"ESSENTIAL" AND "NONESSENTIAL" AMINO ACIDS IN THE URINE OF SEVERELY BURNED PATIENTS[1]

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The negative nitrogen balance of thermal trauma is a well recognized phenomenon (1, 2). The meaning and significance of this to body metabolism are poorly understood. Partition of urinary nitrogen may assist in interpreting the significance of nitrogen loss. Walker (3) and Taylor, Levenson, Davidson, and Adams (4) found an increase in nonprotein urinary nitrogen of burned patients. This was chiefly due to undetermined nitrogen, the latter defined as the difference between the total nonprotein nitrogen and the sum of urea, uric acid, creatinine, and alpha-amino nitrogen. To analyze this partition further we have studied the urinary amino acid excretion of severely burned patients. Amino-aciduria has been found after severe thermal trauma. A similar amino-aciduria has been observed after comparable surgical trauma, suggesting that the phenomenon is nonspecific. Similar studies on patients with hypercorticism (Cushing's disease) suggest that the adrenal gland may be operative in the amino-aciduria of thermal trauma.

MATERIALS AND METHODS

The paper chromatographic technic described by Martin and Synge (5), Consden, Gordon, and Martin (6), and Dent (7) was utilized to study the urinary excretion of free amino acids. Hydrolysis of urine was not carried out, and so the role of peptiduria cannot be evaluated.

Aliquots of 24-hour urine collections were analyzed for nitrogen (Kjeldahl) and volumes corresponding to 250 micrograms of total nitrogen were chromatographed two-dimensionally. The first solvent consisted of phenol and water; and the second, of butanol, propionic acid, and water (8). The papers were developed by spraying lightly with 0.1 per cent ninhydrin in ethanol.

Using this technic, urine of normal people is found to contain mainly glycine and alanine with occasional traces of other "nonessential" amino acids. In general, only the lower molecular weight, "nonessential" amino acids are found in urine (9–11).

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Five members of our laboratory personnel served as "normals." Figure 1 represents the amino acid pattern of the person in this group showing the greatest excretion of amino acids. It also illustrates our charting conventions. The amino acids have been arbitrarily classified as "essential" and "nonessential" in terms of the latest available information regarding the necessity of their presence in the diet of normal adult human beings in order to maintain positive nitrogen balance (12). Within both groups the amino acids have been arranged in order of increasing molecular weight.

The severely burned

Six severely burned patients were studied. All were brought to the hospital shortly after injury except Case No. 52–51, who was transferred from another hospital 48 hours after injury. All had suffered extensive thermal trauma involving from 15 to 65 per cent of the total body surface.

All patients were in good general health prior to being burned. Treatment was similar in each instance. Plasma and blood were used for colloid replacement according to a standard formula (13). Penicillin and streptomycin were administered in every case. All the burned areas were treated with occlusive dressings. Administration and calculation of oral intake were supervised by a special dietitian. Twenty-four hour urine samples were collected in bottles containing thymol and toluol by means of an indwelling catheter. Aliquots were used for analyses.

The unburned

Similar observations were done on patients undergoing conventional surgical procedures. In order of increasing magnitude these procedures were: excision of fissure in ano, hemorrhoidectomy, herniorrhaphy, total hysterectomy, and subtotal gastrectomy. All operations were elective and all patients in good general and nutritional condition. Gas-oxygen-ether anesthesia was used in all but the first two cases, where spinal anesthesia was utilized. Postoperatively, saline, glucose, and water were administered intravenously until the patient's oral intake was adequate.

