Urinary Peptide Excretion in the Burned Patient

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Pronounced changes in nitrogen metabolism are known to occur as the result of a serious burn (1-8). Although considerable information is available on these changes, little attention has been given to the quantitative excretion of peptides in the urine of the burned patient. Such an assay has interesting theoretical possibilities. The nitrogenous products in the urine come from ingested food and body tissues. Since it is unlikely that urinary peptide excretion is direct from digested food (9, 10), it was felt that they probably originate in body tissues. Thus, liberation of peptides may be a reflection of tissue destruction or cellular disorganization, and this in turn could conceivably be a reflection of a major factor in the physiology of a burned patient.

In this report the urinary peptide loss will be correlated with the clinical estimate of the seriously burned patient. In so doing we will attempt to show how this relatively simple determination may be used to provide important information concerning the clinical condition of such a patient.

Material and Methods

Studies were made on 38 patients admitted to the Surgical Research Unit for the treatment of burns. These burns ranged from minimal to lethal, and 16 of the 38 did not survive. Since normal values are not available on children (11), it was not possible to adequately interpret the

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results on those who were burned. Thus, the lower age limit of patients dealt with in this report was held to 17 years.

Therapy was essentially in accordance with that given by Reiss and associates (12). During the first several days following a burn, administration of fluids is intensive to ensure a normal blood pressure. This is of importance to the present study in that it encourages adequate urine formation, since an analysis of urine would be of little value in a patient with near renal failure.

The Burn Index (13) was used to define the over-all seriousness of a burn with both second- and third-degree involvement. This index weights a second-degree burn as being one half as serious a clinical entity as a third-degree burn. Estimates of the per cent of body area involved and the degree of burn were usually averaged from the independent observations of at least two surgeons experienced in the handling of burned patients.

Urinary peptides were assayed by the biuret method of Balikov and Castello (11). Analyses were done on aliquots of 24-hour collections (8:00 A.M. to 8:00 A.M.) and are reported in terms of glutathione (GSH) equivalents. Normal values for adults are from 1.1 to 4.7 Gm. of peptide/24 hours as GSH. Specimens that were not assayed on the day of arrival (as on week-ends) were stored in a refrigerator. No samples were stored for more than 3 days.

Clinical evaluations as in diagnosing septicemia or excessive wound purulence were made available through the generous cooperation of the Bacteriology Section and the surgeons of the Clinical Ward of the Surgical Research Unit.

RESULTS

Figure 1 illustrates the trend from elevated peptide excretions following the burn injury to normal values as the patient recovers. Each dot represents the average excretion for a 10-day period on a patient who survived. When two successive averages were normal, and the patient was considered clinically well, no further values were plotted. Results on 19 seriously burned patients (Burn Index 15 to 51) are represented. By the 70- to 80-day period only 1 patient (J. W., Burn Index 44) still had an average abnormal peptide excretion. With one exception, this patient had the most serious burn of those who lived. In no case was an abnormal value obtained on a patient who was completely healed. Two values seem exceptionally high for their period; viz., 23.8 in the 40- to 50-day period and 14.9 in the 60- to 70-day period. In the first instance, the patient was receiving protein hydrolysate, orally, and in the second instance, the pa-
The correlation between the urinary peptide excretion and days postburn on patients who recovered. Each dot represents the average excretion within its 10-day period. Normal values were not plotted after two successive averages were normal, and the patient was considered clinically well. (Urinary peptides are in terms of Gm. per 24 hours as GSH.)

Tient had a septic arthritic condition and incipient congestive heart failure in addition to her burn. These were exceptional circumstances which could conceivably have an effect on peptide excretion.

The daily pattern of peptide excretion is shown in Fig. 2 for C. A. with a Burn Index of 15, and J. W. with a Burn Index of 44, both of whom recovered. These patients had relatively uncomplicated recoveries and so were deemed most suitable to illustrate the basic differences between mild and severe burns on survivors. It can be noted that some of the values in Fig. 2 are considerably higher than any values in Fig. 1. This is due to a phenomenon which can be termed "rhythmic spiking." Such spikes lasted only a few days and then subsided. When values are combined over a 10-day period as in Fig. 1, it is apparent that these spikes are masked in the over-all average. Figure 2 also illustrates three characteristics of the more serious burns: (1) higher spikes, (2) increased frequency of spiking, and (3) a longer time before normal values are obtained.

Figure 3 illustrates the daily pattern of peptide excretion for G. M.
Fig. 2. The circles connected by a solid line represent the daily urinary peptide excretion pattern of C. A. who had a Burn Index of 15. The dots connected by a dash line represent this pattern for J. W. who had a Burn Index of 44. (Urinary peptides are in terms of Gm. per 24 hours as GSH.)

with a Burn Index of 38, and F. E. D. with a Burn Index of 47, both of whom died. Although in patients who recover, the highest spike is usually the initial spike reached within approximately 10 days postburn (Fig. 2), subsequent spikes reaching successively higher levels have been characteristic of patients whose condition was deteriorating.

