SERUM PROTEIN CHANGES IN THERMAL TRAUMA. I. ELECTROPHORETIC ANALYSIS AT pH 8.6

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It is a well established fact that the circulating proteins of the human are profoundly affected by surgical, accidental and combat trauma. The medical literature on this topic emphasizes the remarkable quantitative and qualitative changes in intracellular and extracellular protein constituents occurring after and in response to injury. Fundamental information concerning these metabolic alterations as well as shifts in body water and electrolytes are of paramount importance in the development of basic concepts of treatment.

This study concerns itself with the quantitative changes in the serum electrophoretic pattern of patients who had sustained a thermal injury. Although there is a large body of literature pertaining to the alterations in the various serum electrophoretic protein components in human disease (1-4), there are relatively few reports dealing with postsurgical experiences (5, 6) and thermal trauma (7, 8).

METHODS AND MATERIALS

I. Laboratory methods. A. Collection of specimens. Blood was obtained by venipuncture of the antecubital fossa of normal individuals and from the most accessible site in burned patients. In six of the cases studied, specimens were obtained within seven hours following injury. Otherwise, all blood samples were collected in the fasting state every other day postburn and no specimens were obtained immediately after a blood transfusion or surgical procedure. All chemical and electrophoretic analyses were performed on nonhemolyzed serum. Blister bleb fluid was collected by needle aspiration and analyzed following coagulation.

B. Analysis of serum total protein. Serum protein was measured by the Biuret method of Wolfson and Cohn (9).

C. Moving-boundary electrophoresis. In preliminary studies, some of which will be mentioned herein, sera were analyzed by moving-boundary electrophoresis at pH 8.6 in the Tiselius Apparatus according to methods outlined by Reiner, Fenichel and Stern (10). Several electrophoretic measurements were also carried out in acetate buffer pH 4.5 in the same system (11). In general, filter paper electrophoresis was found to be better adapted to this type of study because of the difficulty in obtaining blood from severely burned patients and this technique was employed throughout the major portion of this investigation.

D. Filter paper electrophoresis. Sera were analyzed by electrophoretic separation on filter paper in veronal buffer at pH 8.6 for 16 hours using a Spinco Model M Apparatus. Details of the method as well as the procedure used in dyeing the paper strips are available elsewhere (12). The dye stained strips were scanned with a Spinco Model RA Analytrol Densitometer and the per cent composition of the electrophoretic components calculated from the tracings of the automatic integrator. The per cent composition of each serum fraction was then multiplied by the serum total protein level to obtain an estimate of the protein concentration of each component. No correction was made for albumin "trailing" or differences in dye binding capacity of the various fractions. A statistical analysis of replicate serum specimens obtained from normal individuals and burned patients demonstrated a reproducibility consistent with that reported by others (13) using this method.

The mean concentration and standard deviation (in gram per cent) for serum total protein and the electrophoretic components of sera collected from 25 young military personnel in good health were as follows: albumin, 3.4 ± 0.2; alpha-1 globulin, 0.38 ± 0.03; alpha-2 globulin, 0.83 ± 0.18; beta globulin, 1.01 ± 0.2; gamma globulin, 1.45 ± 0.25; * total protein, 7.04 ± 0.16.

II. Clinical material. A summary of the pertinent clinical features of the patients studied in this investigation is presented in Table I. Cases have been tabulated in order of increasing burn severity as estimated on the basis of the burn index. The burn index (B.I.) was

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* When the normal equivalent deviate (14) was plotted against the concentrations of each set of individual components, the alpha-2 and gamma globulin components were found to present a curve of positive skewness indicating non-normal distribution. A similar finding was obtained with the moving-boundary data. This is perhaps not surprising in view of the fact that a large series of serum total globulin determinations on healthy individuals demonstrated non-normal distribution (15).
SERUM PROTEINS IN THERMAL TRAUMA

TABLE I
Summary of cases studied

<table>
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<th>Patient no.</th>
<th>Sex</th>
<th>Age</th>
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<th>Survival‡</th>
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* B.I. = per cent area third degree burn + ¼ area second degree burn.
† All estimates of per cent and area of burn are final estimations.
‡ S, survived; E, expired.

calculated in a manner similar to that of Bull and Squire (16) by the formula:

B.I. = per cent area third degree burn

+ ¼ area second degree burn.