In an attempt to evaluate the role of the adrenal glands, three patients with hypercorticism due to fullblown Cushing's disease were studied. Twenty-four hour urine collections were carried out before and after radical subtotal adrenalectomy. A minimum of fourteen days was permitted to elapse after surgery so that any effects due to

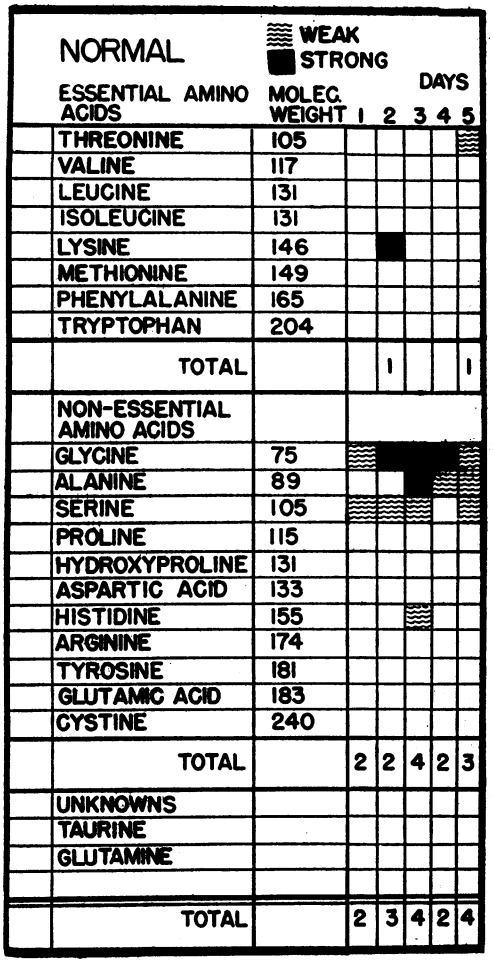

FIGURE 1

surgical trauma *per se* (as seen in the previous group) might be minimized.

RESULTS

The burned

The findings in the burned patients are reported in order of increasing severity. There are two charts for some patients. The first represents the amino acid excretion patterned after Figure 1; the second corresponding chart, suffixed by the letter A, shows the simultaneous over-all nitrogen balance and gross amino-aciduria for the same patient. Since volumes of urine containing equal amounts of nitrogen were chromatographed daily, the significance of the "A" chart becomes obvious. If the amino-aciduria represents a constant percentage of the nitrogen excretion, then chromatography of urine containing equal amounts of nitrogen should result in equal amounts of total amino acid on the chromatogram.

A 19-month-old boy with deep second degree burns of 17 per cent of his body surface (Figure 2, Case 52–88) showed a pattern of amino acid excretion only slightly greater than normal. If taurine and glutamine were to be excluded, the pattern might be considered at the upper limit of our "normals."

A similar pattern is seen in the amino acid excretion of a 42-year-old man who suffered a thermal burn of 30 per cent of his body surface; about half of this was full thickness (Figure 3, Case 52–70). Amino-aciduria subsided somewhat after the first four weeks.

Figure 4 (Case 52–51) demonstrates the amino acids present in the urine of a 30-year-old lineman who suffered deep, high voltage, electrical burns of the entire right arm and both thighs. The arm was amputated at the shoulder and both legs were radically debrided at the time of admission, 48

