Altered Reactivity to Skin Homografts in Severe Thermal Injury *

FELIX T. RAPAPORT,** M.D., JOHN MARQUIS CONVERSE, M.D., LEIF HORN, PH.D., DONALD L. BALLANTYNE, JR., PH.D., JOHN H. MULHOLLAND, M.D.

From the Department of Surgery and the Institute of Reconstructive Plastic Surgery, New York University Medical Center, New York

THERE has been a general impression that severe burns may be accompanied by alterations in the subjects' responsiveness to antigenic stimulation. This association has been limited, however, by a paucity of information on the response of severely burned individuals to well-characterized antigens in precisely defined immunological systems.[4] This has also been true of studies of skin homograft rejection patterns in severely burned humans and experimental animals.

Holman,[28] Dempster and Lennox,[18] Kay,[33] Branch, Wilkins, and Ross,[14] and Blocker[13] have found prolongation of survival of skin homografts in severely burned human subjects. Ashley and associates[5] observed accelerated rejection of skin homografts applied to scalded rats. These studies did not, however, consider known variables such as graft dosage, effects of prior blood transfusion, or of application of grafts to burned areas in the recipients. Each of these factors, and particularly that of graft dosage [9, 35, 39, 52, 56] might have contributed significantly to the results observed. It has therefore been difficult to assign a specific immunologic significance to these conflicting observations.

It is the purpose of this study to report the response of severely burned animals to skin homografts in an experimental setting where a deliberate attempt has been made to limit the bearing of such variables upon the results observed.

Materials and Methods

Adult male open-stock Wistar rats weighing 250 to 300 Gm. were used. The animals were clipped on the day before burning. The technic of burning has been described in detail elsewhere.[29, 38] Briefly, it consists of applying, under ether anesthesia, a thermal-regulated metal surface maintained at 250° C. to a precalculated body surface area of the rat (30%) through a measured asbestos frame. Control of the duration of exposure and of the pressure applied produces a standard full-thickness burn in the exposed area.

Each rat received a skin homograft from a normal donor, either at the time of burning, or within the first 15 days after the burn. The skin grafts were applied to normal areas in the recipient, in constant dosage similar to that used in previous studies of the immune response to skin homografts in this species.[9, 35, 52] The skin transplants were full-thickness specimens measuring 1.69 cm.^{2}, applied to an intact area on the lateral aspect of the dorsum of the animals. The method of transplantation has been described previously.[9] Control rats received skin homografts from the same donors, applied to similar body areas. Periodic gross and stereomicroscopic observations pro-

* Submitted for publication May 3, 1963.

** Career Scientist Award of the Health Research Council of the City of New York, Contract #I-349, Department of Surgery.

Supported by a grant from the John A. Hartford Foundation, Inc.

The authors wish to take this opportunity to express their deep gratitude to Dr. H. Sherwood Lawrence for his continued guidance, encouragement and editorial comment.

vided an objective evaluation of graft viability in burned and in control animals.[35, 52]

Animals in this study received no treatment other than ether anesthesia, burning, and application of skin homografts.

Experimental Results

Selection of a 30 per cent body surface area third degree burn as the standard thermal injury resulted from a preliminary study of mortality rates associated with the technic of burning employed. It is noted in Figure 1, in 210 rats exposed to 40 per cent and 30 per cent burns, that the mortality resulting from 40 per cent burns was 90 per cent by the third day after burning. When only 30 per cent of the body surface was burned, the mortality was reduced to 37 per cent. As this value appeared to offer a suitable compromise between the aim at a maximum severity burn and survival of a sufficient number of animals for homograft studies, it was utilized for this study.

The results are outlined in Table 1. Three groups of burned animals were studied. The time of graft application did not appear to have a significant effect upon subsequent observations—provided that the grafts were applied within the first 15 days after burning. Following application of homografts, the animals were observed for 13 days in the first group, for 18 days in the second, and for 21 days in the third group. One hundred and thirty-three animals were studied in the course of the experiment. Of these, 42 animals served as controls, and 91 rats were homografted after a 30 per cent body surface area burn.

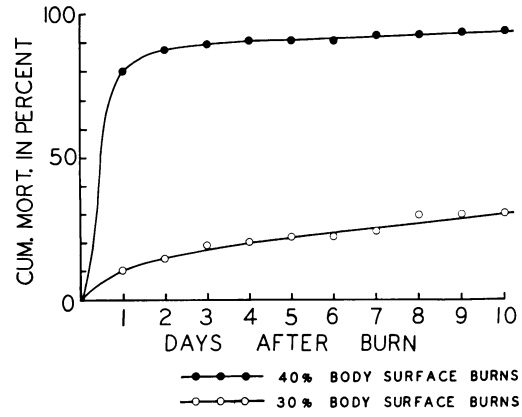

FIG. 1. Comparative mortality of 30 and 40 per cent body surface area full-thickness skin burns in open stock Wistar rats.