This formula has been used in similar studies of serum protein levels in burns (17) and has been demonstrated to be the best index in the evaluation of the nitrogen metabolism following thermal injury (18).

The per cent of burn area was estimated clinically by the rule of nines (19) in which the body surface is divided into areas representing 9 per cent or multiples of 9 per cent: the head and neck, 9 per cent; anterior trunk, 2 × 9 or 18 per cent; posterior trunk, 18 per cent; each lower extremity, 18 per cent; each upper extremity, 9 per cent; and the perineum, 1 per cent. Since initial estimates of burn involvement are commonly overestimated, all values given are based on the final estimation of the patient's injury after excision, grafting, and so forth.

For purposes of tabulation and discussion, the patients listed in Table I have been arbitrarily broken down into three groups. Group I includes those patients who sustained a third degree injury of less than 10 per cent and whose B.I. was less than 10. Group II consisted of nine patients having a range in B.I. of 12 to 21. Cases in Group III were the most severely injured, having a range in B.I. of 24 to 53. Of these 12 cases, 9 had proven or clinical septicemia (75 per cent) and 6 expired (50 per cent). All patients were studied within a 10 month period so that established methods of treatment were essentially similar in each case (20).

RESULTS

Figures 1 through 4 present data on the alterations of the serum levels of total protein and electrophoretic components of 14 patients who had sustained a third degree thermal injury of greater
than 12 per cent of the body surface. Albumin and alpha-2 globulin levels on 4 less severely burned patients (Group I patients) are included in Figures 2 and 3. Values obtained on Day 0 represent the first serum specimen drawn after the injury. Otherwise, all values were obtained on specimens collected each morning on the day indicated in the figures.

From the data presented in Figure 1, it is seen that there is a sharp reduction in the serum total protein level in the immediate postburn period. Although analyses were performed on six patients, within 45 minutes to seven hours after injury, there was no evidence of serum hyperproteinemia immediately after the burn as has previously been reported by others (21). At about the first to the fifth day, the serum total protein level in the majority of patients was in the neighborhood of 5 Gm. per cent, irrespective of the severity of the burn. This observation has also been made by others (20) on patients under similar therapy at this Unit where fluid and electrolyte administration in the immediate postburn period is emphasized.

That the decrease in albumin is mainly responsible for the initial fall in serum total protein concentration is indicated in Figure 4. However, percentage-wise, many of the electrophoretic patterns were normal at Day 0 or Day 1 indicating that secondary dilution from shifts of water to vascular spaces as well as fluid therapy also account for low levels of circulating protein. From Day 2 to 5, the decreased level of albumin is almost wholly responsible for the low total protein since during this time several of the other serum protein components are normal or elevated. Between Day 5 and 6, albumin concentration reaches a minimum and remains at a very low level in most instances for two to three months following a burn. Serum total protein on the other hand, gradually starts to increase toward normal levels about Day 6. Inspection of the changes in globulin components (Figures 3 and 4) indicates that this increase in total protein level is due to an increase in certain of the globulin components, particularly gamma globulin and alpha-2 globulin which constitute the majority of the globulin fraction.

In Figure 3, the comparatively rapid increase in the alpha globulins beginning about the second
postburn day is indicated. The accurate electrophoretic quantitation of alpha-1 globulin is difficult due to its comparatively low concentration and dependence upon separation from albumin. However, in spite of considerable variation, the data presented in Figure 3 indicate that alpha-1
globulin parallels the level of alpha-2 globulin following the thermal injury.

