THE ROLE OF HUMORAL ANTIBODIES IN REJECTION OF SKIN HOMOGRAFTS IN RABBITS

I. Passive Transfer of Isoimmune Serum to Conditioned Hosts

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Plates 48 and 49

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The immunologic nature of homograft rejection is well established (1, 2). The antigen in this reaction is contained in the cell nucleus (3, 4) and more specifically in nuclear deoxyribonucleic acid, as suggested by Medawar in 1946 (5) and shown experimentally by Billingham *et al.* in 1956 (6). The type of antibody involved in graft rejection, or better the part played by humoral and cell-bound antibodies in destruction of homografts, is less clearly defined. Although humoral antibodies against homografts can be detected in animals of several species that have recently rejected tissue homotransplants (7–9), such antibodies are usually considered to play a minor (if any) role in homograft rejection (1, 2, 10, 11). The weight of evidence apparently suggests that cell-bound antibodies are primarily responsible for homograft rejection (12–15). Yet, a series of observations would favor the view that humoral antibodies may also be significant in this respect.

(*a*) Subjects with agammaglobulinemia fail to reject skin homografts (16, 17) although they are capable of developing delayed immune responses (17, 18). Patients with hypogammaglobulinemia will reject skin homografts, but require a longer period than normal subjects to do so (17).

(*b*) Woodruff (13) observed that homografts placed in millipore chambers within the host, and therefore subject only to humoral influences, were destroyed sooner when they came in contact with the cells of the host than homografts that had not been sensitized by humoral factors.

(*c*) Isolated, viable epidermal cells placed in contact with serum sensitized against them failed to grow when returned to an appropriately prepared donor's bed (19).

(*d*) Early reports (12, 13, 20, 21) of experiments with diffusion chambers indicated that homologous cells were not destroyed in immunized hosts unless host cells had access to the chamber. More recently, however, Amos and Wakefield (22) and Algire (23), working with tumor cells, have shown that grafts grown in cell-impenetrable chambers are destroyed rapidly in immunized hosts.

(*e*) Although there seem to be at least four different types of antibody activity in the serum of animals bearing homografts (24), cytotoxic antibodies appear as those more directly related to rejection. These antibodies will destroy the cells responsible for their formation as

well as other elements with identical genetic structure (25). *In vitro* studies of this phenomenon have been published by Stetson and Jensen (24), Terasaki *et al.* (25, 26), Merrill *et al.* (27), and others (28). Their results are in opposition, perhaps because of different techniques, to those previously reported by Medawar (29) and Allgöwer *et al.* (30). Jensen and Stetson (31) believe that hemagglutinating and cytotoxic activities of isoimmune sera represent manifestations of a single class of antibodies, capable of agglutinating donor cells or of sensitizing them for complement lysis.

(*f*) Passive transfer of humoral antibodies has been used to test their effect in homograft rejection *in vivo*. Negative results in mice bearing skin homografts and injected with pooled antiserum prepared in other mice, members of the same inbred strain, were reported by Billingham *et al.* (3, 33). Stetson and Demopoulus (32) showed that while systemic administration of isoimmune serum to mice bearing established homografts was without effect, the local injection of such serum produced breakdown of the homograft. Stetson and Jensen (24) gave intravenous injections of antisera of high cytotoxic activity to mice with skin homografts from mice whose cells were destroyed *in vitro* by such antibodies without observing any difference in the test grafts. Nevertheless, when the injection of antisera was followed by local application of bromobenzene or xylene on the graft, rejection occurred in the painted areas. These authors attribute their results to the breakdown of hypothetical "blood-graft barrier" (34).

(*g*) Harris *et al.* (35), working with transfer of homologous lymph node cells in rabbits, observed that suppression of transferred lymphoid elements occurred regularly after injection of serum pooled from groups of rabbits which had been injected with leukocytes pooled from the blood of 60 to 70 rabbits. Recently, Garver and Cole (36) reported that specific antisera prevented the protective effect of homologous bone marrow transplantation in lethally irradiated mice.

(*h*) An extensive literature is available on the *in vitro* and *in vivo* cytotoxic effect of antitumor humoral antibodies (37). Of special interest here is the repeated observation that resistance to tumor growth can be passively transferred by isoimmune serum.

