THE BACTERIAL INDUCTION OF HOMOGRAFT SENSITIVITY

I. Effects of Sensitization with Group A Streptococci*

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(Received for publication, May 17, 1965)

The sharing of antigenic substances by widely separated phylogenetic groups received its first experimental demonstration in the work of Ehrlich and Morgenroth (1) who, in 1901, demonstrated the formation of goat and sheep hemolysins in rabbits immunized with ox erythrocytes. In 1904, Ballner and von Sagasser (2) reported that sensitization of rabbits with red yeast resulted in the formation of antisera which agglutinated a wide variety of Gram-negative organisms as well as red yeast. Soon thereafter, Forssman (3) described the development of sheep hemolysins after immunization of rabbits with guinea pig tissues, heralding a period of intensive study of heterologous antigens in nature. The Forssman antigens have since that time been described in a wide variety of organisms and tissues, including Gram-positive and Gram-negative bacteria, yeasts, and tissues of certain mammals, birds, and fish (4, 5). Significant steps have been made towards gaining an understanding of the chemical nature of these antigens (6).

The Forssman antigens do not, however, represent the only type of heterophile relationship existing between bacterial and mammalian species. Other heterophile antigen systems, which have been found to be independent of the Forssman antigens and of each other, have been described. They include antigens shared by human erythrocytes of all types and the *Shiga bacillus* (7), antigens present in human erythrocytes of Group O and the *Shiga bacillus* (8), antigens common to human erythrocytes of Group A and the Paratyphoid B bacillus (9), and antigens shared by human erythrocytes of Group A and the pneumococcus (10). Antigens shared by Group A red blood cells and pneumococci have subsequently been associated with pneumococcus Type XIV (11, 12). Schiff's original description of shared antigens between human erythrocytes of Group

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

‡ Supported in part by National Institutes of Health grant HE-03919 and by basic sciences training grant 26466, United States Public Health Service.

§ Career Scientist of the Health Research Council of the City of New York (I-349), Department of Surgery.

O and the *Shiga bacillus* has been extended by Springer and his associates, and by Iseki, to include a wide variety of other Gram-negative bacteria (13, 14).

The demonstration of cross-reactions of Group A streptococcal antigens with human and rabbit heart and skeletal muscle by Kaplan and associates (15–20), and by Zabriskie, Freimer, and Seegal (21) constitutes an important addition to the list of known heterophile antigen systems. These observations have been further supported by the report of Markowitz and Lange (22) that nephritogenic streptococci and human glomeruli may possess antigens in common. The possible relevance of these findings to the role of Group A streptococci in the pathogenesis of rheumatic heart disease and glomerulonephritis (23, 24) prompted an evaluation of the effects of sensitization with Group A streptococci in another form of mammalian altered tissue response, the homograft rejection reaction.

Preliminary observations have demonstrated that Group A streptococci induce in the guinea pig a state of altered reactivity to skin homografts similar to that observed following sensitization of the recipient with a first-set skin homograft (25). The present report confirms and extends this observation, and demonstrates that the type of homograft response obtained following sensitization of guinea pigs with streptococci is indistinguishable grossly and histologically from that observed after pretreatment with homologous guinea pig tissues. Effects of sensitization of guinea pigs with 6 other Lancefield types of Group A streptococci are described and discussed in the light of their pertinence to the localization of the factor(s) in the streptococcus concerned with sensitization to skin homografts.

Materials and Methods

Experimental Animals and Bacterial Preparations.—Outbred male guinea pigs of the Hartley strain, fed on a standard Purina pellet diet, were used in this study. The animals were between 2 and 4 months old, and weighed 250 to 350 gm.

Bacterial strains used were Rockefeller University stock streptococcal strains Group A, Type 4 (T4/95/RB5), Type 5 (T5B/126/1), Type 6 (S4B/100/4), Type 11 (T11/81/2), Type 12 (T12/36/4), Type 14 (T14/46/5), Type 49 (B737/71/1), grown in dialysate media prepared by the method of Wannamaker (26). Modified dialysate media prepared by substituting phytone (BBL) (Papain digest of soy beans) for peptone, and bacto-asparagine (Difco Laboratories, Inc., Detroit) and bacto-yeast (3 per cent) (Difco Laboratories) for dialysate of beef heart infusion was used to eliminate animal tissue derivates from the preparative media.

Overnight cultures grown at 37°C were collected and washed twice in sterile saline; they were resuspended in saline and heat-killed in a 56°C water-bath for 45 minutes (27). The heat-killed organisms were resuspended in media 199 (Microbiological Associates, Bethesda, Maryland) and emulsified in Freund's incomplete adjuvant (Difco Laboratories). The final bacterial concentration in this preparation was 12.5 mg (dry weight) per ml. The animals were given 0.1 ml of this emulsion in each foot-pad. Bacteria grown in modified dialysate media were treated in a similar fashion.

Control animals included untreated guinea pigs, as well as recipients injected with similar volumes of Wannamaker's media, media 199 (Microbiological Associates), Freund's incomplete

adjuvant (Difco Laboratories), or emulsions of these materials in Freund's incomplete adjuvant.

Eleven to 14 days after injection, the guinea pigs were challenged with skin homografts obtained from normal unrelated donors. Simultaneously, untreated control recipients received first-set skin grafts from the same donors.

Method of Grafting.—The grafting techniques employed throughout this study were based upon the method of Billingham and Medawar (28), and of Sparrow (29). They consisted of the delineation of an 11 mm circular defect on the thoracoabdominal surface of the recipients with a Castroviejo trephine, followed by complete excision of the host dermis by sharp dissection, exposing the panniculus carnosus and the overlying vascular channels. A fitted, full-thickness skin homograft was applied to the graft bed, and apposed to the host skin with interrupted 5–0 silk sutures. A pressure dressing was applied, and changed on the 3rd postoperative day. After this time, the grafts were examined and dressed daily. Surgical procedures were performed under parenteral nembutal anesthesia (20 mg per kg body weight). Strict sterile precautions were observed.