Sera obtained routinely from six patients were analyzed at pH 4.5 in the Tiselius apparatus (11). At this pH the majority of the serum proteins are positively charged and migrate cathodically with the exception of the more acidic glycoproteins of the alpha globulins fraction. The glycoprotein components having a mobility of M-1 and M-2 as described by Mehl, Golden and Winzler (11) were found to be markedly elevated at about Days 4 to 6 following injury. Similar findings have been obtained by chemical and immunological methods (22, 23) which appear to suggest that increases in the glycoprotein concentrates following injury are partially responsible for the increase in alpha globulin components of serum. Increases in alpha globulin occur in a variety of wasting diseases and in most instances of hypalbuminemia. High levels of alpha-2 globulin have previously been reported in burns (7, 8, 24) although there is no information on alpha-1 globulin changes. It thus appears evident that in many types of stress including burns, increase in alpha-globulin components occurs irrespective of the type of injury sustained (25).

Very little evidence was obtained that any change takes place in the level of the beta globulins following a burn (Figure 4). With the exception of a few instances in which this component was slightly decreased in the immediate postburn period, no radical change in beta globulin was noted by either filter paper or moving-boundary electrophoretic techniques. This is perhaps a little surprising in view of the marked alterations in lipoprotein levels reported in laboratory animals following injury (24), since the beta globulin component includes the majority of these high molecular weight substances. These changes would not necessarily be evident by filter paper electrophoresis in which only protein components are detected by the protein staining procedure, but one would expect the beta globulin area as measured by the moving-boundary method to be increased. This did not occur, however.

The lack of any detectable change in the level of beta globulin lipoprotein as measured electrophoretically in the patients studied seems to indi-
cate that the alterations in lipoprotein levels following injury to laboratory animals (26) may be due to changes in the alpha-1 lipoproteins. It is entirely possible that the small increases in alpha-1 globulin protein noted in Figure 3 could be responsible for the marked changes in the lipid moiety of this component (27). However, such an interpretation is difficult because of the separate properties of lipoproteins which are measured by electrophoretic, ultracentrifugal and chemical techniques.

The second half of Figure 4 presents the levels of serum gamma globulin in Group II and Group III patients following a burn. Since there was no significant deviation from the normal range in Group I patients, these data were not included in the figure.

Following thermal injury, there appears to be a tendency toward hypogammaglobulinemia during the first six days postburn irrespective of the extent of the burn. The initial reduction of gamma globulin followed by an increase in this component above the normal range at about the second week following injury took place in almost every burn case studied. No particular relationship could be found between the change in level of gamma globulin following injury and the predisposition or occurrence of septicemia in the patients studied. However, it was noted that in four patients with septicemia in whom gamma globulin data were collected at about the time of a positive blood culture or onset of symptoms, the level of gamma globulin appeared to be abnormally low. An example of the gamma globulin level in two such patients, one with early sepsis and one with late sepsis, is presented in Figure 5.

The present study supports the observations of Balikov, Artz and Solometo who noted a tendency toward post-traumatic hypogammaglobulinemia in a study of 19 burn patients admitted to the Clinical Division of this Unit (17). These workers drew attention to the fact that patients with a B.I. greater than 5 usually had a gamma globulin level that was low both in concentration and in percent of the total serum protein. It is interesting to note that although their technique and normal values for gamma globulin were different from the present study, the observed results were essentially similar (Figure 4).
TABLE II
Comparison of the distribution of electrophoretic components in serum and blister fluid (BF) collected simultaneously in the same patient

<table>
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<tr>
<th>Patient</th>
<th>Specimen</th>
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* Total protein.
† FP, filter paper; MB, moving-boundary.

DISCUSSION

A number of explanations have been offered for the hypogammaglobulinemia following burns (17). It appears from our own observations and related data in the literature, however, that this phenomenon can be explained on the basis of shifts in body water and the resulting dilution of protein following burns.