In this paper a series of experiments is reported in which the role of humoral antibodies in skin homograft rejection has been studied by passive transfer of immune serum to cortisone-conditioned and non-conditioned rabbits bearing skin grafts of donors to which the serum was previously sensitized.

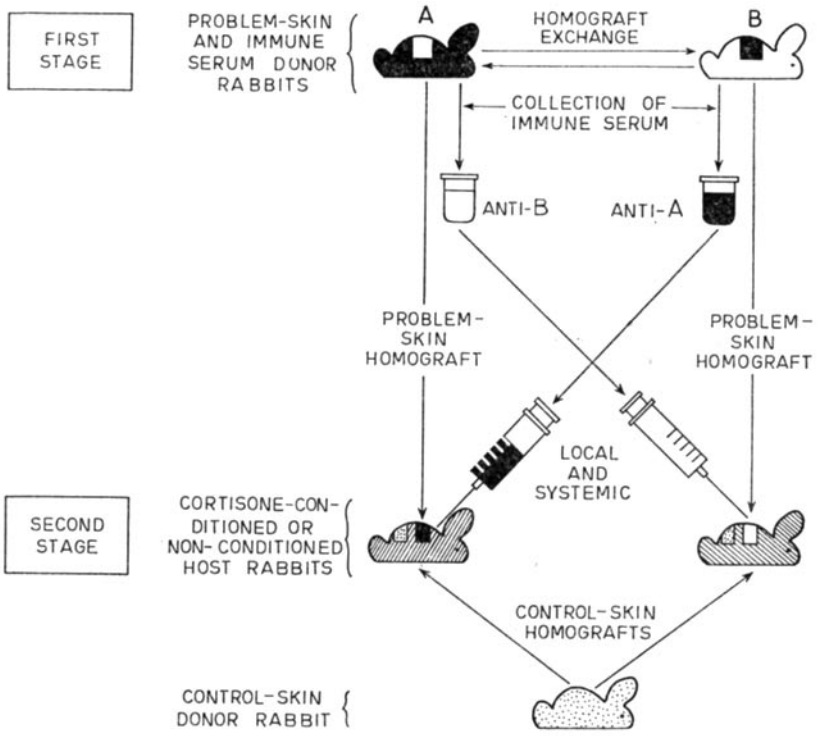
Materials and Methods

The experiments were carried out in rabbits with an average weight of 1500 gm. The rabbits were obtained from various colonies in order to insure absence of inbreeding. All homografts performed in these experiments consisted of square full-thickness skin grafts measuring 2 cm by side; they were always placed on the back of the hosts and maintained there with a continuous cotton suture.

The general design of the experiments is depicted in Text-fig. 1. In the first stage of the procedure skin homografts were exchanged between pairs of donor rabbits. 5, 10, and 15 days after grafting the animals were bled from the marginal vein of the ear after intramuscular injection of 1000 u of sodium heparin. Approximately 25 cc of blood was obtained every time from each rabbit. Sera obtained from the three bleeding sessions from the same animals were separated by centrifugation, pooled, and stored at 4°C in sterile containers.

The second stage of the procedure consisted in grafting skin from rabbits used in the first stage to host animals, which were then separated into two groups: one group was immediately

started on 5 mg of cortisone[1] every other day, with half the doses given intramuscularly and the other half locally (38). The other group received no cortisone. The steroid was given in order to depress the host's immune response and prolong the survival of homografts (39). Caudally to the problem-skin homograft a control-skin homograft was placed in each animal, obtained from donors which were not used in the first stage of the procedure. Both skin grafts were placed simultaneously on the back of the host under general ether anesthesia, separated by a 1 cm bridge of tissue. All operated animals received 50,000 u of penicillin every other day throughout the experiment.



TEXT-FIG. 1. General design of experiments. See text for description.

The immune serum was given by two different routes, namely systemic and local. For systemic administration 5 cc of undiluted serum were given intraperitoneally at the time of grafting, and 5 and 10 days later. Local administration of serum was carried out at the time of grafting and 5 days later, in the form of subcutaneous injections of 0.75 cc of undiluted serum in the bridge of tissue separating the two grafts. This 10:1 ratio of systemic to local doses has been suggested as adequate for transfer studies (40). Local administration of immune serum (24, 33) was included in these experiments because of the possibility of failure of systemic administration due to factors such as dilution, initial low concentration of antibodies, or local circulatory blocking by the "blood-graft barrier" (34). Obviously, the serum given to an animal was the one supposed to carry antibodies against the problem-skin graft.

