Experimental Studies with Reference to Antigen-Antibody Phenomena Following Severe Extensive Burns *

PETER MATTER, M.D., KENNETH CHAMBLER, M.D., BRUCE BAILEY, M.D.,
S. R. LEWIS, M.D., T. G. BLOCKER, JR., M.D.,
VIRGINIA BLOCKER, M.D.

From the Department of Surgery, the University of Texas Medical Branch, Galveston, Texas

CLINICAL trial of convalescent burn serum, which followed the experimental studies of Feodorov and his co-workers,[10] has stimulated considerable interest in recent years in reappraisal of the problem of whether or not toxemia following thermal trauma is a specific entity and, if so, whether it occurs as an immune phenomenon or is related to other causative factors. Meanwhile, despite Soviet[11] and Czech[8] experience and occasional reports of therapeutic use of the serum in other areas, including a few cases following the Chicago School fire,[28] it was concluded in November, 1961 at a workshop conference on *Immunotransfusion in the Treatment of Burns* sponsored by the Subcommittee on Plasma, the National Academy of Sciences, National Research Council,[15] that available data do not indicate whether or not convalescent serum, blood, or plasma surpass other methods of treatment of acute burns to a statistically reliable degree. Should the concept of immunotransfusion be valid, it was further questioned whether the benefits of such therapy might not in fact be related to antibodies against antigens of bacterial origin rather than to specific substances produced in heat-damaged tissues, including elements of the blood. Finally, the point was raised that the published mortality figures on burns in the Soviet Union by Pushkar,[24] Petrov,[23] and others indicate that death during the first 48 hours following injury is still a major problem in recognized burn centers as it was in this country 20 years ago, and that more variables might exist to confuse the issue than were apparent at first glance.

Sevitt[36] has discussed the background of burn toxemia in detail and Malm, who has completed an historical review of the subject with 234 references in a monograph as yet unpublished,[17] summarized his findings at the 1960 International Congress on Research on Burns,[18] reporting as the earliest references Wertheim's work in 1868,[40, 41] and Avdakoff's[2] in 1876, both of whom studied the effects of injections of blood from burned into unburned animals. By 1925, more than 1,000 references had been collected by Harkins[14] in his Textbook on burns, but with developments in surgical physiology and bacteriology, articles both *pro* and *con* began to appear more infrequently in the literature. Brancati,[7] in 1923, had advanced the theory of an antigenic toxin elaborated in burned tissue on the basis of anaphylactic shock in guinea pigs subjected to small experimental burns, and this concept was developed by Simonart[39] and a number of workers including Sol Ray Rosenthal,[26, 27] who, in 1937, reported both a specific burn toxin and a neutralizing antitoxin. Schütz in 1935[32] at a meeting of the Fifteenth International Congress in Leningrad mentioned what appears to be the first therapeutic use of convalescent

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serum and summarized an interesting series of experiments in twice-burned animals. Subsequently Segal and Uzdin,[33] in 1940, published the results of four years' experience with convalescent serum in clinical patients. In the middle 1950's there appeared the work of Feodorov and Skurkovich,[10] Simonart's article on autointoxication after burns,[39] and additional publications by Rosenthal and his coworkers.[29, 30]

The Soviets demonstrated *in vivo* evidence for specific antigens produced from skin injured by thermal trauma and for the development of burn toxin antibodies as an autoimmunization phenomenon. Guinea pigs sensitized by extracts of burned skin were desensitized with normal serum and skin extracts and then subjected to further injection of burned skin extract with resultant anaphylactic shock.

The antigen was characterized as being a thrombin-like material, heat-labile, incapable of passing through a Seitz filter, and not species-specific.

In the presence of antigen from burned skin, serum of burned dogs showed, according to these workers, an ability to fix complement, by the prolonged cold complement fixation technic, whereas tests with normal skin extracts from the same animals were weak or negative. Complement fixation tests became positive about the 7th post-burn day and reached a maximum titer between 20 and 40 days. Activity of the serum could be destroyed on heating to 65° or 70° C. for 30 minutes.

Following standardized burns in unanesthetized dogs produced by flaming alcohol sponges it was also reported that improvement in toxemic symptoms and in early mortality was obtained from the use of convalescent burn serum and *isoimmune* serum taken from animals who had received repeated injections of blood from acutely burned dogs. As a result of these laboratory experiments convalescent burn serum was adopted in the U. S. S. R. for routine therapeutic use in a number of institutes treating acute burns.