Survival times of grafts applied to control animals were consistent with previous observations made in open stock Wistar rats.[9] In 42 control grafts, 33 (79%) survived seven days after transplantation. Only five grafts (12%) were viable at the end of the second week, and two grafts (5%) by the eighteenth day. All grafts were rejected by the twenty-first day.

TABLE 1. *Survival Times of Skin Homografts Applied to Normal Recipient Areas in Open Stock Wistar Rats Following 30% Body Surface Area Full-thickness Skin Burn*

Experimental Conditions	No. of Rats	No. Skin Homografts Surviving at: 7 Days		14 Days		18 Days		21 Days	
		No. Rats	%	No. Rats	%	No. Rats	%	No. Rats	%
Controls	42	33	79	5	12	2	5	0	0
Short-term burn study (14 days)	45	45	100	43	98	—	—	—	—
Long-term burn study (18 days)	34	34	100	32	94	32	94	—	—
Long-term burn study (21 days)	12	12	100	10	83	10	83	10	83

In the 91 burned animals, all grafts survived at the end of the first week following transplantation (as compared with 79% control grafts). After 14 days, 85 grafts were still viable (i.e., 93% grafts were intact, as compared with 12 per cent control grafts); after 18 days, 42 of 46 grafts observed had survived (i.e., 90% as compared with 5% control grafts); by the end of the third week, when all controls had been rejected, 10 of 12 homografts in burned animals studied for that period continued to survive.

Discussion

The burned subject's response to thermal injury is associated with a wide variety of pathophysiologic changes.[3] The effects of stress and other endocrinologic responses have been described [2, 20, 21, 30] and the resulting metabolic alterations have been documented.[17, 27, 36, 41, 42, 53] Secondary anemia and delays in wound healing have also been noted.[31, 37] The marked decrease in reactivity of severely burned subjects to skin homografts could be interpreted as support for the possibility that the generalized disturbances observed are also associated with some impairment of immunologic responsiveness.

With few exceptions, however, there is little experimental evidence to support the conclusion that dermal burns induce a loss of specific immunologic function.[40, 54] The studies of Balch [6, 7] in man have demonstrated that severe injury, or nutritional impairment does not result in reduction of resistance to infection, or in loss of the capacity to form serum antibody. Recently, Balch has reported similar findings in severely burned patients.[8] His observations suggest that thermal injury may actually be associated with an increase in phagocytic activity of leukocytes, and elevations in the level of serum complement. Langohr, Owen, and Cope have also reported that burned subjects retain the ability to form serum antibody in response to challenge with Staphylococcal extracts.[34] This finding, as has been noted by Balch,[8] also receives support from the low incidence of tetanus in immunized burned patients who have received a booster injection of tetanus toxoid at the time of injury.

In view of the lack of evidence of impairment of any specific immunologic mechanism studied thus far, it would appear that functions other than the capacity to make serum antibody should be explored for their relevance to the prolonged survival of skin homografts after severe burns. One possible explanation has been sought in the increased production of adrenocortical steroids in severely burned patients and experimental animals. There is no clear-cut evidence, however, that levels of adrenocortical steroids elaborated in stressed experimental animals are sufficient to inhibit skin homograft rejection.[10, 11] This possibility is further weakened by the demonstrated ineffectiveness of adrenocortical steroids and of ACTH to inhibit skin homograft rejection in human subjects.[15, 55]

In a search for alternate explanations for the prolonged skin homograft survival observed in severely burned patients and experimental animals, recent concepts derived from well defined immunological systems may be particularly pertinent. These concepts include: 1) competition of antigens; 2) immunological paralysis; and 3) sharing of tissue transplantation antigens.

Competition of Antigens: Adler [1] has observed that the immune response to an antigen can be impaired by the previous, simultaneous, or subsequent injection of an additional antigen. This effect, termed competition of antigens, may result in varying degrees of suppression of the host's response to the first antigen. It appears to affect the early events of the immune response, and its intensity is related to the potency of the antigenic stimulus afforded by the second competing antigen.