[1] Methicorthelone, Schering Corporation, Bloomfield, New Jersey.

Thus, cortisone-treated and untreated hosts bearing problem-skin and control-skin homografts received serum of animals sensitized against problem skin. Differences in the time of rejection of problem skin, as compared with control-skin homografts, would indicate specific participation of passively transferred humoral antibodies. Simultaneous earlier rejection of *both* homografts in rabbits given immune serum, as compared with animals receiving no humoral antibodies (donors of skin homografts in the first stage of the procedure), would suggest a non-specific role of serum factors in homograft rejection.

Evaluation of results was based on both gross and microscopic changes. The latter were examined only in a small group of animals specially prepared for this purpose since it was considered undesirable to interfere with the spontaneous course of homografts by repeated biopsies. Daily observations of grafts were tabulated according to the following features: (*a*) consistency, (*b*) preservation of healthy edges, (*c*) mobility on underlying tissues, (*d*) color, (*e*) temperature, (*f*) hair, and (*g*) size. Special emphasis was placed on the first five characteristics, which were soon appreciated to reveal the earliest stages in the onset of rejection. According to Dammin (41) microscopic examination of homografts was carried out in this series at a critical day, when gross features of rejection were fully established. This was found to be represented by 5-day-old homografts in cortisone-conditioned rabbits with local serum application. Therefore, four animals were prepared under these conditions, the homografts and surrounding tissues were removed and fixed in 10 per cent formalin, and small blocks were embedded in paraffin, cut at 10 μ and stained with hematoxylin eosin and Gömöri's aldehyde fuchsin method for elastic fibers.

Tabulation of results was carried out considering the day after grafting when *most* gross features of rejection were present. It was found that as soon as the first signs of rejection appeared all others became visible 24 to 36 hours later, so a possible deviation of 1 day should be admitted. Actual elimination of homografts was very variable and depended on accidental tearing or other conditions unrelated to immune rejection. When the number of animals available permitted it, differences in time of rejection between the various groups were tested for statistical significance on a t table at a p level of 0.01.

RESULTS

The day of rejection for each animal in these experiments appears in Table I. Average results with standard deviations for the various groups are graphically shown in Text-fig. 2. The average rejection time of skin homografts in donor rabbits was 8.0 days. Control-skin homografts in non-conditioned animals treated with intraperitoneal immune serum were rejected in an average of 8.3 days, and those of non-conditioned animals given serum locally showed rejection in an average of 7.7 days. It was postulated that the differences between the latter two results were not significant, so they were averaged and 8.0 days was considered as the critical day of rejection for control homografts in these experiments, a figure in close agreement with results in untreated donor rabbits.

Problem-skin homografts in non-conditioned animals treated with intraperitoneal immune serum were rejected at 6.3 days, but the difference with 8.0 days was not tested statistically because of the small number of animals. In non-conditioned rabbits receiving immune serum locally the critical day of rejection was 4.0 days, but again the number of animals was too small for statistical analysis.