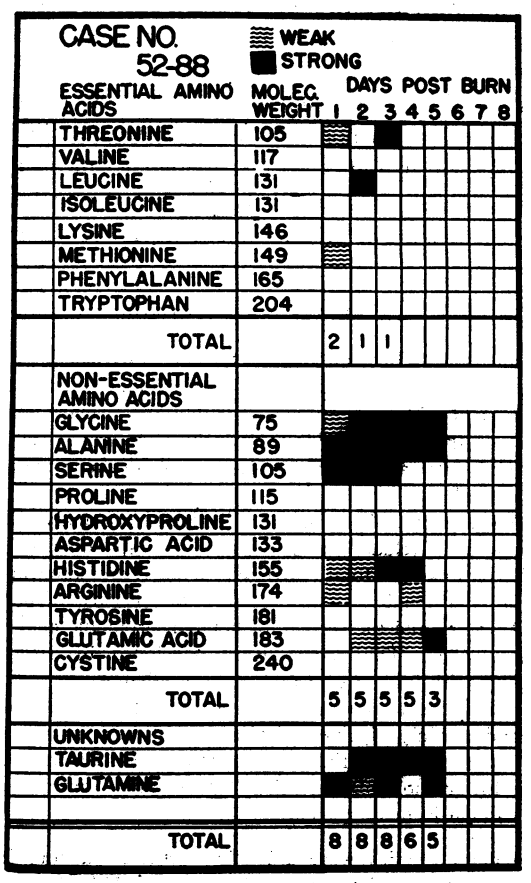

FIGURE 2

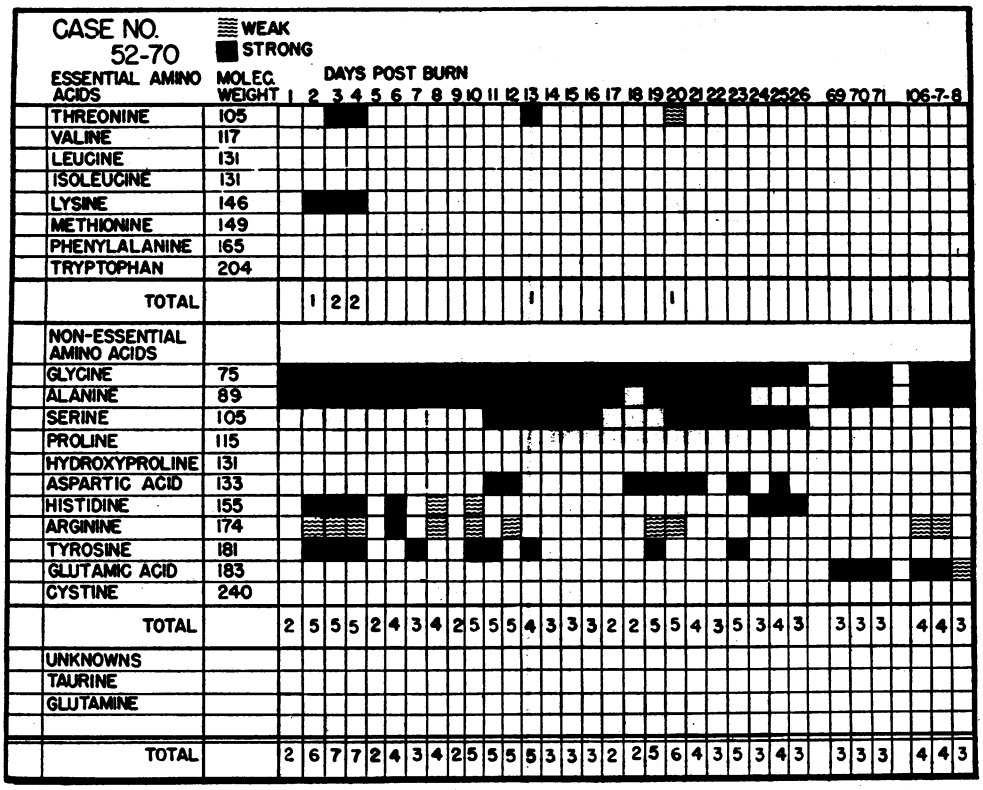

FIGURE 3

hours after injury. His convalescence was uneventful. Taurine and glutamine were again present. The over-all amino acid excretion appeared greater than normal throughout the period of observation. In Figure 4A this patient's nitrogen balance has been charted synchronously with the number of amino acids excreted. Nitrogen balance was calculated by subtracting the grams of nitrogen (Kjeldahl) in the urine, feces, and dressings from grams of nitrogen administered by mouth and parenterally. It is of interest to note the relative constancy of the amino-aciduria despite the shifts in nitrogen balance.

Figure 5 (Case 52–90) represents the amino-aciduria of a 23-year-old man who suffered thermal burns of 40 per cent of his total body surface, most of which were full thickness. Amino-aciduria was still present after forty days, at which time many areas were still unhealed. After 139 days, when grafting had been completed, reduction in amino acid excretion, particularly as regards the "essential" amino acids, is evident.