Method of Graft Observation.—The gross appearance, vascular status, and histologic characteristics of test and control skin homografts were studied at daily intervals after the 3rd postoperative day. The method of Taylor and Lehrfeld (30) was employed for stereomicroscopic observation of the graft surface, utilizing a Bausch and Lomb stereomicroscope at 19, 45, and 90 magnifications. This method made possible visualization of the superficial vessels of the graft, and of blood flow through their lumina. Graft biopsies for histologic study were fixed and stained with hematoxylin and eosin.

Criteria for the Determination of Homograft Rejection.—The behavior of first-set and of repeat-set skin homografts from the same donor has been described in detail in the guinea pig by Sparrow (29) and by Bauer (31); their observations served as background for this study. Skin grafts rejected before the 6th postoperative day were considered to have undergone accelerated rejection, giving evidence of a state of homograft sensitivity. This criterion was applied to white grafts and to accelerated rejection reactions. Grafts surviving for 6 days or more were considered to have evoked a first-set type of response; *i.e.*, to have given no evidence of host sensitization. The determination of homograft rejection was based upon gross, stereomicroscopic, and histologic criteria.

Gross criteria: In the case of the first-set type of homograft response, criteria of rejection included progressive graft edema and cyanosis, petechial hemorrhages, confirmed by subsequent escharification.

Three types of responses were noted as evidence of accelerated rejection. The first was the *white graft reaction* (31), characterized by a parchment-white surface, epidermal ulceration, and non-adherence of the graft to the recipient bed. The second was the *accelerated rejection reaction*, in which rapid progression of edema, cyanosis, and hemorrhage occurred between the 3rd and 5th postoperative day. The third type of response included reactions intermediate between white graft and accelerated rejection. Such reactions presented features of white grafts, but also exhibited hemorrhagic changes associated with partial vascularization (32).

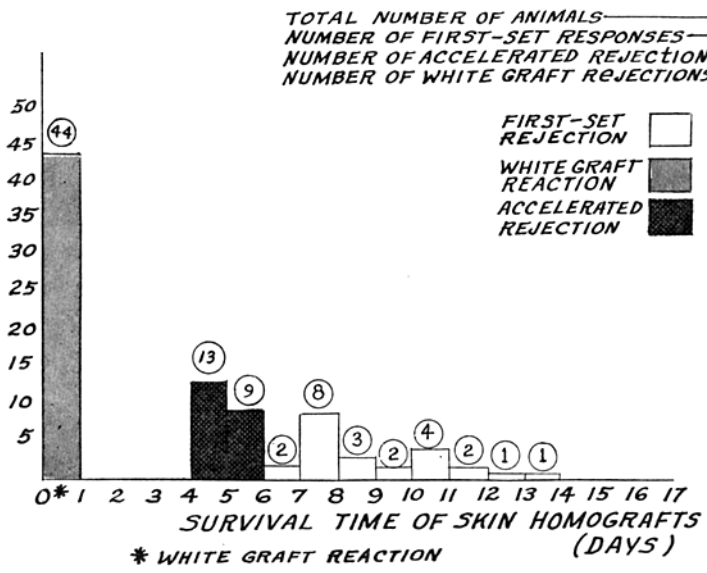
Stereomicroscopic criteria: The determination of homograft rejection by observation of vascular changes at the graft surface was described by Taylor and Lehrfeld in the rat (30), and has been used to study homograft behavior in other species (33, 34). Recent reports indicate good correlation between stereomicroscopic and histologic determinations of homograft rejection (32, 33). In vascularized grafts, early dilatation of graft vessels, followed by cessation of blood flow, provided a reliable standard of rejection. This was confirmed by the rapid appearance of punctate thrombi along the course of the vessels, vessel breakdown, and diffuse hemorrhages.

White graft reactions and intermediate or mixed white graft reactions were characterized

by the absence of vascularization and parchment-white color, coupled, in the case of mixed white graft reactions, with spotty areas of hemorrhage and thrombosis.

Gross and stereomicroscopic determinations of homograft rejection were confirmed in all instances by (*a*) escharification and sloughing of the graft, or (*b*) histologic examination of the graft.

Histologic criteria of homograft rejection: The studies of Sparrow (29) and of Bauer (31) formed the basis for the evaluation of skin homograft responses in this study. First-set grafts (Figs. 1 to 4) showed the usual pattern of early vascularization and epidermal proliferation, with the appearance of mononuclear cell infiltrates and progressive vascular congestion by the 5th or 6th postoperative day. Subsequent epidermal necrosis, disorganization of dermal ele-



TEXT-FIG. 1. Homograft response in guinea pigs sensitized with Group A Type 12 streptococci.

ments, and diffuse hemorrhages provided a reliable index for the determination of graft rejection. White graft reactions were recognized by the absence of vessels and necrosis of the graft. Such grafts stained diffusely eosinophilic and exhibited epidermal necrosis, fragmentation of dermal collagen fibers, and minimal cellular infiltrates. The line of contact of graft and host bed was marked by the "black line" of Bauer (31), a dense network of mononuclear, spindle-shaped cells, which also filled the interstices of host dermal collagen fibers in apposition with the graft. Numerous areas of hemorrhage were also noted in this region (Figs. 5 to 8).