Simonart's studies included injection of *in vitro* heat-denatured serum proteins and of commercial preparations of polypeptides into laboratory animals. No toxic reactions were noted on intravenous injections, but morbid or lethal effects occurred on subcutaneous administration into rabbits or into the ventral lymph sac of the frog. It was his theory that the toxicity resulted from hydrolysis by a proteolytic enzyme present in lymph fluid, and he noted that the euglobulin factor extracted from peptone-produced edema provoked the same result when injected into other animals. This study and that of Godfraind,[12] working with Simonart, who advanced the hypothesis that the pathological changes in acute burns were related to increased protease activity, have been challenged in particular by Allgöwer,[1] who failed to duplicate their results under conditions of rigid bacteriological control. Allgöwer has, however, emphasized the fact that greater *toxicity results* when animals are subjected to higher levels of thermal damage and has reported that blood heated to 96° C. produces a 30 per cent mortality in rabbits within 48 hours when injected intraperitoneally (50% in eight days), whereas no morbidity is noted from blood heated to 80° C. Whether or not specific toxic agents are involved is still a moot question.

Rosenthal and his associates, in a series of publications including a presentation at the International Congress on Research in Burns,[31] have reported evidence for the presence of toxins in acute burn sera as demonstrated by inhibition of HeLa cell tissue culture growth, hemolysis of red cells of acutely burned individuals, and precipitinogens against healed burn sera. These effects have been neutralized by *antitoxins* present in the blood of healed burned individuals. Graber[26] at the Surgical Research Unit at the Brooke Army Medical Center, utilizing Rosenthal's technics with HeLa

cell cultures, was unable to duplicate his results. Sell,[34] at the Tissue Bank of the U. S. Naval Medical School in Bethesda, and Miller,[28] at the Naval Medical Research Unit at Great Lakes, in a controlled double-blind study of a large number of specimens in 1961, found confirmation only when there was hemolysis of specimens or when there had been exposure to sunlight. Since Rosenthal's technic included storage of the serum with the clot and since controlled data had been impossible to obtain during the study of the Chicago School fire patients, Miller has stated that no positive conclusions can be drawn from the work conducted in his laboratory at that time and that data obtained were "consistent with" but "in no way confirmatory of a toxin-antitoxin concept." Meanwhile, at the Burns Unit in Prague under the direction of Dobrkovský, Pávková and Doležalova,[21] clinical studies have been in progress for several years. Convalescent burn serum is employed in clinical subjects, and clinical signs and symptoms are correlated with a characteristic curve of serum antibody levels employing a collodion agglutination method against antigens obtained from both involved and uninvolved skin of burn patients. These are believed to be disintegration products of a polypeptide or mucopolysaccharide nature.

Malm and his associates[17, 18] have studied extensively the effect of convalescent serum in burned rats, and his experiences have paralleled rather closely those of our laboratory,[6] with preliminary enthusiasm for convalescent burn serum dampened by later failure to obtain significant differences in survival rates in the early period. In his case when a change was made to pathogen-free rats at the Walter Reed Army Institute of Research, a high enough mortality rate could not be obtained in the control series against which to evaluate the immediate effects of convalescent serum therapy although slight protective effects were noted in terms of ultimate survival when very extensive burns were inflicted.

Sanford Rosenthal[25] at the Peru Project in Lima has reported that use of convalescent serum in burned mice has produced results "only slightly more effective" than normal gamma globulin. Koslowski,[16] in investigating the same problem in burned rats, has found no improvement in over-all mortality.

Recent attempts by Moyer's group,[20] by Sell,[35] and by our laboratory to reproduce the original experiments of Feodorov in guinea pigs have been unsuccessful. Although delayed anaphylactoid reactions have been noted, no true anaphylaxis has been observed.

Additional facets of investigation with reference to antigen-antibody phenomena following thermal trauma, which have given inconclusive and inconsistent results, have included the use of Schultz-Dale tests by Chambler and Matter in our group and by Sell;[35] serological technics by Graber[13] and by Chambler, and gel-precipitation (Ouchterlony plates) and immunoelectrophoretic studies by Chambler and Matter which have been confirmed by Sell.[35] We have also utilized a latex particle fixation test as a substitute for the collodion particle solution of Pavkova in an effort to demonstrate antibody titers to burned skin in the serum of clinical patients.