Immunological Paralysis: Felton [22, 23] noted that massive dosages of antigen (pneumococcal polysaccharide) did not induce serum antibody formation in adult mice, and that such treatment inhibited subsequent attempts to elicit a serum antibody response to this antigen. Felton attributed this effect to the saturation of antibody-producing cells with a poorly metabolized antigen over a prolonged period of time. Kaplan, Coons and Dean,[32] Freund,[24] and Dixon, Maurer and Weigle[19] confirmed these findings, and suggested that, in view of the large excess of available antigen, any serum antibody might be neutralized as rapidly as it was formed. Recent studies of Sercarz and Coons,[50] Gitlin, Monckeberg and Janeway [26] and Brooke and Karnovsky [16] have secured evidence, however, that the phenomenon of immunological paralysis is not due to a continuous neutralization of antibody, but rather to an induced state of specific immunologic unresponsiveness.

The concepts of competition of antigens and of immunological paralysis may serve to suggest experimental approaches to the further study of possible causes of prolonged skin homograft survival in severely burned subjects. In this regard, reports of the wide variety of potentially antigenic materials released into the general circulation from burned tissues [48, 49] and the possible antibody response reflected by the associated serum hyperglobulinemia [12, 43, 44] may be particularly pertinent.

Sharing of Tissue Transplantation Antigens: A third observation of more direct relevance to the prolonged survival of skin homografts in burned subjects is the real possibility that unrelated individuals may share tissue transplantation antigens. This possibility is based on recent evidence of cross-sensitization to skin homografts from unrelated individuals in human recipients sensitized to some other individual donor's skin. It is further supported by the prolonged survival of skin homografts exchanged between individual donors who induced cross-reactions to each others' skin homografts in various unrelated recipients.[25, 45-47] Similar evidence has also begun to appear in the course of studies of experimental animals, where skin homografts were exchanged between individuals stemming from outbred lines of unknown genetic backgrounds.[51]

Some of the antigens released from the host's burned tissues may bear a similarity to those present in skin homografts from certain individual donors applied to the burned recipient. If such antigens are released in sufficient concentrations, a partial or complete suppression of the response to skin homografts obtained from such compatible donors may result. Such a phenomenon could be mediated through either of the mechanisms discussed above (competition of antigens; immunological paralysis). The reported variations in survival times of skin homografts obtained from different donors and applied to the same burned recipient [3] are consistent with this interpretation.

It is probable that no single factor, immunological or otherwise, can be implicated in the prolonged survival of skin homografts in severely burned individuals. The present state of knowledge concerning this complicated state also makes it probable that such an over-simplification would serve no useful purpose. Nevertheless, the mechanisms considered above may provide an approach to the isolation of those factors that are amenable to experimental analysis. Although the observations relating to competition of antigens, immunological paralysis, and the sharing of tissue transplantation antigens have been made in otherwise normal subjects, they suggest a method of study of similar mechanisms operative in the state of altered tissue reactivity associated with thermal injury.

Summary and Conclusions

Application of a 30 per cent body surface area full-thickness skin thermal injury results in prolongation of skin homograft survival in open stock Wistar male rats.

This result is discussed in the light of recent findings which suggest an experimental approach to the analysis of the state of altered tissue reactivity associated with thermal injury. These findings include antigen competition, immunological paralysis, and the sharing of tissue transplantation antigens by randomly selected unrelated individuals.