TABLE I

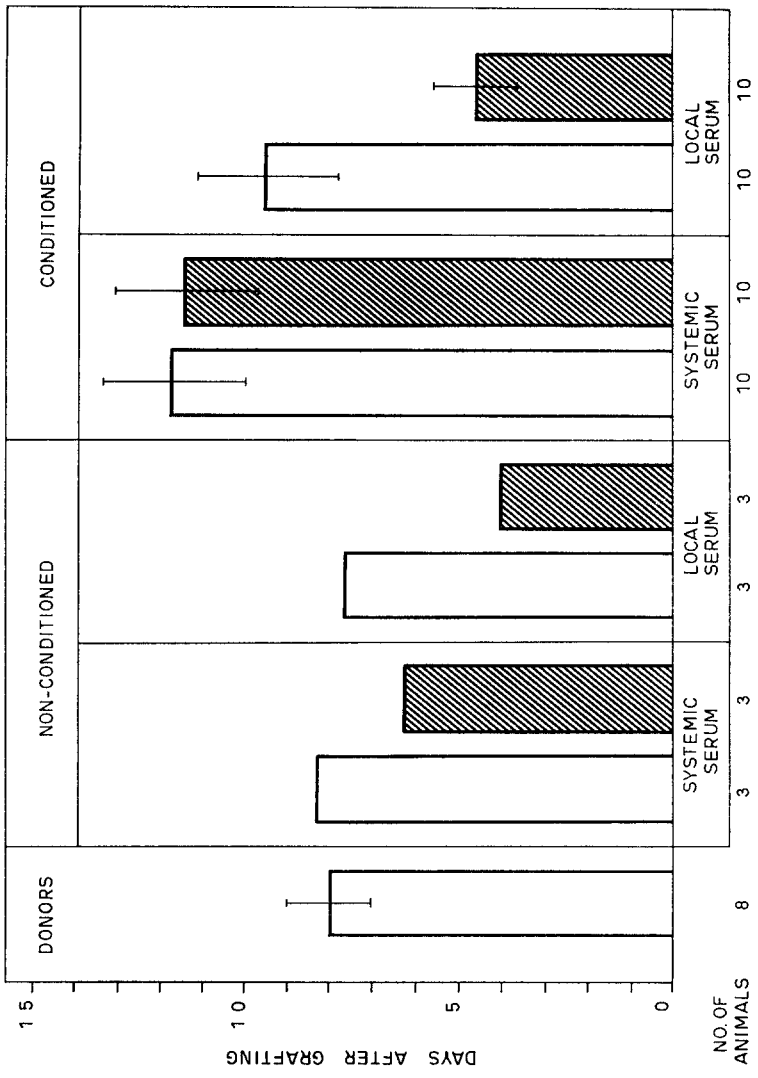
Critical Day of Homograft Rejection

A. First Stage of the Experiment

Controls (donors)	A	8
	B	9
	C	8
	D	7
	E	7
	F	8
	G	8
	H	9

B. Second Stage of the Experiment

Animal group		Animal No.	Critical day of rejection	
			Control graft	Problem graft
Non-conditioned	Systemic serum	1	8	6
		2	9	6
		3	8	7
	Local serum	4	8	4
		5	7	4
		6	8	4
Conditioned	Systemic serum	7	14	14
		8	10	9
		9	10	10
		10	9	9
		11	11	10
		12	13	13
		13	12	12
		14	13	12
		15	13	12
		16	12	12
	Local serum	17	4	4
		18	9	6
		19	10	6
		20	8	5
		21	11	4
		22	10	3
		23	10	5
		24	11	4
		25	11	4
		26	11	5



TEXT-FIG. 2. Average day of rejection of control- and problem-skin homografts in the various groups.

Control-skin homografts in cortisone-conditioned animals treated with systemic immune serum were rejected in an average of 11.7 days, while those of cortisone-conditioned rabbits given serum locally revealed rejection at 9.5 days. These data failed to be significantly different, so the average of 10.6 days was compared with the figure of 8.0 days obtained from non-conditioned animals. This difference proved to be significant at the level of $p = 0.01$, showing that conditioning had been effective in prolonging survival of skin homografts.

Problem-skin homografts in cortisone-conditioned animals treated with intraperitoneal injections of immune serum revealed their critical day of rejection at 11.4 days, which was almost the same as for control-skin homografts in both conditioned groups. On the other hand, problem-skin homografts in cortisone-conditioned animals given immune serum locally were rejected at an average of 4.6 days, which was significantly different from the average of 10.6 days found for control-skin homografts.

Microscopic examination of both control- and problem-skin homografts in cortisone-conditioned rabbits given immune serum locally and followed for 5 days after grafting revealed the following differences: control homografts were better preserved, showed little or no vascular thrombosis, acute inflammation and necrosis were almost absent, and epithelial cells were clearly visible. Problem-skin homografts, on the other hand, showed almost complete obliteration of structure, many thrombosed vessels, and intense inflammation and extensive necrosis; the epithelium was barely distinguishable as a darker layer on top of the homogenized and destroyed dermis (Figs. 1 and 2).

DISCUSSION

The results of this work indicate that in rabbits in which the immune response has been delayed by cortisone, rejection of skin homografts is accelerated in a highly significant manner by the local administration of serum obtained from animals immunized against the graft. In non-conditioned rabbits the situation seems to be similar, although the number of animals used was too small for statistical analysis. Acceleration of rejection of homografts was not observed when the serum was given intraperitoneally. Failure of systemic immune serum administration may be due to several causes: first, it may be a factor of dilution, brought about by mixture of the immune serum with the circulating fluids of the host; second, antibodies may exist in a very low titer to begin with; third, antibodies may exist in adequate concentrations but are unable to reach the graft because of local circulatory difficulties, such as described by Amos (34). Finally, antibodies may exist in low titers, dilution and circulatory blockade participating in different degrees to lower their concentration below effective levels for rejection. Perhaps adequate preparation of serum by repeated skin homografts from the same donor might increase the titer of

antibodies in immune serum and the different reasons for their failure can be overcome.