Plasma protein circulating in the vascular network is diminished following a burn due to withdrawal of water from unburned tissue and an increase in capillary permeability (21, 28). These effects would be responsible for the reduction in total protein and the various electrophoretic components in thermally injured laboratory animals (29, 30) who have received no treatment and probably to a greater extent in humans receiving fluid therapy. The interstitial space in moderate and severe burns increases quite markedly following a burn, even in adequately treated patients (31). That this body compartment is responsible for trapping the protein lost from the vascular compartment is demonstrated by the fact that lymph draining from the burn area shows an increased protein content (32). Although such lymph shows a preponderance of albumin over that in serum, it also contains appreciable globulin (21) and indicates the large concentration of this fraction dissolved in interstitial water. An analysis of blister blebs (which can be regarded as interstitial fluid) in seven of the patients in this study amply confirms this fact (Table II). It will be noted in the table that in each instance the per cent distribution of albumin is greater than that for globulin. This might be expected since the transfer rates from vascular to extravascular space in the normal is slightly greater for albumin than gamma globulin (33). However, in spite of the differential permeability to albumin, the globulin concentration is quite appreciable when one considers the expanded interstitial volume in which it is dissolved. It is this abnormally distended plasma protein reser-
voir which is responsible for the low total protein
level existing in the plasma immediately following
a burn. This might be expected to be obtained
even in normal persons undergoing fluid infusions
(33, 34).

Figure 6 presents a three dimensional diagram
of the mean levels of the various filter paper elec-
trophoretic components of 14 patients following
thermal injury. What appears to be a selective
reduction in gamma globulin during the first six
days following a burn may actually be the result
of several factors. The alpha globulins have a
very high turnover rate (35) and are apparently
rapidly mobilized by the liver in response to stress
(25). As a result, at about the third postburn day
gamma globulin appears depressed in relation to
the alpha globulins which have returned to normal
levels or are elevated. From the fifth to the sixth
postburn day, diuresis usually occurs in burned
patients (36). It will be noted in Figure 6 that
this is the time during which gamma globulin re-
turns to normal levels and subsequently over-
compensates for its previous low level. The rate
of reinfusion of protein from interstitial to vas-
cular compartments during this time is probably
different from what it was from vascular to inter-
stitial (37). This is known to be particularly so
for gamma globulin from acute plasmapheresis
experiments (38).

From the foregoing remarks it appears entirely
plausible that the shifts in gamma globulin follow-
ing burns can be explained on the basis of shifts
in body water and to some extent dilution. Cer-
tainly this appears the most obvious answer. The
relationship between the gamma globulin level
and septicemia is not clear. Only a small per-
centage of gamma globulin represents antibody
protein (39) and it would appear that burn pa-
tients have a normal total body gamma globulin
although of different distribution from that found
in healthy individuals.

Summary

This study concerned itself with the quantita-
tive changes in the serum electrophoretic pattern of
28 patients who had sustained a thermal injury.
The methods utilized included moving-boundary
and filter paper electrophoresis at pH 8.6. The
latter technique was found to be more adaptable
to this type of study and as a result, the data ob-
tained on 18 patients using this method are pre-
sented in more detail.

Following thermal injury having a burn index
of less than 10 there is little evidence that any
marked or definite change takes place in any of
the serum components. Above this burn index,
_i.e.,_ greater than 12, however, the pattern pre-
sented with time postburn appears to result ir-
respective of the area or severity of the injury.
The changes in each of these electrophoretic com-
ponents with time following injury are discussed
in relation to underlying biochemical mechanisms.

No particular relationship was noted between
the extent of hypogammaglobulinemia following
injury and either the predisposal or occurrence
of septicemia. Although there was some evidence
(four patients) that septicemia occurred at a time
of hypogammaglobulinemia, the data are insuf-
ficient to permit any conclusion. A reduction in
gamma globulin following injury occurred to some
extent in every patient with a burn index greater
than 10 and it is suggested that this phenomenon
is the result of a shift in the compartmentalization
of this component.

No alteration was noted in the beta globulin
component following injury. However, the lack
of any observed change in this component may be due to the method of analysis employed.

REFERENCES