In the line graphs of both Figs. 2 and 3 there are areas where no daily values are shown, as for example in Fig. 2 between days 33 and 39 on the dash line. Results were unavailable for several reasons, one of which was the formation of a yellow precipitate during the analytic procedure. This precipitate apparently takes up most of the copper of the biuret reagent since, when centrifuged, the supernatant is almost colorless. It is believed to be a cuprous oxide produced by a reducing substance, such as glucose (11). At other times, results were unavailable due to the presence of preservatives which were added in preparation of the urine for other analyses and whose effect on the peptide assay was unknown.
Fig. 3. The circles connected by a solid line represent the daily urinary peptide excretion pattern of G. M. who had a Burn Index of 38, and who died on his thirty-third postburn day. The dots connected by a dash line represent this pattern for F. E. D. who had a Burn Index of 47 and who died on his twentieth postburn day. (Urinary peptides are in terms of Gm. per 24 hours as GSH.)

Associated Conditions and Therapy

An increased peptide excretion was found to accompany the onset of septicemia. Fourteen patients in this study were diagnosed as having septicemia sometime during their course of treatment. Of these, 8 had peptide values available on the day preceding and the day of onset of septicemia. The average increase of urinary peptide excretion in this 24-hour period was 7.5 Gm. as GSH. Increases ranged from 3.1 on a patient (M. L.) with a positive blood culture but without a spiking temperature, chills, or prostration, to 14.7 on a patient (J. C.) who died 4 days later. Patient M. L. had a temperature up to 104.6° 8 days after the positive blood culture was found. His peptide excretion increased 9.0 Gm. from the day before.

Table 1 illustrates the moderate increase in peptide excretion to be expected as the result of an operative procedure such as grafting. (“Post-
Table 1. The Effect of Grafting on Peptide Excretion
Postgraft excretion minus pregraft excretion

<table>
<thead>
<tr>
<th>Burn Index</th>
<th>Days Postburn</th>
<th>0 to 30</th>
<th>Over 30</th>
</tr>
</thead>
<tbody>
<tr>
<td>15-24</td>
<td>Average difference in Gm. of peptide/24 hrs. as GSH.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25-34</td>
<td>3.6</td>
<td>1.8</td>
<td></td>
</tr>
<tr>
<td>Over 35</td>
<td>4.6</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.8</td>
<td>2.7</td>
<td></td>
</tr>
</tbody>
</table>

graft” excretion refers to the day of the operation and “pregraft” excretion refers to the day before the operation.) There appears to be a trend for this increase to be of lesser magnitude if the operation occurs after 30 days postburn. In fact, 6 of the 57 values used to compile these averages showed a decrease in peptide excretion all occurring in the period after 30 days postburn. This may be due to smaller grafting procedures or an increased ability of the patient to cope with an operative procedure, or both, since most patients are well on their way to recovery by this time. No clear-cut relation between the extent of burn and increase in peptide excretion resulting from an operative procedure is evident.

When they were almost healed, 2 patients were given growth hormone (Armour’s). A course of therapy consisted of 50 mg. intramuscularly every 6 hours for 5 days. A total of seven such courses of therapy were given. In all but one instance, there was an increase in peptide excretion with maximum values usually exhibited 3 to 5 days after the onset of therapy. These maximum values showed an average increase of 4.0 Gm. over the excretion the day before therapy was begun. This effect of growth hormone is difficult to interpret. There was a concomitant weight loss of about 4 pounds over the 5-day period of medication in each of the six instances with elevated peptide excretion. In the one case of diminished excretion, the patient gained about 5 pounds during the course of therapy. Members of the Surgical Research Unit presently investigating the effect of growth hormone on the burned patient, are of the opinion that its effect on weight and nitrogen balance depends, at least in part, on nitrogen intake which was uncontrolled in this study.

Two patients received protein hydrolysate1 intravenously. This resulted in an increase in peptide excretion of from 5 to 10 Gm. as GSH per liter of hydrolysate. This is not surprising since the molecular size of most of the fractions would permit easy passage through the kidney. It has been mentioned by Christensen (9) that there is large urinary wastage upon injection of peptides.

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1 Five per cent bovine plasma digested to free amino acids and polypeptides of which at least 50 per cent of the total nitrogen was present as free α-amino nitrogen.
Tarry and bloody stools indicative of gastrointestinal bleeding were noted on 4 patients. The day on which bleeding was noted showed a slight increase in peptide excretion (average 2.0 Gm.) compared with the day before. However, maximum values were usually reached 2 to 3 days later, at which time the increase over the day before bleeding began averaged 12.6 Gm. The range of maximum increases varied considerably from 3.4 on a patient with possible kidney damage to 32.1 on a patient who died 4 days later with a diagnosis of bleeding duodenal ulcer, peritonitis, and hemorrhage of the pancreatic duodenal artery.