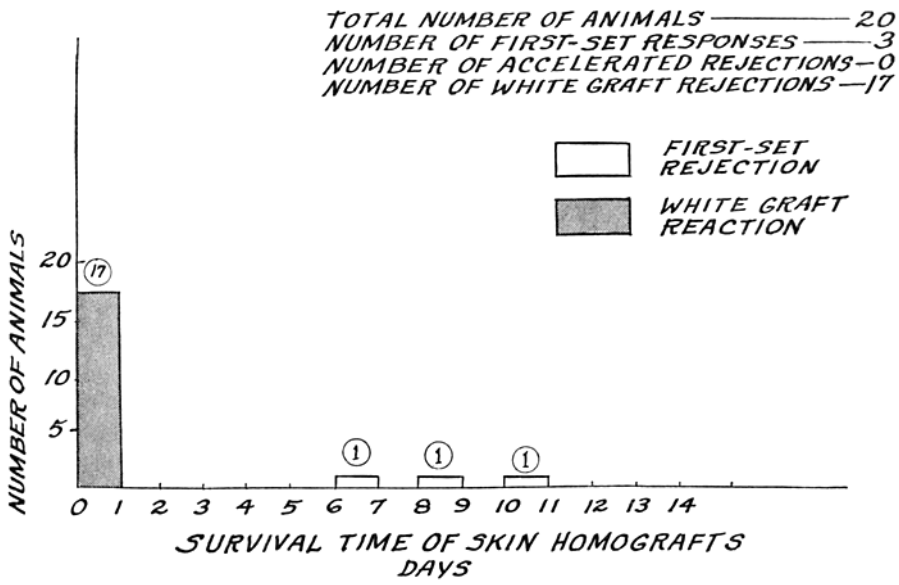
It is of interest that only a minimal degree of host cell invasion occurred in the white graft reactions. This criterion provided a difference between the white graft, and mixed white graft reactions, in which partial vascularization of the deeper layers of the graft was associated with varying degrees of polymorphonuclear leucocyte infiltration. This infiltrate was particularly noted in the subepidermal layers (32). In addition, there was evidence of vessel breakdown and hemorrhage in the deeper dermal layers of such grafts (Figs. 9 to 12).

Accelerated rejections (Figs. 9 to 12) were characterized by graft vascularization and epi-

dermal proliferation, followed rapidly by massive hemorrhages, infiltration of the graft with mononuclear cells, and epidermal necrosis. These changes occurred by the 3rd or 4th postoperative day. Accelerated rejection, as well as white graft reactions, differed markedly from the first-set type of response, which, during this same period, gave no evidence of graft damage.

EXPERIMENTAL RESULTS

Effects of Sensitization with Group A Type 12 Streptococci.—Text-fig. 1 illustrates the response of 89 consecutive guinea pigs to sensitization with Group A Type 12 streptococci. Sixty-six recipients gave altered responses to first-set skin



TEXT-FIG. 2. Homograft response in guinea pigs sensitized with Group A streptococci grown in modified dialysate media.

homografts. The latter included 44 white graft reactions, and 22 accelerated rejections at 4 to 5 days. Twenty-three guinea pigs had first-set responses, with survival of the grafts extending from the 6th to the 14th postoperative days.

As noted in Figs. 1 to 8, histologic patterns of graft response observed in animals exhibiting either accelerated rejection or white graft reactions were identical to those described for such reactions after sensitization of the recipients with guinea pig tissues (29, 31). First-set rejections in this group of animals did not differ from those noted in untreated animals (Figs. 1–4).

In order to exclude the possible participation of culture medium constituents derived from animal tissue in the induction of the observed reactions, guinea pigs were pretreated with streptococcal cells grown in a modified dialysate

media. Text-fig. 2 illustrates the response to skin homografts in 20 such animals. Seventeen of the 20 recipients exhibited white graft reactions, and 3 animals gave first-set responses at 7, 9, 19 days, respectively.

Effects of Sensitization with Other Types of Group A Streptococci.—Six additional Lancefield types of Group A streptococci were examined for their ability to induce homograft sensitivity. As noted in Table I, 72 animals were sensitized with heat-killed streptococcal cells. There were 58 altered responses, including 38 white graft reactions, and 20 accelerated rejections at 4 to 5 days. Fourteen animals rejected their grafts in first-set fashion, at 6 to 11 days. There was no

TABLE I

Homograft Response in Guinea Pigs Sensitized with Other Group A Streptococcal Types

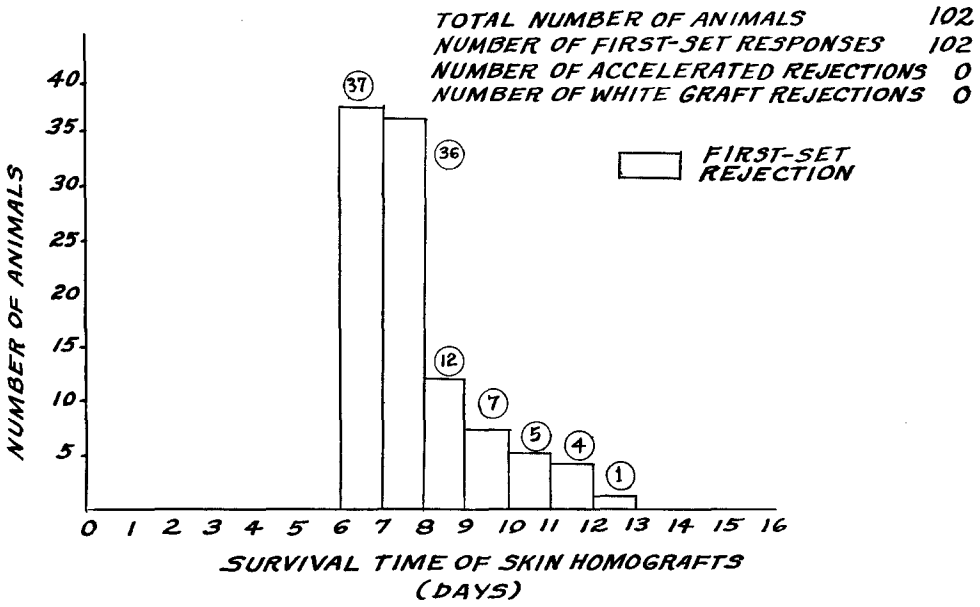
Group A streptococcal type used	No. of animals studied	Response of recipients to first-set skin homografts		
		First-set rejection	Accelerated rejection (No. of animals)	White graft reaction
Type 4	9	3	0	6
Type 5	17	4	6	7
Type 6	17	2	11	4
Type 11	10	3	3	4
Type 14	9	2	0	7
Type 49	10	0	0	10

Total number of animals studied, 72.
First-set responses elicited, 14.
Accelerated rejections and white graft reactions, 58.

significant difference between the ability of different types of Group A streptococci to induce homograft sensitivity in guinea pigs.