It has been shown beyond reasonable doubt that cell-bound antibodies are at least partly responsible for the homograft rejection phenomenon (12–15). The present experiments seem to prove that in the skin-homograft-rabbit system humoral antibodies also play a definite role in rejection of homografts. It is therefore permissible to suppose that homograft rejection is the result of the interaction of both types of antibodies. Extreme situations, in which there seems to be complete lack of either of the two forms of antibody, result in tolerance of homografts. Agammaglobulinemia would represent an instance of absolute humoral deficiency (16, 17) while Algire's early millipore experiments (12) would provide examples of complete absence of cell-bound antibodies. In any of these situations, addition of the missing element will result in homograft rejection, as in hypogammaglobulinemia, where there appear to exist small amounts of humoral antibodies, or as in Woodruff's experiment (13) with millipore chambers, where destruction of homografts was accelerated when cells of the host came in contact with foreign tissues previously exposed to immune serum. The present work represents a successful passive transfer of humoral antibodies to rabbits in which the immune response had been depressed by cortisone, which thus recovered their full ability to reject specific skin homografts. On the other hand, transfer of sensitized lymph node cells to animals with acquired tolerance for skin homograft will reestablish their capacity to reject otherwise tolerated grafts (42). This experiment, however, fails to discriminate between the effects of both types of antibodies, since sensitized lymph node cells are chiefly lymphocytes, which have been shown to be involved both in the synthesis of humoral antibodies (43–45) and to carry cell-bound antibodies (46, 47). The possibility of achieving passive transfer of specific anti-homograft humoral antibodies by means of sensitized lymphoid cells within millipore chambers introduced into conditioned hosts is presently under study in this laboratory.

SUMMARY

Gross and microscopic observations of skin homograft rejection carried out in cortisone-conditioned and non-conditioned rabbits seem to indicate that humoral antibodies play an important role in the phenomenon. Thus, local administration of isoimmune serum to animals bearing skin homografts resulted in a significantly earlier rejection of that particular test graft without modifying the course of a neighboring control-skin graft. This result appears to support the idea that homograft rejection is not only due to cellular antibodies but to a combination of both humoral and cellular immune responses, which should not be regarded as completely unrelated.

BIBLIOGRAPHY

1. Brent, L., Tissue transplantation immunity, *Progr. Allergy*, 1958, **5**, 271.
2. Merrill, J. P., Transplantation of normal tissues, *Physiol. Rev.*, 1959, **39**, 860.

