73	5.75	1.14	10.67

† Each period represents an average of 5 days.
* Food intake reduced because of loss of appetite.
** Urine and feces pooled because of diarrhea.

lections were terminated, but there was no marked gain in weight until the food intake was increased.

DISCUSSION

It has been suggested (1) that the negative nitrogen balance following burns may be due, at least in part, to a reduced nitrogen and caloric intake. Since the loss of nitrogen in these animals occurred on an intake sufficient to maintain a positive nitrogen balance previous to burning, the nitrogen deficit would seem to indicate an accelerated catabolism. This view is in agreement with the studies on patients with typhoid fever (8), fractures (9), and burned patients (10).

The loss of nitrogen after burning was marked. Dogs 3, 4, 7, and 8 lost respectively 52.00, 34.65, 43.40, and 33.45 grams of nitrogen. In dog 4, the nitrogen loss was equivalent to 216 grams of protein, or in terms of muscle, to 1080 grams (calculated as 20 per cent protein). Since this dog weighed 10.91 kgm., 1080 grams represents approximately 10 per cent of the total body weight. If the nitrogen loss is calculated in terms of plasma containing 6 grams of protein per 100 ml., dog 4 lost the equivalent of 3600 ml. of plasma or roughly 4 times her normal plasma volume. Since nitrogen loss in the wounds is known to occur (11), the total nitrogen loss was even greater than has been indicated.

In this study, no attempt was made to determine the exact nature of the lost protein. During the control periods, the urea nitrogen averaged 80 to 85 per cent and the ammonia nitrogen 6 per cent of the total urinary nitrogen output. After the burn, however, dogs 3 and 4 showed a 10 per cent decrease in the percentage of total nitrogen excreted as urea; the percentage of ammonia nitrogen remained the same. This change was not seen in dogs 7 and 8. Total excretion of creatine and creatinine in dogs 3 and 4 showed an increase, but in terms of percentage of total urinary nitrogen, the values were similar to those of the control periods. The marked decrease in the percentage of nitrogen excreted as urea reported by others (12) was at no time encountered in this study, even in samples of urine taken at 24 and 48 hours following the burn. In experiments on burned calves, Glenn, Muus, and Drinker (13) reported increased values in the blood non-protein nitrogen. Their results showed that it is possible, at least under certain conditions, to demonstrate that creatine is released in the burned area. They found that "undetermined nitrogen" did not increase proportionately more than the total non-protein nitrogen except in one case. Whatever the nature of the loss of nitrogen, it is evident that there is an accelerated breakdown of protein following burns, and thus an increased requirement.

Several means of correcting the nitrogen loss suggest themselves, namely, feeding increased amounts of protein, increasing the nitrogen-sparing foods, particularly carbohydrate, and the administration of hormones known to have a nitrogen-retaining effect (anterior pituitary growth principle or testosterone). Shaffer and Coleman (14) have reported that a high carbohydrate diet with a liberal intake of protein can correct or diminish the nitrogen loss encountered during the febrile stage of typhoid fever. Cuthbertson's work (9) on patients with fractures indicates that it may not be possible to stem completely the loss of body tissue during the period of maximum tissue catabolism, but that it can be diminished by feeding large amounts of protein and carbohydrate. Even though the loss of nitrogen after burns cannot be entirely prevented, it is evident that the protein intake should be increased above the normal requirement after the catabolic phase has subsided. Such a conclusion seems justified, since our experimental animals failed to regain their body weight on an intake that had been adequate during the control period. A significant nitrogen retention in one normal man and in 3 patients with gastric carcinoma was reported (15) with the use of parenterally administered testosterone propionate.

The effect of this nitrogen loss and the treatment of it are now being studied in dogs under the same conditions as reported in this paper.

SUMMARY

Changes in nitrogen balance and body weight were studied in 4 adult female dogs before and after burning. For about 15 days following the burn, there was a marked deficit of nitrogen on an intake that had been sufficient to maintain nitrogen equilibrium and body weight previous to

the burn. No marked alteration occurred in the percentage of nitrogen excreted as urea and ammonia. The animals did not regain their weight, even though retention of nitrogen began, until the diet was increased.

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BIBLIOGRAPHY

1. Cope, O., Nathanson, I. T., Rourke, G. M., and Wilson, H., Symposium on management of Cocoanut Grove burns at Massachusetts General Hospital; metabolic observations. Ann. Surg., 1943, **117**, 937.
2. Taylor, F. H. L., Levenson, S. M., Davidson, C. S., Browder, N. C., and Lund, C. C., Problems of protein nutrition in burned patients. Ann. Surg., 1943, **118**, 215.
3. Davidson, E. C., Sodium chloride metabolism in cutaneous burns and its possible significance for a rational therapy. Arch. Surg., 1926, **13**, 262.
4. Lucido, J., Metabolic and blood chemical changes in a severe burn; case report. Ann. Surg., 1940, **111**, 640.
5. Cowgill, G. R., and Drabkin, D. L., Determination of a formula for the surface area of the dog. Am. J. Physiol., 1927, **81**, 36.
6. Ma, T. S., and Zuazaga, G., Micro-Kjeldahl determination of nitrogen; a new indicator and an improved rapid method. Indust. and Engin. Chem., Anal. Ed., 1942, **14**, 280.
7. Van Slyke, D. D., and Cullen, G. E., A permanent preparation of urease, and its use in the determination of urea. J. Biol. Chem., 1914, **19**, 211.
8. Coleman, W., and DuBois, E. F., Clinical calorimetry; calorimetric observations on the metabolism of typhoid patients with and without food. Arch. Int. Med., 1915, **15**, 887.
9. Cuthbertson, D. P., Further observations on the disturbance of metabolism caused by injury, with particular reference to the dietary requirements of fracture cases. Brit. J. Surg., 1936, **23**, 505.
10. Hirshfeld, J. W., Abbott, W. E., Pilling, M. A., Heller, C. G., Meyer, F. L., Williams, H. H., Richards, A. J., and Obi, R., Metabolic alterations following thermal burns. III. Effect of variations in food intake on the nitrogen balance of burned patients. Arch. Surg., 1945, **50**, 194.
11. Hirshfeld, J. W., Williams, H. H., Abbott, W. E., Heller, C. G., and Pilling, M. A., Significance of the nitrogen loss in the exudate from surface burns. Surg., 1944, **15**, 766.
12. Taylor, F. H. L., Levenson, S. M., Davidson, C. S., and Adams, M. A., Abnormal nitrogen metabolism in patients with thermal burns. New England J. Med., 1943, **229**, 855.
13. Glenn, W. W. L., Muus, J., and Drinker, C. K., Observations on the physiology and biochemistry of quantitative burns. J. Clin. Invest., 1943, **22**, 451.
14. Shaffer, P. A., and Coleman, W., Protein metabolism in typhoid fever. Arch. Int. Med., 1909, **4**, 538.
15. Abels, J. C., Young, N. F., and Taylor, H. C., Jr., Effects of testosterone and of testosterone propionate on protein formation in man. J. Clin. Endocrinol., 1944, **4**, 198. Correction, Ibid., 1944, **4**, 611.