Control Studies.—One hundred fifty-three guinea pigs served as concurrent controls. One hundred two animals were tested with skin homografts in the absence of any other form of treatment. Text-fig. 3 illustrates the patterns of homograft responses observed in this group. Such grafts had survival times extending from 6 to 13 days, with a mean survival time of 8.10 days. There were no white grafts or accelerated rejections.

Table II illustrates the response of 51 animals to bacteria-free constituents of the suspensions used. These included Incomplete Freund's adjuvant, media 199 and Wannamaker's media, and emulsions of the latter two substances in incomplete Freund's adjuvant. All recipients gave first-set types of homograft responses, with rejection times extending from the 6th to the 14th postoperative days, and a mean survival time of 8.98 days. No white grafts or accelerated rejections were observed.



TEXT-FIG. 3. Behaviour of first-set homografts in untreated guinea pigs.

TABLE II

Homograft Response in Guinea Pigs Treated With Bacteria-Free Reagents

Type of reagent used	No. of animals studied	Response of recipients to first-set homografts		
		First-set rejection	Accelerated rejection (No. of animals)	White graft reaction
Media 199	10	10	0	0
Incomplete Freund's adjuvant	12	12	0	0
Wannamaker's media	8	8	0	0
Emulsion of media 199 in Freund's incomplete adjuvant	12	12	0	0
Emulsion of Wannamaker's media in Freund's incomplete adjuvant	9	9	0	0

Total number of animals studied, 51.
Number of first-set responses elicited, 51.
Number of accelerated rejections or white graft reactions elicited, 0.

DISCUSSION

Results of this study indicate that, under the experimental conditions described, heat-killed Group A streptococci have the ability to induce in the guinea

pig a state of altered reactivity to skin homografts which is indistinguishable on gross and histologic examination from that observed after sensitization with skin homografts. An interesting and unexplained component of this observation is the apparent lack of individual specificity of the response in that skin grafts obtained from any randomly selected donor elicited this response. This differs from homograft sensitivity induced by mammalian tissues, which has been reported to exhibit individual specificity (35, 36). Studies of the effects of hyperimmunization of human recipients with skin homografts or other sources of transplantation antigens may have some relevance to this problem. Application of repeated skin homografts from the same donor, or intradermal injection of transplantation antigens, have induced in such recipients accelerated rejection of skin homografts from the leucocyte donor, as well as of grafts obtained from other unrelated subjects (37–43). This observation raises the possibility that a similar state of hyperimmunization may be involved in the apparent lack of individual specificity of the responses noted in this study.

Skin autografts applied to recipients at the same time as test homografts did not share the fate of such homografts. Rather, they became vascularized, and assumed the appearance of the surrounding normal skin. This observation lends support to the impression that the bacterial induction of homograft sensitivity is not the result of a non-specific form of altered vascular reactivity, but may constitute another example of tissue reactions associated with sensitization to Group A hemolytic streptococci. In this respect, the induction of homograft sensitivity in guinea pigs by pretreatment of the recipients with streptococcal cells may be pertinent to the studies of Schwentker and Comploier (44), Cavelti (45, 46), and Jaffe and Holz (47), who have described tissue damage after treatment with emulsions of homologous tissue and Group A streptococci. In the present instance, however, the response observed did not require the use of homologous tissue in pretreatment of the subject.

The results of this study are in keeping with the observations of Murphy and Swift (48, 49), Robinson (50), Kirschner and Howie (51), and Glaser *et al.* (52), on the induction of cardiac lesions in rabbits after repeated infection with Group A streptococci. The report of Markowitz, Armstrong, and Kushner (53) on the induction of glomerular lesions in rats after intraperitoneal implantation of diffusion chambers containing nephritogenic streptococci, and the induction of runt-type disease is neonatal mice by repeated injections of Group A streptococci described by Ekstedt and Nishimura (54) may also be of relevance to the present observations.

The ability of Group A streptococci to induce homograft sensitivity in guinea pigs was not limited to Type 12, but was found in all streptococcal types tested. This observation suggests that the streptococcal factor(s) concerned with the induction of homograft sensitivity are not related to the M protein moiety of the streptococcal cell wall (55). Such factors may reside in other cell wall com-

ponents, in the cell membrane, or in the internal portion of streptococcal cells. This possibility is pertinent to the reports of Kaplan and associates (15–20), Zabriskie, Freimer, and Seegal (21), and Markowitz and Lange (22), who have described antigens cross-reacting with mammalian tissues in these components of Group A streptococci.

The relevance of the bacterial induction of homograft sensitivity to other tissue responses to Group A streptococci awaits elucidation of the mechanisms whereby this response is mediated and the isolation and characterization of the factor(s) responsible for its induction. Such studies, as well as the evaluation of the ability of streptococci to induce altered homograft reactivity in other mammalian species are currently under investigation.

SUMMARY

Heat-killed Group A hemolytic streptococci can induce in guinea pigs a state of altered reactivity to skin homografts which is indistinguishable from that which results from sensitization with homologous tissues. Challenge of suitably prepared recipients with first-set skin homografts obtained from unrelated randomly selected donors elicits white graft reactions or accelerated rejection of such grafts. The gross and histologic appearance of these grafts is identical with that observed in similar reactions obtained in guinea pigs sensitized with homologous tissues. The ability of Group A hemolytic streptococci to induce homograft sensitivity in the guinea pig is a property shared by Types 4, 5, 6, 11, 12, 14, and 49 of Group A streptococci.

The authors express their appreciation to Mr. Wilbur Matson, Miss Sally Short, Mrs. Dorothy Stern, and Miss Arline Zisman, for the excellence of their technical assistance. We are also grateful to Dr. Alex C. Solowey and Dr. Donald Wood-Smith for professional assistance.