3. Billingham, R. E., Brent, L., and Medawar, P. B., Quantitative studies on tissue transplantation immunity. II. The origin, strength and duration of actively and adoptively acquired immunity, *Proc. Roy. Soc. London, (Series B)*, 1954, **143,** 58.
4. Billingham, R. E., Brent, L., and Medawar, P. B., Quantitative studies on tissue transplantation immunity. III. Actively acquired tolerance, *Phil. Tr. Roy. Soc. London, (Series B)*, 1956, **239,** 357.
5. Medawar, P. B., Immunity to homologous grafted skin. II. The relationship between the antigens of blood and skin, *Brit. J. Exp. Path.*, 1946, **27,** 15.
6. Billingham, R. E., Brent, L., and Medawar, P. B., The antigenic stimulus in transplantation immunity, *Nature*, 1956, 178, 514.
7. Simonsen, M., Buemann, J., Gammeltoft, A., Jensen, F., and Jorgensen, K., Biologic incompatibility in kidney transplantation in dogs. I. Experimental and morphologic investigations, *Acta Path. et Microbiol. Scand.*, 1953, **32,** 1.
8. Bollag, W. S., Demonstration of antibodies following homografts, *Transplant. Bull.*, 1956, **3,** 43.
9. Amos, D. B., Gorer, P. A., Mikulska, B. M., Billingham, R. E., and Sparrow, E. M., An antibody response to skin homografts in mice, *Brit. J. Exp. Path*, 1954, **35,** 203.
10. Medawar, P. B., The immunology of transplantation, *Harvey Lectures*, 1958, **51,** 144.
11. Hildemann, W. H., and Medawar, P. B., Relationship between skin transplantation immunity and the formation of humoral isoantibodies in mice, *Immunology*, 1959, **2,** 44.
12. Algire, G. H., Weaver, J. M., and Prehn, R. T., Studies on tissue homotransplantation in mice, using diffusion-chamber methods, *Ann. New York Acad. Sc.*, 1957, **64,** 10.
13. Woodruff, M. F. A., Cellular and humoral factors in the immunity to skin homografts. Experiments with a porous membrane, *Ann. New York Acad. Sc.*, 1957, **64,** 1014.
14. Berrian, J. H., and Brent, L., Cell-bound antibodies in transplantation immunity, *Ann. New York Acad. Sc.*, 1958, **73,** 654.
15. Lawrence, H. S., Homograft sensitivity, *Physiol. Rev.*, 1959, **39,** 811.
16. Good, R. A., and Varco, R. L., Studies on agammaglobulinemia. I. Successful homograft in skin in a child with agammaglobulinemia, *J. Am. Med. Assn.*, 1955, **157,** 713.
17. Good, R. A., Varco, R. L., Aust, J. B., and Zak, S. J., Transplantation studies in patients with agammaglobulinemia, *Ann. New York Acad. Sc.*, 1957, **64,** 882.
18. Porter, H. M., The demonstration of delayed-type reactivity in congenital agammaglobulinemia, *Ann. New York Acad. Sc.*, 1957, **64,** 932.
19. Billingham, R. E., and Sparrow, E. M., Studies on the nature of immunity to homologous grafted skin, with special reference to the use of pure epidermal grafts, *J. Exp. Biol.*, 1954, **31,** 16.
20. Weaver, J. M., Algire, G. H., and Prehn, R. T., The growth of cells *in vivo* in diffusion chambers. II. The role of cells in the destruction of homografts in mice, *J. Nat. Cancer Inst.*, 1955, **15,** 1737.
21. Algire, G. H., Weaver, J. M., and Prehn, R. T., Growth of cells *in vivo* in diffusion

chambers. I. Survival of homografts in immunized mice, *J. Nat. Cancer Inst.*, 1954, **15,** 493.