Soon after the publication of Feodorov's work a series of studies was carried out in our laboratory employing exchange transfusions from acutely burned dogs into normal animals. Results were rather inconsistent in terms of mortality and toxic symptoms, and the experiments were abandoned for lack of suitable monitoring devices. The present investigations were initiated in 1960.

Experiences on our service at the University of Texas Medical Branch during the past two years may be summarized as follows:

In Vivo Studies

In an effort to study the therapeutic effects of convalescent burn serum in rats, before directing attention to the clinical patient, a series of experiments was set up originally similar to those of Malm and his co-workers. These were conducted by Bailey, who had previously devised and tested instrumentation for standardized burns in which the degree of body weight immersion could be correlated with the extent of burn inflicted by hot water with great accuracy, the two functions of dipping-and-weighing and dipping-and-burning being separated in the process.

In experiments which involved several thousand animals published by Bailey in 1961,[3] it was noted that standard scalds of 65 per cent weight immersion at 90° C. for 15 seconds in 200-Gm. female Holtzman albino rats resulted in 100 per cent mortality between 20 and 40 hours with an average of 27 hours, discarding anesthetic deaths from Nembutal. In convalescent-serum treated groups the average survival time was 60 hours with a range, for the most part, of 40 to 80 hours. The convalescent serum was prepared from animals which had survived scalds at 55 per cent weight immersion (approximately 35% surface area) at 90° C. for 15 seconds inflicted three months previously—a group of 100 of 1,000 animals. Serum was prepared after intravenous injection of 20 cc. of normal saline containing 5,000 units of penicillin and 5.0 mg. of streptomycin and was stored in sterile flasks at −10° C. The significance of this technic has not been evaluated, but sera drawn at shorter intervals following burning, two to eight weeks, proved ineffective in prolonging early mortality.

Intravenous injections of crude extracts of both normal and burned skin into healthy animals were immediately fatal; subcutaneous injection of scalded skin extract and intravenous administration of 5.0 cc. of acute burn serum produced variable results, toxic as a rule, rather than lethal.

Additional experiments were conducted by Bailey to assess the effect of prior scalding on subsequent reburning of the laboratory rats, other types of trauma being employed for control purposes. Results suggested that this type of *pretreatment* prolonged ultimate survival and indicated some protective influence as a simple response.

In the course of further *in vivo* experiments under Chambler with convalescent burn serum in Holtzman rats it was noted that a group of control animals not only survived longer than six days but that many lived longer than those treated with convalescent serum. Since the deaths in the latter series were suspected of being related to the anesthesia, Nembutal 3.0 mg./100 Gm., and since animals had been discarded previously because of failure to regain consciousness following experimental burns, it was decided to change from barbiturate to ether anesthesia. At the same time animal quarters were moved, and with warmed temperatures of 22° to 24° C. and in a draft-free room, without an air-conditioning unit in the vicinity, the rats used as controls lived for longer periods of time than previously. It was therefore decided to evaluate first the factors in the laboratory which might have affected the over-all burn mortality.

Experiment I. Two groups of 10 female Holtzman rats weighing 200 Gm. ± 10 were given a standard burn of 65 per cent body weight immersion in water of 90° C. for 20 seconds. Group A was anesthetized with pentobarbital, Group B with ether. The rats were kept in separate cages with water and food *ad lib.* in a room with a constant temperature of 23° C. The animals of the second group recovered from their ether anesthesia in less than 15 minutes, some of them drinking water by this time. None of the pentobarbital anesthetized rats recovered earlier than 90 minutes after the burn. Two rats in this group died during the first six hours without regaining consciousness after the burn.

Rectal temperature measured by thermocouple showed an average decrease three hours postburn from 36° to 32° C. in Group A in contrast to an average drop of only 1.0° C. in Group B. The average fluid intake in the first 24 hours was 28 cc. in Group A and 48 cc. in Group B which indicated that prolonged recovery time from anesthesia was a definite factor in postburn therapy.

Since all remaining 18 rats survived for a prolonged period of more than 60 hours, it was assumed that results obtained previously were due in part to the effect of the anesthetic pentobarbital and partly to the influence of the immediate environment with respect to temperature and drafts.