BIBLIOGRAPHY

1. Ehrlich, P., and Morgenroth, J., Ueber häemolysine, *Berl. Klin. Woch.*, 1901, **38,** 569, 598.
2. Ballner, F., and Von Sagasser, R. R., Ueber die bildung von homologen und heterologen agglutininen im tierkörper, *Arch. Hyg.*, 1904, **51,** 245.
3. Forssman, J., Die herstellung hochwertiger spezifischer schafhämolysine ohne verwendung von schafblut. Ein Beitrag zur Lehre von heterologer Antikörperbildung, *Biochem. Z.*, 1911, **37,** 78.
4. Buchbinder, L., Heterophile phenomena in immunology, *Arch. Path.*, 1935, **19,** 841.
5. Bailey, G. H., and Shorb, M. S., Heterophile antigen in pneumococci, *Am. J. Hyg.*, 1931, **13,** 831.
6. Goebel, W. F., Shedlovsky, T., Lavin, G. I., and Adams, M. H., The heterophile antigen of pneumococcus, *J. Biol. Chem.*, 1943, **148,** 1.
7. Eisler, M., Ueber ein gemeinsames antigen in den zellen des menschen und in shigabazillen, *Z. Immunitätsforsch.*, 1931, **67,** 38.

8. Schiff, F. and Adelsberger, L., Ueber blutgruppenspezifische antikörper und antigen, *Z. Immunitätsforsch.*, 1934, **81,** 46.
9. Landsteiner, K., Individual differences in human blood, *Science*, 1931, **73,** 403.
10. Bailey, G. H., and Shorb, M. S., Immunological relationships of pneumococci and other heterophile antigens and biological significance in pneumococcus infections, *J. Exp. Med.*, 1933, **17,** 358.
11. Bullowa, J. G. M., The management of the pneumonias, New York, Oxford University Press, 1937, 316.
12. Finland, M., and Curnen, E. C., Agglutinins for human erythrocytes in type XIV anti-pneumococcic horse serums, *Science*, 1938, **87,** 417.
13. Springer, G. F., Williamson, P., and Brandes, W. C., Blood group activity of gram-negative bacteria, *J. Exp. Med.*, 1961, **113,** 1077.
14. Iseki, S., Blood group substances in bacteria, *Gunma. J. Med. Sc.*, 1952, **1,** 1.
15. Kaplan, M. H., and Meyeserian, M., An immunological cross-reaction between Group A streptococcal cells and human heart tissue, *Lancet*, 1962, **1,** 706.
16. Kaplan, M. H., and Meyeserian, M., Immunologic studies of heart tissue. V. Antigens related to heart tissue revealed by cross-reaction of rabbit antisera to heterologous heart, *J. Immunol.*, 1962, **88,** 450.
17. Kaplan, M. H., Immunologic relation of streptococcal and tissue antigens. I. Properties of an antigen in certain strains of group A streptococci exhibiting an immunologic cross-reaction with human heart tissue, *J. Immunol.*, 1963, **90,** 595.
18. Kaplan, M. H., Svec, K. H., and Arana-Sialer, J., Role of streptococcal infection in induction of auto-antibodies to heart in rheumatic fever., *J. Clin. Inv.*, 1963, **42,** 946.
19. Kaplan, M. H., and Suchy, M. L., Immunologic relation of streptococcal and tissue antigens. II. Cross-reaction of antisera to mammalian heart tissue with a cell wall constituent of certain strains of Group A streptococci, *J. Exp., Med.*, 1964, **119,** 643.
20. Kaplan, M. H., and Svec, K. H., Immunological relation of streptococcal and tissue antigens. III. Presence in human sera of streptococcal antibody cross-reactive with heart tissue. Association with streptococcal infection, rheumatic fever and glomerulonephritis, *J. Exp. Med.*, 1964, **119,** 651.
21. Zabriskie, J. B., Freimer, E. H., and Seegal, B., An immunological relationship between streptococcal membranes and human heart tissue, *Fed. Proc.*, 1964, **23,** 343.
22. Markowitz, A. S., and Lange, C. F., Jr., Streptococcal related glomerulonephritis. I. Isolation, immunochemistry and comparative chemistry of soluble fractions from Type 12 nephritogenic streptococci and human glomeruli, *J. Immunol.*, 1964, **92,** 565.
23. Rammelkamp, C. H., Jr., and Weaver, R. S., Acute glomerulonephritis, the significance of the variations in the incidence of the disease, *J. Clin. Inv.*, 1953, **32,** 345.
24. Wertheim, A. R., Lyttle, J. D., Loeb, E. N., Earle, D. P., Seegal, B. C., and Seegal, D., The association of type specific hemolytic streptococci with acute glomerulonephritis, *J. Clin. Inv.*, 1953, **32,** 359.

25. Rapaport, F. T., and Chase, R. M., Jr., Homograft sensitivity induction by Group A streptococci, Science, 1964, **145,** 407.
26. Wannamaker, L. W., Electrophoretic studies of the extracellular products of Group A streptococci, *J. Exp. Med.*, 1958, **107,** 783.
27. Lancefield, R., A micro precipitin technique for classifying hemolytic streptococci and improved methods for producing antisera, *Proc. Soc. Exp. Biol. and Med.*, 1938, **38,** 473.
28. Billingham, R. E., and Medawar, P. B., The technique of free skin grafting in mammals, *J. Exp. Biol.*, 1951, **28,** 385.
29. Sparrow, E. M., The behavior of skin autografts and skin homografts in the guinea-pig, with special reference to the effect of cortisone acetate and ascorbic acid on the homograft reaction, *J. Endocrinol.*, 1953, **9,** 101.
30. Taylor, A. C., and Lehrfeld, J. W., Determination of survival time of skin homografts in the rat by observation of vascular changes in the graft, *Plastic. Reconstruc. Surg.*, 1953, **12,** 423.
31. Bauer, J., Jr., Histocompatibility in inbred strains of guinea pigs., *Ann. New York Acad. Sc.*, 1958, **73,** 663.
32. Marshall, D. C., Friedman, E. A., Goldstein, D. P., Henry, L., and Merrill, J. P., The rejection of skin homografts in the normal human subject. Part I., Clinical observations, *J. Clin. Inv.*, 1962, **41,** 411.
33. Ballantyne, D. L., Jr., Platt, J. M., and Converse, J. M., Comparative study of the vascularization of white grafts and typical second-set skin homografts in the rat., *Surg. Forum*, 1963, **14,** 472.
34. Converse, J. M., and Rapaport, F. T., The vascularization of skin autografts and homografts, an experimental study in man, *Ann. Surg.*, 1956, **143,** 306.
35. Rapaport, F. T., and Converse, J. M., The immune response to multiple-set skin homografts, an experimental study in man, *Ann. Surg.*, 1958, **147,** 273.
36. Lawrence, H. S., Homograft sensitivity, an expression of the immunologic origins and consequences of individuality, *Physiol. Rev.*, 1959, **38,** 811.
37. Brent, L., Tissue transplantation immunity, *Progr. Allergy*, 1958, **5,** 271.
38. Rapaport, F. T., Thomas, L., Converse, J. M., and Lawrence, H. S., The specificity of skin homograft rejection in man, *Ann. New York Acad. Sc.*, 1960, **87,** 217.
39. Rapaport, F. T., Thomas, L., Converse, J. M., and Lawrence, H. S., Variations in the specificity of skin homograft reactions in man, *Fed. Proc.*, 1961, **20,** 36.
40. Friedman, E. A., Retan, J. W., Marshall, D. C., Henry, L., and Merrill, J. P., Accelerated skin graft rejection in humans pre-immunized with homologous peripheral leukocytes, *J. Clin. Inv.*, 1961, **40,** 2162.
41. Rapaport, F. T., Lawrence, H. S., Thomas, L., and Converse, J. M., Biological properties of leucocyte fractions in the induction and detection of skin homograft sensitivity in man, *Fed. Proc.*, 1962, **21,** 40.
42. Rapaport, F. T., Lawrence, H. S., Thomas, L., Converse, J. M., Tillett, W. S., and Mulholland, J. H., Cross-reactions to skin homografts in man, *J. Clin. Inv.*, 1962, **41,** 2166.
43. Rapaport, F. T., Lawrence, H. S., Converse, J. M., and Mulholland, J. H., Leucocyte fractions as skin homograft antigens in man, *Surg. Forum*, 1963, **14,** 146.