Excessive wound purulence uncomplicated by septicemia or bloody stools is usually (11 of 12 instances) accompanied by a measurable increase in peptide excretion. Since the extent of wound purulence is usually noted at a dressing change, increases are measured from the day of the last dressing change at which purulence was not noted to the day on which purulence is noted. The average increase in this period was 3.0 Gm. The maximum increase during this period was 3.5 Gm.

Several other conditions appeared to be associated with increased urinary peptide excretion but occurred too infrequently to generalize on relationship. They were: (1) 2 patients with low plasma calcium levels; (2) 2 patients on heparin therapy for the treatment of thrombosis; (3) 2 patients with tachycardia; (4) 1 patient who in addition to a bacteremia, developed a yeast growth in his blood; and (5) 1 patient who developed incipient congestive heart failure.

Temperature rises unaccompanied by septicemia, purulent wounds, etc., had no apparent effect on peptide excretion. An increase of 1° F. or more was accompanied by ten instances of increased and ten of decreased peptide excretion. Limiting the correlation to temperature increases of at least 2° F. showed six instances of increased and five of decreased excretion.

Nine healthy, normal male soldiers were put on bed rest and fed varying amounts of protein as part of a concurrent nitrogen balance study. The urinary excretion of peptides was followed on these men. Their intake varied from 5.6 to 337 Gm. of protein in any particular 24-hour period. The correlation between this varied intake and the urinary peptide excretion was determined by the regression analysis technic described by Snedecor (14). Based on \( n = 53 \), a \( t \) value of 1.26 was obtained which is equivalent to a \( p \) value between 0.2 and 0.3. There is apparently no significant relation between protein ingestion and peptide excretion in the normal person at bed rest.

A correlation was sought between protein ingestion and the urinary peptide excretion in a burned patient. J. W. with a Burn Index of 44, was
Table 2. The Relation Between Protein Intake and Urinary Peptides in a Severely Burned Patient

<table>
<thead>
<tr>
<th>Postburn day</th>
<th>Protein intake (Gm./24 hr.)</th>
<th>Urinary peptides (Gm./24 hr. as GSH)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>39.4</td>
<td>31.4</td>
</tr>
<tr>
<td>7</td>
<td>44.2</td>
<td>27.7</td>
</tr>
<tr>
<td>8</td>
<td>44.2</td>
<td>18</td>
</tr>
<tr>
<td>9</td>
<td>44.2</td>
<td>13.4</td>
</tr>
<tr>
<td>10</td>
<td>44.2</td>
<td>20.6</td>
</tr>
<tr>
<td>11</td>
<td>16.9</td>
<td>22.2</td>
</tr>
<tr>
<td>12</td>
<td>16.9</td>
<td>19.7</td>
</tr>
</tbody>
</table>

placed on controlled protein intakes and was studied from his sixth through twelfth postburn day. The results shown in Table 2 make it unnecessary to confirm by statistical analysis the lack of correlation between protein intake and peptide excretion in this severely burned patient.

DISCUSSION

It must be recognized that this method for assay of urinary peptides yields results in terms of GSH equivalents and not as the absolute amount of peptide or peptide nitrogen (11). Thus, for example, an excretion of 20 Gm./24 hours as GSH does not necessarily mean that the equivalent of 20 Gm. of protein has been excreted since the relation between these two values has not yet been elucidated.

The increase in peptide excretion which accompanies septicemia was always noted with the first signs (15) of this condition whether bacteriologic or clinical. In 4 of the 8 cases studied, the urinary peptide increased the day before the blood showing the positive culture was drawn. It is therefore suggested that a sudden unexplained increase in peptide excretion stimulate concern over the possibility of septicemia being present.

A serious burn is rarely an uncomplicated insult. It is always accompanied by surgical procedures. In addition, septicemia, wound purulence, gastrointestinal ulcers, etc., are not uncommon. All these complicating phenomena give rise to a "hyperpeptiduria" unless, of course, there is concomitant renal failure. On the other hand, oral protein intake and uncomplicated temperature fluctuations have no effect on the excretion of urinary peptide. This information is consistent with the theory that the source of the peptides in the urine is endogenous and that "hyperpeptiduria" is a reflection of tissue destruction or cellular disorganization. The assay of urinary peptides may thus serve as an objective laboratory aid to the physician in his evaluation of the condition of a burned patient.
SUMMARY

The pattern of peptiduria in serious burns has been presented. This pattern has been found to vary, depending upon whether the patient's condition deteriorates or improves. The changes in this pattern resulting from various complications often seen in an extensive burn have been noted.

Evidence has been presented supporting the theory of the endogenous origin of urinary peptides.

REFERENCES