In order to observe the influence of convalescent serum on mortality following standardized burns under ether anesthesia and in warmer environment, Bailey's experiments were next repeated using first the remaining convalescent serum from his studies and later serum collected after three months from survivors of rats subjected to a 40 per cent weight immersion burn (about 32% of the body surface area) in hot water of 90° C. for 20 seconds. The animals were exsanguinated by a carotid cut but without prior injection of saline and antibiotics.

Experiment II. Female Holtzman rats of 170 to 180 Gm. were epilated and anesthetized with ether in a glass chamber until unconscious. As soon as they were attached to the rat frame on the burn machine anesthesia was reinforced with open-drop ether applied by a cotton wool pad. All experimental rats were awake and moving five to 10 minutes following burning. The following categories were studied in groups of 10 animals each:

Group 1. Burning, no therapy except food and water *ad lib.* (controls).

Group 2. Burned rats treated with 3.0 cc. of dextrose-saline intraperitoneally immediately after the burn and at four and eight hours. Food and water *ad lib.* (controls).

Group 3. Burned rats treated as in Group 2 with the addition of 1.5 cc. of normal rat serum given intravenously very slowly immediately postburn (controls).

Group 4. Burned rats treated as in Group 2 with addition of 1.5 cc. of convalescent burn serum administered as in Group 3, slowly and immediately postburn.

Results: In Group 1 controls five animals (50%) survived longer than six days. Death occurred in these at 5, 7, 8, 10, and 38 hours after the burn. In Group 2 controls there were only three survivors (30%) after six days. Times of death were at 3, 4, 5, 22, 24, 38, and 53 hours. In Group 3 controls were five six-day survivors (50%) with deaths as follows: 20 hours (due to hemoperitoneum); 29 hours; 45 hours (due to pneumonia); 59 hours; and 5 days. In Group 4 were eight survivors (80%); one death occurred 70 hours postburn and one at 5 days.

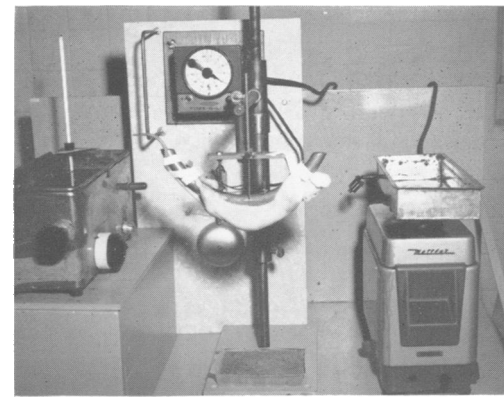

FIG. 1. Apparatus for inflicting standardized scald according to percentage of weight immersion (Bailey [3]).

It was obvious from this study that early mortality differed from results obtained in Bailey's experiments; as a matter of fact, a large number of the animals survived indefinitely, irrespective of initial therapy. Since two of the deaths in Group 3 could be explained sufficiently by postmortem findings aside from specific effects of the burn, it was believed that there was insufficient difference between Groups 4 and 5 to demonstrate any evidence of a protective effect of convalescent serum. Subsequent repetition of these studies showed essentially the same results—approximately 50 per cent mortality at the end of a week or 10 days, regardless of the type of therapy. Since late deaths were associated with local infection or with immobility from constricting effect of the burn eschar which resulted in inadequate intake of fluids and food, it was believed the test was not suitable for estimation of the influence of the sera on ultimate survival and that if a specific burn toxin should exist, it must be related to morbid rather than lethal factors,

at least under the conditions of the experiment with Holtzman albino rats in the indicated weight range.

Efforts were directed, next, at producing a more severe burn which would result in a high mortality during the early postburn period and furnish a test animal for evaluation of the protective effects of convalescent burn serum and *immune* serum. For this purpose a series of experiments was conducted in which rats with burns of 30, 45 and 60 per cent were exposed to scalds at 90° C. for periods of time varying from 15 to 45 seconds. Previous studies made in association with development of the Bailey Burner had shown that a 65 per cent weight immersion scald, corresponding to approximately 50 per cent of body surface in extent, was the feasible upper limit which could be utilized without involving the genitalia, the head and neck, or the extremities of the animal. At 15 seconds all animals survived in all categories; at 45 seconds all animals died, including those treated with normal and convalescent serum, indicating either that the burn inflicted for this length of exposure was overwhelming or that convalescent serum had no protective effect. Below 45 seconds no predictable results could be obtained in the controls.