Bibliography

1. Adler, F. L.: Competition of Antigens, in Mechanisms of Hypersensitivity. Intern. Symp. Henry Ford Hosp. J. H. Shaffer, G. A. LoGrippo and M. W. Chase, Editors, Boston. Little, Brown Co., 1959, p. 539.
2. Arney, G. K., E. Pearson and A. B. Sutherland: Burn Stress Pseudodiabetes. Ann. Surg., **152**:77, 1960.
3. Arturson, G., Pathophysiological Aspects of the Burn Syndrome. Acta Chirurg. Scand., Suppl. 274, 1961.
4. Artz, C. P. and E. Reiss: The Treatment of Burns, W. B. Saunders Co., Philadelphia, 1957.
5. Ashley, F. L., E. G. McNall, R. F. Sloan, J. Taylor and E. N. Garcia: Studies on Mammalian Homotransplants of Skin Following Thermal Burn. Proc. First Intern. Congr. on Research in Burns, C. P. Artz, Ed., 1960, p. 378.
6. Balch, H. H.: The Effect of Severe Battle Injury and of Post-traumatic Renal Failure on Resistance to Infection. Ann. Surg., **142**: 145, 1955.
7. Balch, H. H.: Nutrition and Resistance to Infection. Ann. Surg., **147**:423, 1958.
8. Balch, H. H.: Resistance to Infection in Burned Patients. Ann. Surg., **157**:1, 1963.
9. Ballantyne, D. L. and J. M. Converse: The Relation of Hair Cycles to the Survival Time of Suprapannicular and Subpannicular Skin Homografts in Rats. Ann. N. Y. Acad Sci., **64**:958, 1957.
10. Billingham, R. E., P. L. Krohn and P. B. Medawar: Effect of Cortisone on Survival of Skin Homografts in Rabbits. Brit. Med. J., **1**: 1157, 1951.
11. Billingham, R. E., P. L. Krohn and P. B. Medawar: Effect of Locally Applied Cortisone Acetate on Survival of Skin Homografts in Rabbits. Brit. Med. J., **2**:1049, 1951.
12. Birke, G., S. O. Liljedahl and L. Troell: Studies on Burns. The Serum Protein Pattern and Nitrogen Metabolism. Acta Chirurg. Scand., Suppl. 228, 1957.
13. Blocker, T. G.: Burns in Reconstructive Plastic Surgery. J. M. Converse, Ed., W. B. Saunders Co., 1963.
14. Branch, C. D., G. F. Wilkins and F. P. Ross: The Coagulum Contact Method of Skin Grafting in the Treatment of Burns and Wounds. Surg., **19**:460, 1946.
15. Brent, L., in Progress in Allergy, S. Karger, Basel, Vol. V, 1958, p. 271.
16. Brooke, M. S. and M. J. Karnovsky: Immunological Paralysis and Adoptive Immunity. J. Immunol., **87**:205, 1961.
17. Co-Tui, A. M. Wright, J. H. Mulholland, I. Barcham and E. S. Breed: The Nutritional Care of Extensive Burns. Ann. Surg., **119**: 815, 1944.
18. Dempster, W. J. and B. Lennox: An Experimental Approach to the Homotransplantation Problem in Plastic Surgery: the Use of Multiple Donors. Brit. J. Plast. Surg., **4**:81, 1951.
19. Dixon, F. J., P. H. Maurer and W. O. Weigle: Immunologic Activity of Pneumococcal Polysaccharide Fixed in the Tissues of the Mouse. J. Immunol., **74**:188, 1955.
20. Evans, E. I. and W. J. H. Butterfield: Stress Response in Severely Burned. Ann. Surg., **134**:588, 1951.
21. Feller, I.: A Second Look at Adrenal Cortical Function in Burn Stress. Proc. First Intern. Conf. on Research in Burns, C. P. Artz, Ed., 1962, p. 1963.
22. Felton, L. D.: The Significance of Antigen in Animal Tissues. J. Immunol., **61**:107, 1949.
23. Felton, L. D., G. Kauffmann, B. Prescott and B. Ottinger: Pneumococcal Antigenic Polysaccharide Substances from Animal Tissues. J. Immunol., **74**:17, 205, 1955.
24. Freund, J., In: The Nature and Significance of the Antibody Response. A. M. Pappenheimer Jr., Ed., Columbia Press, N. Y., 1953, p. 46.
25. Friedman, E. A., J. W. Retan, D. C. Marshall, L. Henry and J. P. Merrill: Accelerated Skin Graft Rejection in Humans Pre-immunized with Homologous Peripheral Leucocytes. J. Clin. Investig., **40**:2162, 1961.
26. Gitlin, D., F. Monckberg and C. A. Janeway: Effects of Pneumococcus Capsular Polysaccharides on the Degradation of Rabbit

Antibodies in Mice. J. Immunol., **86**:627, 1961.