The amino acid pattern of a 45-year-old woman with 50 per cent body surface burns, over half of which were full thickness, is summarized in Figure 6 (Case 52–92). She died seven days after burning, while in oliguria. The serum nonprotein nitrogen had risen from a value of 31 mg. per cent at the time of admission to 135 mg. per cent two days before death. Figure 6A demonstrates that the period of amino-aciduria corresponded roughly to the period of greatest nitrogen negativity. However, amino-aciduria persisted after restoration of positive nitrogen balance, suggesting that the quantitative and qualitative excretion of amino acids was independent of the over-all nitrogen balance.

A 64-year-old woman with a thermal burn of 64 per cent of her body surface, more than half of which was full thickness, died on her tenth hospital day. Amino-aciduria persisted, though subsiding, until the time of death (Figure 7, Case 52–83). In Figure 7A the amino acid excretion again fluctuates independently of over-all nitrogen balance. The terminal reduction of amino acid excretion may have been due to a reduced glomerular filtration.

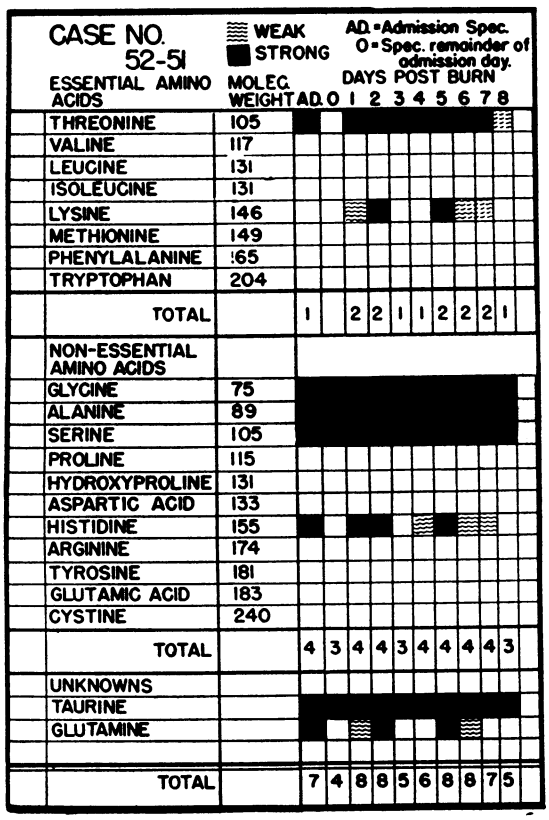

FIGURE 4

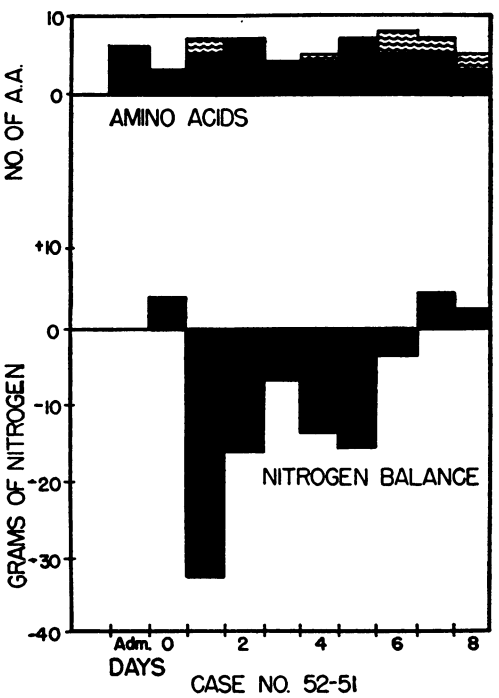

FIGURE 4A

The unburned

Chromatography of the preoperative urine specimens of the five patients undergoing surgery revealed a normal pattern in every case. Minor procedures such as hemorrhoidectomy and excision of fissure in ano resulted in little if any deviation from normal. Partial gastrectomy (Figure 8, Case S–3) resulted in somewhat less amino-aciduria than that seen in Figure 5. Hysterectomy and herniorrhaphy resulted in a pattern which lay between that of gastrectomy and hemorrhoidectomy.