Finally, as a preliminary to *in vitro* tests limited experiments were conducted to attempt to confirm the anaphylaxis experiments of Feodorov in both rabbits and guinea pigs, sensitizing with antigen source, (rat burn serum) desensitizing, and subsequently testing animals with injection of antigen and to re-evaluate the effect of injections of burned and normal skin extracts which Bailey had reported. In the first series animals became ill and a number of the guinea pigs had delayed anaphylactoid deaths, but no true anaphylaxis was observed and postmortem examinations were negative. In the second series intraperitoneal, subcutaneous and intravenous injections of skin extracts were employed in the following experiment:

Experiment III. Aqueous extracts were made from normal rat skin and from animals subjected to a 50 per cent burn 18 hours previously. Two burned skin extracts prepared from animals already dead for some hours were discarded, and a third was taken from a moribund animal for testing purposes. Extracts were prepared by mincing, centrifuging and simple filtration with paper filters and water suction only.

a. Injection of 1.0 cc. of normal and burned skin extracts with 1.0 cc. of normal saline had no effect when injected intraperitoneally into a number of healthy rats.

b. Four previously healthy rats were injected subcutaneously with 1.0 cc. of burned skin extract and 1.0 cc. of normal saline mixed. Three of the rats died within 60 seconds. The other rat became inert immediately and then tremulous and convulsive and in one to two minutes was unconscious, but no anaphylactic signs were noted. Over a period of three hours it gradually improved but was found dead 16 hours later. A fifth rat was given 1.0 cc. of burned skin extract mixed with 1.0 cc. of *immune* serum from a rat which had recovered from a severe burn six weeks previously. No ill effects were noted following this injection nor after a subsequent injection in the same dosage.

c. The same burned skin extract was diluted to half-strength with saline, and 2.0 cc. were injected intravenously into both normal and previously burned rats. In both instances the extract had an immediate lethal effect. When injected into 2.5 kg. rabbit, however, death did not result.

d. The extract was cultured for bacteria; tested for hypo- or hypertonicity; and analyzed with respect to alterations in protein content in comparison with normal rat skin and for variations in sodium and potassium content. All tests were normal, and, in addition, intradermal sensitivity tests were conducted in the rat which had recovered following the convulsive episode. Results were negative to both normal and burned skin extract after 24, 48, and 72 hours.

e. It was planned to centrifuge the extract further in order to minimize particulate emboli and then to separate the material into its albumin and globulin components for further testing, but following further clearing of the filtrate the extract proved to be ineffective in producing toxic symptoms when injected either subcutaneously or intravenously.

Morbid and lethal effects of injections of extracts of normal and burned skin noted in the

TABLE 1. *Effect of Anesthesia and Environmental Factors on Recovery of Rats from a Severe Standardized Burn*

Anesthetic Effects	Group A (Phenobarbital)	Group B (Ether)
Time of recovery	1–8 hr.	3–15 min.
Average body temperature decrease 3 hours postburn	4° C.	1° C.
Fluid intake in the first 24 hours	28 cc.	48 cc.

first part of the experiment and apparently also in Bailey's work must be concluded, hence, to be the result of technical failure initially to clear the extracts of particulate matter. This experiment does not furnish evidence, however, refuting the existence of nontoxic burn antigens in burned tissue.

In Vitro Studies

Attention was next directed toward *in vitro* experiments, in which Chambler, Juanita Bray and Matter have participated.

Assuming the existence of circulating antibodies in the serum of subjects recovering from severe thermal trauma—aside from those which have developed in response to antigens associated with bacterial infection—a series of studies was undertaken in an effort to demonstrate the presence of specific burn antigens which might be implicated as toxic factors. It was recognized at the outset that although *in vitro* tests might verify the existence of an antigen-antibody reaction, no information could be obtained as to the type of pathological process involved, or indeed to the existence of pathology.

From available evidence it was considered that any specific toxin must arise from a deep burn, probably full-thickness in degree; that the toxin produced from coagulated burned tissue would be of protein origin or at least a substance capable of eliciting an immune response; and that the altered or new protein should be toxic to the host when absorbed. It was recognized also that the studies would be affected by the mechanism of clearance of absorbed toxin from the circulating blood, that is, by cell fixation, detoxication, or renal excretion, and the time of clearance, whether early or late, as well.