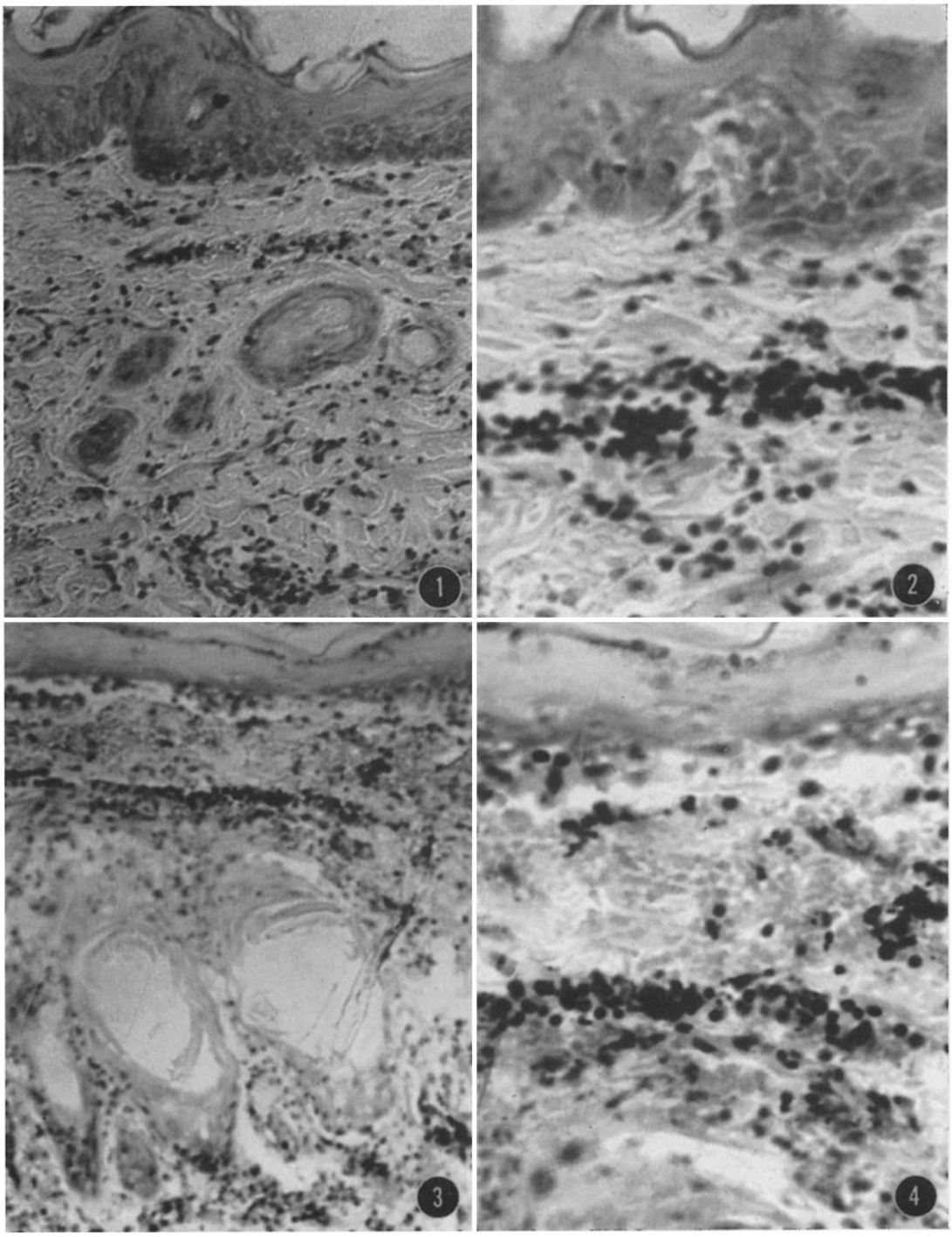
44. Schwentker, F. F., and Comploier, F. C., The production of kidney antibodies by injection of homologous kidney plus bacterial toxins, *J. Exp. Med.*, 1939, **70,** 223.
45. Cavelti, P. A., and Cavelti, E. S., Studies on the pathogenesis of glomerulonephritis. I. Production of autoantibodies to kidney in experimental animals *Arch. Path.*, 1945, **39,** 148.
46. Cavelti, P. A., Studies on the pathogenesis of rheumatic fever. I. Experimental production of autoantibodies to heart, skeletal muscle and connective tissue, *Arch. Path.*, 1947, **44,** 1.
47. Jaffe, R., and Holz, E., Experimental allergic myocarditis, *Exp. Med. and Surg.*, 1948, **6,** 189.
48. Murphy, G. E., and Swift, H. S., Induction of cardiac lesions, closely resembling those of rheumatic fever, in rabbits following repeated skin infections with Group A streptococci, *J. Exp. Med.*, 1949, **89,** 687.
49. Murphy, G. E., and Swift. H. F., The induction of rheumatic-like cardiac lesions in rabbits by repeated focal infections with Group A streptococci, *J. Exp. Med.*, 1950, **91,** 485.
50. Robinson, J. J., Attempts to produce rheumatic carditis in laboratory animals by means of streptococcic injury, *Arch. Path.*, 1951, **51,** 602.
51. Kirschner, L., and Howie, J. B., Rheumatic-like lesions in the heart of the rabbit experimentally induced by repeated inoculation with hemolytic streptococci, *J. Path. and Bact.*, 1952, **64,** 367.
52. Glaser, R. J., Thomas, W. A., Morse, S. I., and Darnell, J. E., The incidence and pathogenesis of myocarditis in rabbits after Group A streptococcal pharyngeal infections, *J. Exp. Med.*, 1956, **103,** 173.
53. Markowitz, A. S.,Armstrong, S. H., and Kushner, D. S., Immunological relationships between the rat glomerulus and nephritogenic streptococci, *Nature*, 1960, **187,** 1095.
54. Ekstedt, R., and Nishimura, E., Runt disease induced in neonatal mice by sterile bacterial vaccines, *J. Exp. Med.*, 1964, **804,** 795.
55. Lancefield, R., The antigenic complex of streptococcic haemolyticus, *J. Exp. Med.*, 1928, **42,** 91.