The three patients with Cushing's disease showed a greater than normal urinary excretion of amino acids before operation. Figure 9 illustrates the typical findings in one of these cases. The patient was a 42-year-old woman with a ruddy face, abnormal fat distribution, hirsutism, and hypertension. A two-stage, radical, subtotal, adrenalectomy was carried out under cyclopropane anesthesia. The postoperative course was uneventful and the dosage of cortisone was gradually reduced from 200 mg. per day to a daily maintenance dose of 30 mg. over a period of two weeks. After this maintenance dose had been established, daily urinary collections were carried out for five days. Despite the possible residual effects of surgery, the

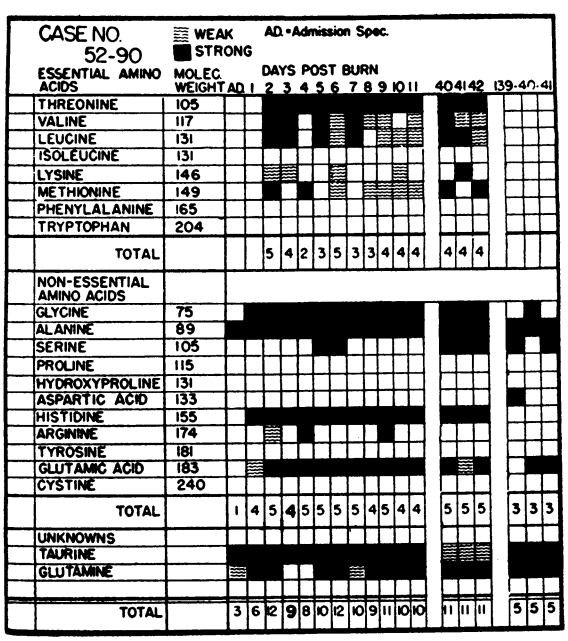

FIGURE 5

CASE NO. 52-92 — ▒ WEAK, ■ STRONG; AD.=Admission Spec. 0=Spec. remainder of admission day.

ESSENTIAL AMINO ACIDS	MOL.EG. WEIGHT	AD	0	1	2	3	4	5	6	7	8	9
THREONINE	105	▒			■	▒						
VALINE	117			▒	■	■		■				
LEUCINE	131			▒	■	■	■	■				
ISOLEUCINE	131											
LYSINE	146				■	■	■	▒	■			
METHIONINE	149											
PHENYLALANINE	165											
TRYPTOPHAN	204											
TOTAL			1	2	4	4	2	3	1			
NON-ESSENTIAL AMINO ACIDS												
GLYCINE	75	▒	▒		▒	▒		■	■			
ALANINE	89	■	■	■	■	■	■	■	■			
SERINE	105											
PROLINE	115											
HYDROXYPROLINE	131											
ASPARTIC ACID	133				■	■	■	■	■	▒		
HISTIDINE	155											
ARGININE	174				■	■		■				
TYROSINE	181											
GLUTAMIC ACID	183	▒	▒			▒		■				
CYSTINE	240											
TOTAL			3	2	4	5	3	4	5	3		
UNKNOWNS			■			■						
TAURINE					■	■	■					
GLUTAMINE						■	■	▒	■	▒		
TOTAL			5	4	10	12	6	8	7	4		

FIGURE 6

CASE NO 52-83 — ▒ WEAK, ■ STRONG; AD.=Admission Spec.