Initially, homologous and heterologous sera tests were conducted as follows:

Experiment IV. Homologous serum was collected by exsanguination under ether anesthesia of female Holtzman rats which had completely recovered and healed from a 30 per cent weight immersion hot-water burn at 90° C. for 15 seconds. Blood was allowed to stand overnight, after which the serum was gently drawn off from the clot. Small amounts of penicillin and streptomycin were added to this pooled primary-response or unboosted serum. Secondary-response or boosted serum was prepared by the same technic 12 days after reburning at 90° C. for 20 seconds (20% weight immersion) a rat which had recovered from a 30 per cent burn as above. This boosted serum presumably should contain a higher concentration of specific antibodies against burn toxin.

Materials to be tested for the presence of antigens included aqueous extracts of burned rat skin prepared with unbuffered saline and excised at one hour and 24 hours postburn; acutely burned rat sera, with animals sacrificed at the time of burning and at 4, 8, 12, 30 minutes, 1, 4, 8, 12, 24, and 48 hours, respectively; and diffusion products of rat muscle. Normal skin extracts and normal serum were employed as controls.

Heterologous sera were prepared in rabbits by sensitization with 1) normal rat serum, to be used for control purposes; 2) serum collected from rats immediately after subjecting them to a standardized burn; and 3) rat serum collected 48 hours after burning. Rabbits were immunized by injection of 0.5 cc. of serum at two-day intervals × 3 and then repeating the procedure after an interval of one week.

TABLE 2. *Homologous Antiserum*

	Ouchterlony Antiserum		Immuno-electrophoresis Antiserum	
	Unboosted Precipitation	Boosted Precipitation	Unboosted Precipitation	Boosted Precipitation
Normal serum	−−	−−	−−	−−
Extract normal skin	−−	−−	−−	−−
Burn serum 2, 4, 6, 8 minutes	−−	+	−−	+
Burn serum 10, 15, 30 minutes	−−	−−	−−	−−
Burn serum 1, 4, 8, 12, 24, 48 hours	−−	−−	−−	−−
Extract skin 24 hours after burning	−−	+	−−	−−

a. *Hemolysin reaction.* An increased hemolytic effect was demonstrated against washed rabbit cells (1/60 suspension between 1/2 and 1/32 dilution), using non-inactivated burn rat serum. This phenomenon has been noted by other workers, in particular by Sell and Graber, with whom results were checked. Its significance is not known. There is, however, a tendency toward hemolysis of normal rabbit cells by normal rat serum which seems to be reinforced in some manner by thermal trauma.

b. *Agglutination reactions.* Numerous experiments were conducted over a period of several months with Boyden's Tanned Cell. Inconsistent results were obtained, and as the test was eventually abandoned because of technical difficulties, no information was obtained as to the presence of agglutinin antigens in burned sera by this method.

Since the reagents employed by Pávková for the collodion particle tests were not available in this country, an attempt was made next to duplicate her results, substituting latex particles in a test which had been employed by Singer and Plotz [38] for the serologic diagnosis of rheumatoid arthritis for collodion particles. The tissue to be used as antigen was homogenized with 1.1 per cent NaCl and frozen. As required it was thawed, centrifuged, and the supernatant was filtered with a Seitz filter and used as antigen. The protein content was determined by absorbence at 280 mu and the equivalent of 2.5 mg. was diluted to 10 cc. with borate-saline buffer, pH 8.2 and mixed with 0.1 cc. stock latex solution (Dow polystyrene latex) 0.81 microns in size. The serum to be tested was diluted serially from 1:2 to 1:520 with borate-saline buffer; 0.5 cc. of the antigen suspension was added to 0.5 cc. of diluted serum, and the mixture was incubated at 37° C. for 18 to 24 hours. The tubes were then centrifuged at 2,300 rpm for ten minutes at 5.0° C. The agglutination indicating a positive test was easily observed by tapping gently on the tubes to resuspend the latex particles. Plain latex without antigen was employed for control.

a. Burned and unburned skin was taken from three patients within 12 hours after injury and used as antigen. No titer could be demonstrated in the serum.

b. Using the same technic, pre- and postburn lymph was collected from the left thoracic duct of three dogs immediately following administration of a standardized burn and at intervals during the first 24 hours in a clinical burn patient as well. Beginning on the fifth day after injury and twice weekly thereafter serum samples were tested by the latex fixation method, but no significant titers could be detected which were related to the burn *per se.* In one dog which had a clinical wound infection, bacterial titers were obtained which indicated that this method is sensitive and reliable.

c. *Precipitation reactions.* For diffusion-in-gel tests (Ouchterlony plates) a seven-well diffusion pattern as described by Feinberg [9] was prepared with a 0.5 per cent special Noble agar. A buffer was not included but 200 mg. of 1.0 per cent methiolate powder was added as a bacteriostatic agent. These reagents were dissolved by stirring in a boiling water bath for 15 minutes. Polished petri dishes previously sprayed with silicone were then filled with 20 cc. of the agar solution.