EXPLANATION OF PLATES

PLATE 52

FIGS. 1 and 2. First-set skin homograft in an untreated control guinea pig on the 5th postoperative day. Note the epidermal proliferation, vascularization of the graft, and infiltration by mononuclear cells. Fig. 1, × 100, and Fig. 2, × 250.

FIGS. 3 and 4. First-set skin homograft in an untreated guinea pig, at the time of rejection of the graft (8th postoperative day). Note the epidermal necrosis, disorganization of dermal components, diffuse hemorrhage, and mononuclear cell infiltrates. Fig. 3, × 100, and Fig. 4, × 250.



(Chase and Rapaport: Bacterial induction of homograft sensitivity. I)

PLATE 53

FIG. 5. White graft reaction induced in a guinea pig by sensitization with homologous skin. Section taken on the 5th postoperative day, illustrating epidermal thinning and necrosis, avascularity, and disorganization of dermal elements. × 100.

FIG. 6. White graft reaction induced in a guinea pig by sensitization with homologous skin. Section taken on the 5th postoperative day, illustrating the network of mononuclear, spindle-shaped cells found at the base of the graft, (black line of Bauer) (31). × 250.

FIG. 7. White graft reaction induced in a guinea pig by sensitization with Group A Type 12 streptococci. Sections taken on the 5th postoperative day, illustrating thinning and necrosis of the epidermis, avascularity of the graft, and disorganization of dermal elements. × 100.

FIG. 8. White graft reaction induced in a guinea pig by sensitization with Group A Type 12 streptococci. Section taken on the 5th postoperative day, illustrating the network of mononuclear, spindle-shaped cells found at the base of the graft (black line of Bauer). × 250.