ESSENTIAL AMINO ACIDS	MOL.EG. WEIGHT	AD	1	2	3	4	5	6	7	8	9	10	11
THREONINE	105		■	■	■	■	■	▒			▒		
VALINE	117		■	■	■	■							
LEUCINE	131		■	■	■	■		▒	▒			▒	
ISOLEUCINE	131												
LYSINE	146	▒	■	■	■	■	■						
METHIONINE	149				▒	▒							
PHENYLALANINE	165												
TRYPTOPHAN	204												
TOTAL			1	4	5	5	5		2	1		1	1
NON-ESSENTIAL AMINO ACIDS													
GLYCINE	75		■	■	■	■	■	■	■	■	■	■	
ALANINE	89		■	■	■	■	■	■	■	■	■	■	
SERINE	105				■	■	■	■	▒		▒		
PROLINE	115												
HYDROXYPROLINE	131												
ASPARTIC ACID	133												
HISTIDINE	155	▒	■	■	■	■	■	▒	▒				
ARGININE	174			▒	■	■					■		
TYROSINE	181												
GLUTAMIC ACID	183	▒	■	■	■	■	■	■	■	■	■	■	
CYSTINE	240												
TOTAL			4	4	6	5	6	5	5	3	3	5	3
UNKNOWNS				2	2	2	2	1	2	1		2	1
TAURINE													
GLUTAMINE													
TOTAL			5	10	13	12	13	6	9	5	3	8	5

FIGURE 7

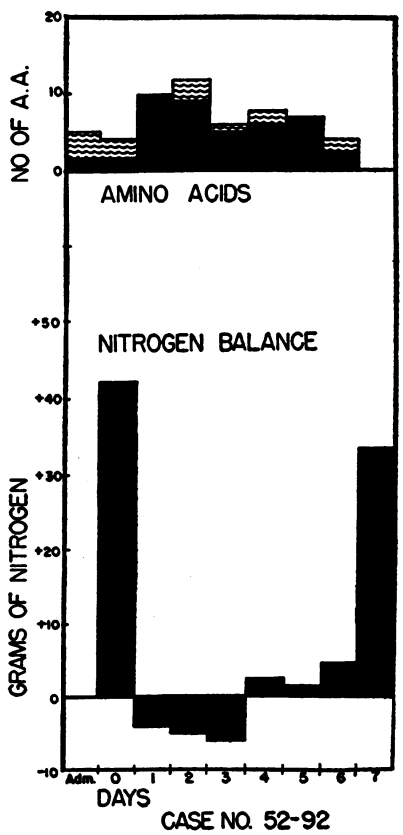

FIGURE 6A

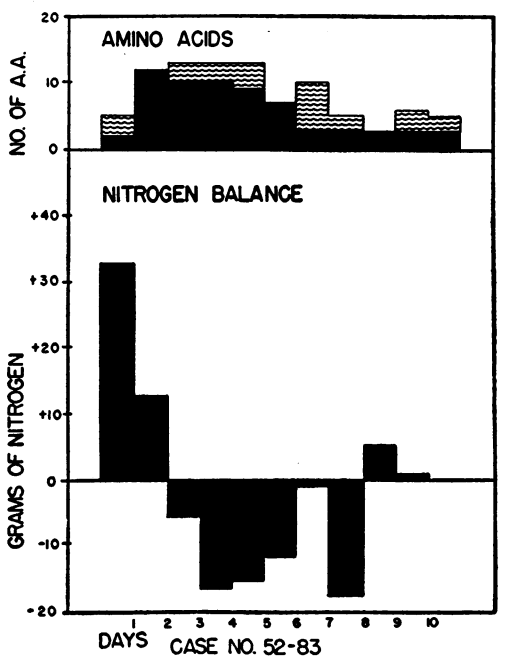

FIGURE 7A

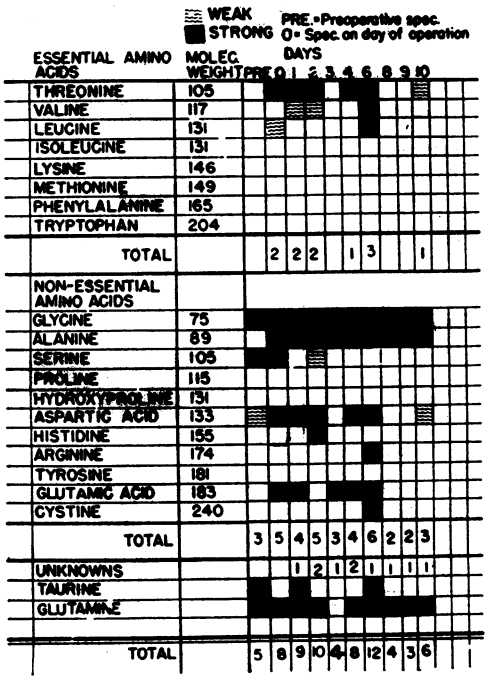

FIGURE 8

minimal amino acid excretion is striking. Figure 9A shows the reduction of amino-aciduria in the postoperative phase as well as the over-all nitrogen metabolism.