27. Harkins, H. N. and C. N. H. Long: Metabolic Changes in Shock After Burns. Am. J. Physiol., **144**:661, 1945.
28. Holman, E.: Protein Sensitization in Iso-skin Grafting; is the Latter of Practical Value? Surg., Gynec. & Obst., **38**:100, 1924.
29. Horn, L. and O. Malm: Report to the Committee on Shock and Trauma. National Acad. Sci., National Research Council, 1958.
30. Hume, D. M., The Neuro-endocrine Response to Injury. Ann. Surg., **138**:548, 1953.
31. James, G. W., O. J. Purnell and E. J. Evans: The Anemia of Thermal Injury. J. Clin. Investig., **30**:191, 1951.
32. Kaplan, M. H., A. H. Coons and H. W. Deane: Localization of Antigen in Tissue Cells. III. Cellular Distribution of Pneumococcal Polysaccharide Types II and III in the Mouse. J. Exp. Med., **91**:15, 1950.
33. Kay, G. D.: Prolonged Survival of a Skin Homograft in a Patient with Very Extensive Burns. Ann. N. Y. Acad. Sci., **64**:767, 1957.
34. Langohr, J. L., C. R. Owen and O. Cope: Bacteriological Study of Burn Wounds. Ann. Surg., **125**:476, 1947.
35. Lehrfeld, J. W. and A. C. Taylor: Dosage Phenomenon in Rat Skin Homografts. J. Plast. & Reconstr. Surg., **12**:432, 1953.
36. Levenson, S. M.: Some Problems of Thermal Injury. Post-Grad. Med., **29**:595, 1961.
37. Levenson, S. M., H. L. Upjohn, J. Preston and A. Steer: Effects of Thermal Burns on Wound Healing. Ann. Surg., **146**:357, 1957.
38. Malm, O. J. and G. J. M. Slawikowski: Mortality After Severe Experimental Burns of Rats. Surg. Forum, **12**:36, 1961.
39. Medawar, P. B.: Behavior and Fate of Skin Autografts and Skin Homografts in Rabbits. J. Anat., **78**:176, 1945.
40. Millican, R. C.: Role of Infection on the Delayed Deaths of Mice Following Extensive Burn Injury. Proc. First Intern. Congr. on Research in Burns, C. P. Artz, Ed., 1962, p. 195.
41. Moyer, C. A.: An Assessment of the Therapy of Burns: a Clinical Study. Ann. Surg., **137**: 628, 1953.
42. Moyer, C. A.: The Metabolism of Burned Mammals and its Relationship to Vaporizational Heat Loss and Other Parameters. Proc. First Intern. Congr. on Research in Burns, C. P. Artz, Ed., 1962, p. 113.
43. Perlman, G. E., W. W. L. Glenn and D. Kaufman: Changes in the Electrophoretic Pattern of Lymph and Serum in Experimental Burns. J. Clin. Investig., **22**:627, 1943.
44. Prendergast, J. J., R. L. Fenichel and B. Daly: Albumin and Globulin Changes in Burns as Demonstrated by Electrophoresis. Arch. Surg., **64**:733, 1952.
45. Rapaport, F. T., L. Thomas, J. M. Converse and H. S. Lawrence: The Specificity of Skin Homograft Rejection in Man. Ann. N. Y. Acad. Sci., **87**:217, 1960.
46. Rapaport, F. T., H. S. Lawrence, L. Thomas, J. M. Converse, W. S. Tillett and J. H. Mulholland: Cross-reactions to Skin Homografts in Man. J. Clin. Investig., **41**:2166, 1962.
47. Rapaport, F. T., H. S. Lawrence, L. Thomas and J. M. Converse: Biological Properties of Leucocyte Fractions in the Induction and Detection of Skin Homograft Sensitivity in Man. Fed. Proc., **21**:40, 1962.
48. Rocha e Silva, M. and S. R. Rosenthal: Release of Pharmacologically Active Substances from the Rat Skin *in vivo* Following Thermal Injury. J. Pharmacol. & Exp. Therapeutics, **132**:110, 1961.
49. Rosenthal, S. R.: Substances Released from the Skin Following Thermal Injury. Burn Toxin. Surgery, **46**:932, 1959.
50. Sercarz, E. and A. H. Coons: Specific Inhibition of Antibody Formation During Immunological Paralysis and Unresponsiveness. Nature, **184**:1080, 1959.
51. Stetson, C. A., Jr.: Skin Homograft Reactions in the Rabbit—Discussion. Ann. N. Y. Acad. Sci., **87**:220, 1960.
52. Taylor, A. C. and J. W. Lehrfeld: Determination of Survival Time of Skin Homografts in the Rat by Observation of Vascular Changes in the Graft. J. Plast. & Reconstr. Surg., **12**:6, 1953.
53. Taylor, F. H., S. M. Levenson, C. S. Davidson, N. C. Browder and C. C. Lund: Problems of Protein Nutrition in Burned Patients. Ann. Surg., **118**:215, 1943.
54. Verder, E. and S. M. Rosenthal: Role of Infection in the Delayed Deaths of Mice Following Extensive Burn Injury. Proc. Soc. Exp. Biol. & Med., **108**:501, 1961.
55. Woodruff, M. F. A.: Post-partum Induction of Tolerance to Homologous Skin in Rats. Ann. N. Y. Acad. Sci., **64**:792, 1957.
56. Zotikov, E. A. and V. M. Budik: Some Peculiarities of the Survival Time of Skin Homografts. Ann. N. Y. Acad. Sci., **87**:166, 1960.