Empty Ouchterlony plates were refrigerated whereas charged plates were kept at room temperature in a chamber of high humidity.

Homologous sera were collected by exsanguination of female Holtzman rats which had recovered completely healed from a 40 per cent weight immersion scald burn at 90° C. for 20 seconds. One hundred thousand U. of penicillin was added to the serum. Boosted serum was prepared by the same technic 12 days after reburning

rats which had recovered from a 40 per cent burn, as above, in order to obtain a higher concentration of any specific antibodies against burn toxin which might be present.

Materials to be tested for the presence of antigen included aqueous extracts of burned rat skin prepared with unbuffered saline and excised at one hour and 24 hours postburn and acutely burned rat sera with animals sacrificed immediately after burning, at five, 10, and 30 minutes, and at 12 and 24 hours postburn. Normal skin extract and normal serum were employed as controls.

Five different unboosted convalescent sera were tested with all of the different presumptive burn toxin sources. None gave precipitation lines on the Ouchterlony plates although some clouding was observed.

With boosted antisera a precipitation line was produced with rat sera collected up to 10 minutes following the burn but not thereafter. This distinct line on the Ouchterlony plate, however, was demonstrated in only one of five boosted convalescent sera which were tested; it might be compared with the nonspecific reactions which have been described recently by Berenbaum and co-workers.[5] Considering the possibility that the antigenic source might have been one or more products of blood hemolyzed following thermal injury, for example, hemolyzed red blood cells, destroyed leukocytes or lysed platelets, tests were made with controls of plain and heated normal unhemolyzed and hemolyzed blood, hemolyzed red cells, and plasma. All of these were negative. Lysed white cells and platelets and necrotic cells were not studied in view of the inconsistent results obtained. At any rate, it was concluded that should the precipitation line represent a true burn antigen it is apparently cell-fixed or at least noncirculating very shortly after the burn.

A further study was carried out using lymph as the presumptive source of burn antigen. Convalescent sera were collected from two dogs which after a complete recovery from a 30 per cent surface area burn had received a second burn. Pre- and postburn lymph was collected after cannulation of the left thoracic duct in five dogs and tested against two different convalescent sera. Neither lymph obtained immediately postburn nor that collected in a period of two hours after the burn formed a precipitation line.

Studies made with heterologous antisera showed no significant differences between normal rat serum and serum taken at various intervals postburn. With skin extracts, more lines resolved from burned tissue preparations than from normal controls.

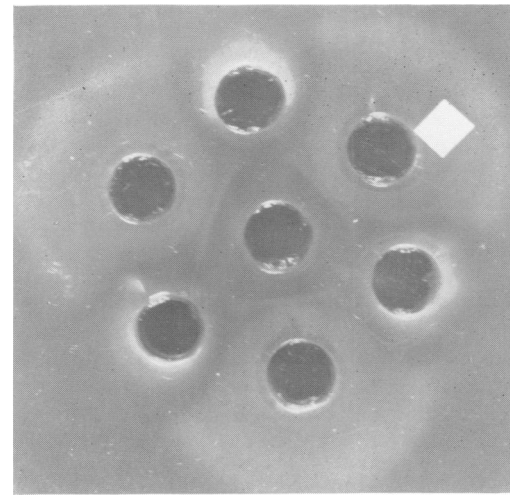

FIG. 2. Precipitation line on Gel-diffusion test (Ouchterlony Plate). Center well contains boosted serum from twice-burned donor rat. Well marked by square contains serum obtained from burned rat less than 10 minutes after injury.

For immunoelectrophoresis studies the method of Graber-Williams was employed with the modifications described by Wienne. In particular, a 1.5 per cent Noble agar, buffered to a pH of 8.2, was prepared. A current of 25 milliamps. at 130 volts applied over 20 minutes was sufficient for good resolution of antigen on a 3×1 inch slide. Essentially the same findings were noted as in the Ouchterlony plates although precipitation lines were somewhat less well defined.