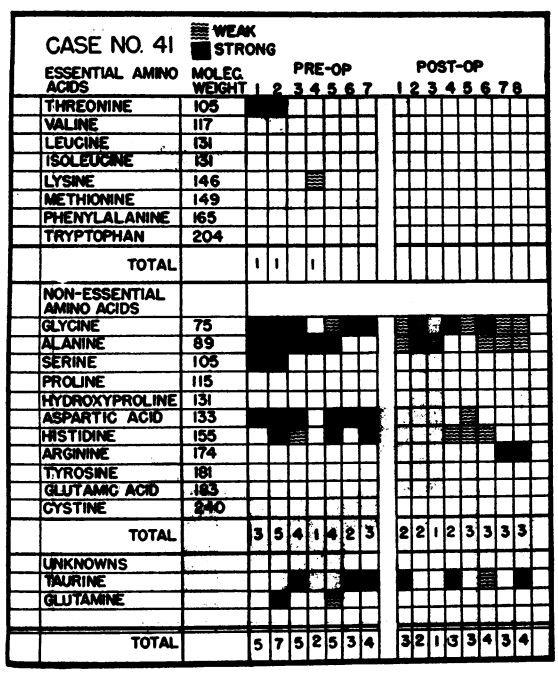

FIGURE 9

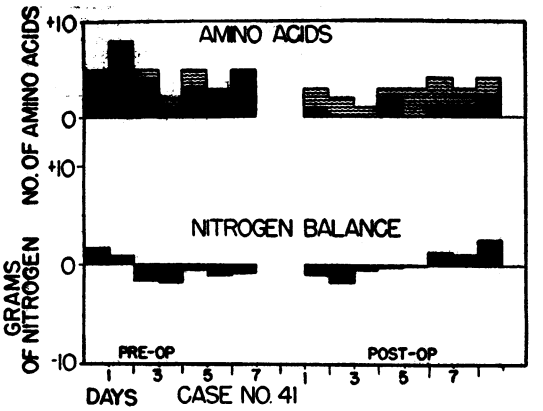

FIGURE 9A

DISCUSSION

Amino-aciduria accompanied the increased urinary nitrogen loss which follows severe thermal trauma. It was also present after major surgical procedures. It consisted of an increase in both the lower molecular weight, "nonessential" amino acids normally found in urine and the higher molecular weight, "essential" amino acids not usually present. There was some suggestion that both the qualitative and quantitative aspects of the amino-aciduria may be proportional to the severity of the trauma. Patients who were not burned severely enough to endanger their lives and patients undergoing minor surgical procedures showed only minimal amino acid excretion with a preponderance of "nonessential" amino acids. Those dying of their burns or undergoing major surgery had a massive excretion of both "essential" and "nonessential" amino acids.

The actual mechanism of this amino-aciduria is not clear. There are three possibilities. Amino-aciduria may result when elevated blood amino acid levels exceed the renal threshold. With normal blood levels, renal damage and failure of tubular reabsorption may cause amino-aciduria. Finally, amino-aciduria may be seen with normal blood levels and "intact" kidneys in certain "metabolic" disorders—*i.e.*, "primary amino-aciduria" as in the de Toni-Fanconi syndrome and cystinuria. Although the available data are inadequate to rule out such a mechanism in thermal trauma, a metabolic disorder of this sort seems unlikely. It would seem most likely, then, that the escape of amino acids observed is due to either elevated plasma amino acid levels or renal dysfunction (al-

though no evidence for the latter has been found), or a combination of these two mechanisms.