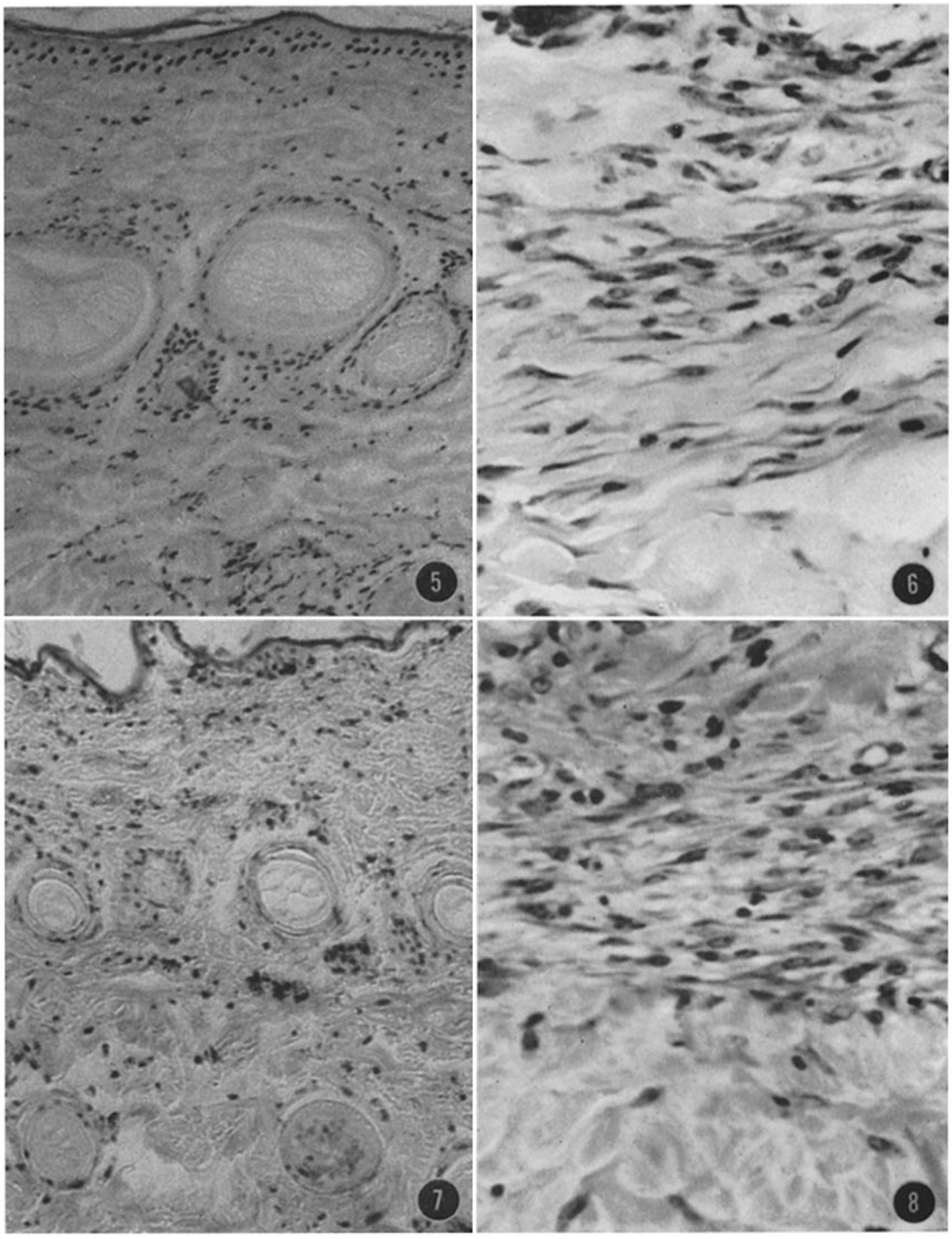


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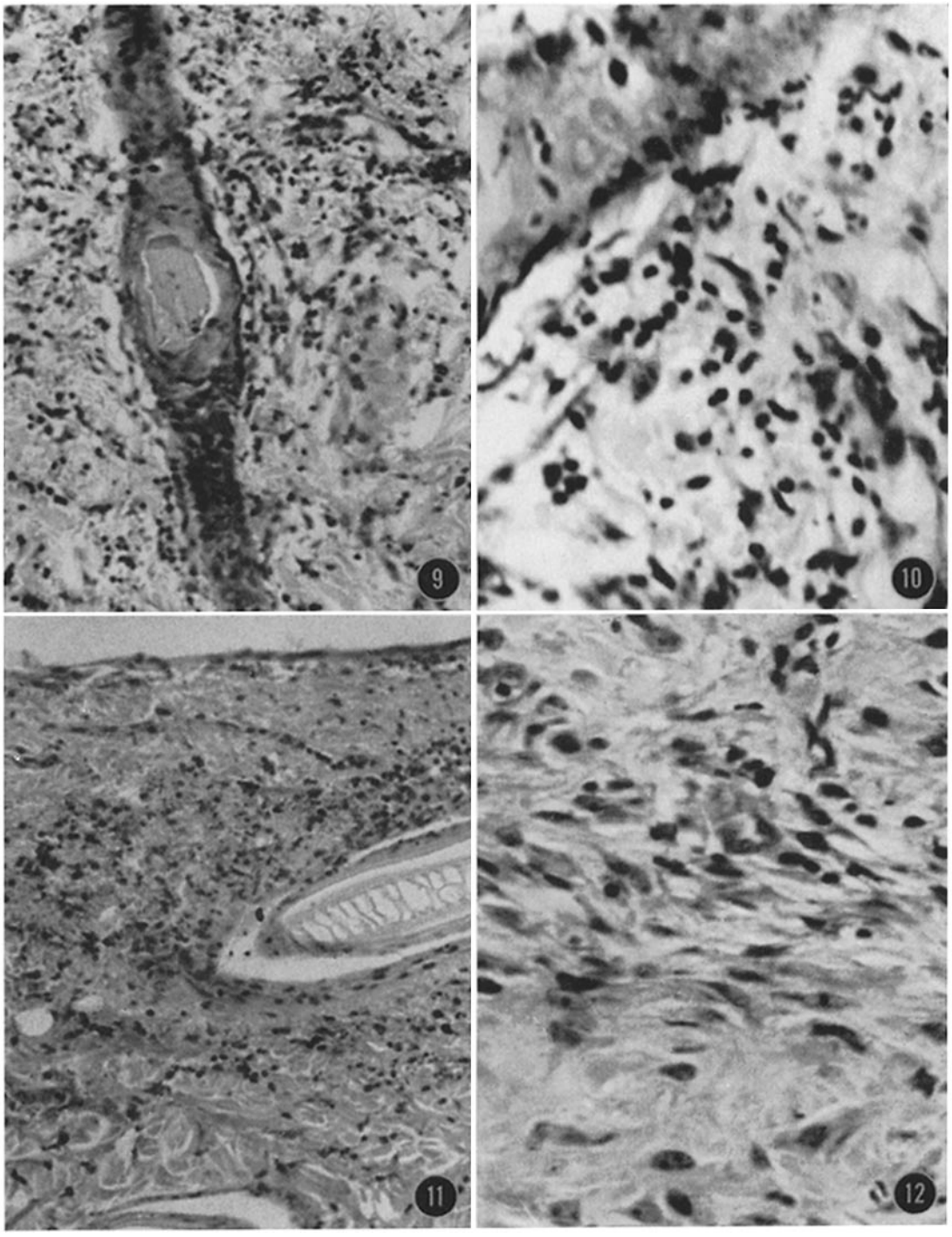
PLATE 54

FIGS. 9 and 10. Accelerated rejection induced in a guinea pig after sensitization with Group A Type 12 streptococci. Sections taken on the 4th postoperative day, illustrating vascularization of the graft, multiple hemorrhages, and prominent infiltration by mononuclear cells. Fig. 9, × 100, and Fig. 10, × 250.

FIG. 11. Mixed white graft reaction induced in a guinea pig after sensitization with Group A Type 12 streptococci. Section taken on the 5th postoperative day illustrates necrosis and thinning of the epidermis, partial vascularization of the graft, areas of hemorrhage and leucocyte infiltration. Many of these cells are polymorphonuclear leucocytes. × 100.

FIG. 12. Mixed white graft reaction induced in a guinea pig after sensitization with Group A Type 12 streptococci. Section taken on the 5th postoperative day, illustrating the network of spindle-shaped cells found at the base of the graft (black line of Bauer). × 250.



(Chase and Rapaport: Bacterial induction of homograft sensitivity. I)