As a final study, the Schultz-Dale test, which depends upon sensitization of smooth muscle with antigen and perfusion with antibody-containing agents to produce a contraction which may be recorded on a drum tracing, was undertaken with the assistance of L. J. Olson,[37] utilizing his apparatus and methodology, and employing both ileum and uterus of the guinea pig sensitized with normal rat serum. In pilot studies two positive reactions were recorded from the ileum in three animals sensitized with acute rat burn serum taken six minutes after injury. Thereafter the following experiment was conducted:

Experiment V. *Series A.* Female guinea pigs were sensitized with rat serum containing presumptive burn antigen. The serum was collected at various intervals after a burn of 40 per cent weight immersion with water of 90° C. for 20 seconds. The serum was diluted with saline (1:5), and three doses of 0.5 cc. were injected intraperitoneally at three-day intervals. The Schultz-Dale experiment was performed only 15 days

after the last injection in an effort to achieve a high antibody titer.

Using the ileum and the uterus of the sensitized guinea pig, a contraction of the smooth muscle with the rat burn serum, after repeated desensitization of the test organ with normal rat serum, should provide evidence of specific antigen-antibody reaction.

Five guinea pigs were sensitized in each group with serum as follows:

Group 1. Rat serum collected 6 minutes post-burn.

Group 2. Rat serum collected 30 minutes post-burn.

Group 3. Rat serum collected 24 hours post-burn.

All guinea pigs were successfully sensitized against rat serum, and no deaths occurred. In no instance could a specific burn-antigen-antibody response be demonstrated.

Series B. In a second series, lymph was collected from the left thoracic duct of five dogs before and immediately following a 30 per cent surface area burn in hot water of 90° C. for 20 seconds. Four guinea pigs were sensitized with the lymph of each of the experimental animals, using the same technic as in Series A. No deaths occurred. The Schultz-Dale tests were again performed 15 days after the last lymph injection. In no instance was a further muscle contraction demonstrable with burn lymph after complete desensitization with preburn lymph of the same dog.

These studies demonstrated the technical difficulties of Schultz-Dale tests and gave further evidence of laboratory inconsistencies.

Conclusion

The consensus at present by the majority of workers in this country with respect to convalescent burn serum, as summarized recently by Pennell,[22] is that while proof is lacking for the existence of a specific burn toxin of antigenic nature and of corresponding specific burn antibodies, "there are indications . . . that specific antimicrobial antibodies may be present in the blood of organisms which have recovered from infected burn wounds. It is within the realm of possibility that beneficial effects might result from transfusion of blood, plasma, or serum from a donor possessing antibodies which react specifically against microorganisms infecting a recently burned organism."

Despite discouraging results obtained to date in our laboratory and by other workers in the field, there is considerable impetus toward continuing laboratory investigation of the problem employing both *in vitro* tests and *in vivo* experimentation in suitable animals, and these are being pursued in addition to studies to evaluate the qualitative factors in convalescent burn serum which would relate to neutralization of bacterial toxins. Meanwhile the results of clinical trial are either not available in sufficient detail for conclusions to be drawn as to the efficacy of convalescent serum therapy or are being conducted without uniform protocols and in insufficient numbers for assessment of its value and the concomitant risks involved, if any exist. Little mention has been made of the logistic difficulties of collecting of serum from convalescent burn patients, although some efforts have been made by national organizations to assist physicians in obtaining donors for clinical trial. This problem has been emphasized by Russian and Czech workers.

It is apparent from experimental studies that antigen-antibody phenomena do occur following thermal trauma but that attempts to reproduce results have been technically difficult with often inconsistent findings, even in the same laboratories. Furthermore, the significance of such reactions is not clear. If they are related to toxic symptoms, statistically valid data are not as yet available for confirmation of a specific burn toxin-antitoxin hypothesis.

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Discussion

Dr. Truman G. Blocker, Jr. (Galveston): After a great deal of thought, I have come to the conclusion that the burn toxin is like the flying saucer: Some have never seen it, but have an open mind about it; some have thought they have seen it but later are not sure or prove to be mistaken; some have never seen it and would not believe it if they did see it; and some have seen it for themselves and even if they were proven to be wrong, could never be convinced otherwise. I hope I stand in the open-minded group.