Elevation of alpha amino nitrogen in the blood of eight of twelve patients with thermal burns in the first 24 hours after injury has been demonstrated (14). However, marked hemoconcentration was present in these patients. Repeated plasma amino nitrogen levels determined in our patients when hemoconcentration was not present were always found to be within normal limits, *i.e.*, less than 5 mg. per cent.

Everson and Fritschel (15) failed to show any rise in plasma amino nitrogen subsequent to anesthesia and surgery. Friedberg and Greenberg (16) and Li, Geschwind, and Evans (17) suggest that cortical extract or adrenocorticotropic hormone may increase the level of plasma amino acids. Greif (18), however, found a significant fall in the plasma amino acid level of a hypopituitary patient receiving adrenocorticotropic hormone. Russell (19) found that the increase in blood urea in nephrectomized rats resulting from intravenously administered amino acids was markedly reduced by this pituitary hormone.

The high protein intake administered to convalescing burned patients might be a factor in elevating the plasma amino acid level. Dent and Schilling (20) showed that after ingestion of proteins large rises occurred in the concentration of many amino acids in the portal blood of dogs, which could be accompanied by amino-aciduria. It is doubtful that a high protein intake played a significant role in our patients, since the amino-aciduria was subsiding as the patients improved clinically and were able to ingest more protein. In addition, the surgical patients received little or no protein during the first days of convalescence.

Certainly all these factors, protein breakdown, shock, and increased steroid production, are operating in the burned and traumatized patients. However, it seems unlikely that increased blood concentration of amino acids alone can account for the observed amino-aciduria.

After administration of ACTH to a patient, Ronzoni, Roberts, Frankel, and Ramasarma (21) found an increase in urinary amino acid excretion, which returned to normal upon cessation of therapy. However, Russell and Wilhelmi (22) demonstrated an improved rate of de-amination in adrenalectomized rats when cortisone was administered. Burnett (23) suggested that steroid administration improved tubular reabsorption in Addison's disease. Cagan, Klein, and Loewe (24) felt that cortisone caused increased tubular reabsorption of intravenously administered amino acids in treated Addisonians. It is possible that one of the adrenal steroids other than cortisone is responsible for failure of tubular reabsorption, but we have no evidence for this at present. Since adrenal hyperfunction is probably present in all our burns, cortisone may be suspected as a causative factor.

The kidney may play a specific role in the amino-aciduria of trauma. Amino acids seem to resemble glucose in their renal behavior. However, the mechanism of tubular reabsorption must differ since phlorizin poisoning does not produce amino-aciduria. Since amino acids filter freely through the glomerulus, amino-aciduria may result from failure of tubular reabsorption. Pitts (25) has shown a competitive tubular reabsorption for creatin and glycine. Since numerous investigators (26 – 28) have shown the presence of creatinuria in burns, a possible explanation for an accompanying amino-aciduria is suggested. However, since such competition exists only at very high plasma levels, this factor is probably of no importance.

Finally, the influence of amino acids themselves on the renal tubules must be considered. Kamin and Handler (29) have shown that the excretion of threonine and histidine was easily effected by the presence of an unusual quantity of any other amino acid in the plasma. Perhaps the presence of a disorderly, albeit not elevated, pattern of amino acids in the renal tubule is enough to initiate a disturbance of tubular reabsorption.

SUMMARY

An abnormal amino-aciduria consisting of excretion of "essential" amino acids not normally present in urine as well as increased quantities of "nonessential" amino acids was found in the urines of severely burned or traumatized patients.

The qualitative and quantitative aspects of this amino-aciduria may be proportional to the severity of the trauma. The amino acid excretion was influenced by but not completely dependent upon the total urinary nitrogen. Hyperactivity of the adrenal cortex as seen in Cushing's disease resulted in a moderate degree of amino-aciduria. The latter effect was not reproduced by a daily

maintenance dose of 30 mg. cortisone after bilateral subtotal adrenalectomy.

This escape of amino acids is most likely due to a failure of tubular reabsorption. The reason for the latter is not clear but may be due to some adrenal steroid other than cortisone, to an abnormal amino acid pattern presented to the tubule, or to some other factor.

ACKNOWLEDGMENT

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