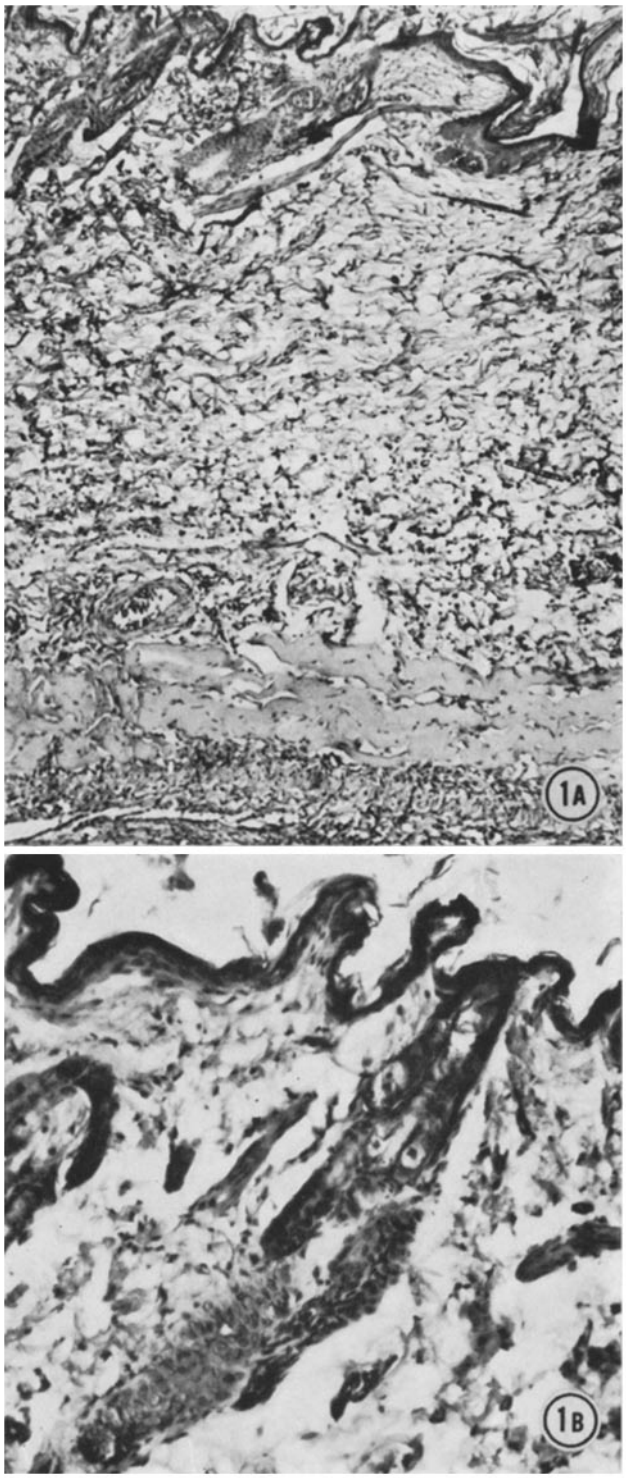
22. Amos, D. B., and Wakefield, J. D., Growth of mouse ascites tumor cells in diffusion chambers. II. Lysis and growth inhibition by diffusible isoantibody, *J. Nat. Cancer Inst.*, 1959, **22,** 1077.
23. Algire, G. H., Growth inhibition of homografts of a plasma-cell neoplasm in cell-impenetrable diffusion chambers placed in hyperimmunized mice, *J. Nat. Cancer Inst.*, 1959, **23,** 435.
24. Stetson, C. A., and Jensen, E., Humoral aspects of the immune response to homografts, *Ann. New York Acad. Sc.*, 1960, **87,** 249.
25. Terasaki, P. I., Cannon, J. A., Longmire, W. P., Jr., and Chamberlain, C. C., Antibody response to homografts. V. Cytotoxic effects upon lymphocytes as measured by time-lapse cinematography, *Ann. New York Acad. Sc.* 1960, **87,** 258.
26. Terasaki, P. I., Antibody response to homografts. II. Preliminary studies of the time of appearance of lymphoagglutinins upon homografting, *Am. Surg.*, 1959, **11,** 896.
27. Merrill, J. P., Hanau, C., and Hawes, M. D., A demonstration of a cytotoxic effect *in vitro* following the rejection of skin grafts by the rabbit, *Ann. New York Acad. Sc.*, 1960, **87,** 266.
28. Hellström, K. E., Cytotoxic effect of isoantibodies on mouse tumor cells *in vitro*, *Transplant. Bull.*, 1959, **6,** 411.
29. Medawar, P. B., Tests by tissue culture methods on the nature of immunity to transplanted skin, *Quart. J. Micr. Sc.*, 1948, **89,** 239.
30. Allgöwer, M., Blocker, T. G., Jr., and Engley, B. W. D., Some immunological aspects of auto- and homografts in rabbits, tested by *in vivo* and *in vitro* techniques, *Plastic Reconstr. Surg.*, 1952, **9,** 1.
31. Jensen, E., and Stetson, C. A., Jr., Humoral aspects of the immune response to homografts. II. Relationship between the hemagglutinating and cytotoxic activities of certain isoimmune sera, *J. Exp. Med.*, 1961, **113,** 785.
32. Stetson, C. A., Jr., and Demopoulos, R., Reactions of skin homografts with specific immune sera, *Ann. New York Acad. Sc.*, 1958, **73,** 687.
33. Billingham, R. E., and Brent, L., Further attempts to transfer transplantation immunity by means of serum, *Brit. J. Exp. Path.*, 1956, **37,** 566.
34. Amos, D. B., The persistence of mouse iso-antibodies *in vivo*, *Brit. J. Cancer*, 1955, **9,** 216.
35. Harris, S., Harris, T. N., and Farber, M. B., Studies on the transfer of lymph node cells. XII. The effect of anti-rabbit leukocyte serum on the transfer of antigen-incubated lymph node cells, *J. Exp. Med.*, 1958, **108,** 411.
36. Garver, R. M., and Cole, L. J., Passive transfer of bone marrow homotransplantation immunity with specific antisera, *J. Immunol.*, 1961, **86,** 307.
37. Gorer, P. A., Some recent work on tumor immunity, *Adv. Cancer Research*, 1956, **4,** 149.
38. Scothorne, R. J., Studies on the response of the regional lymph nodes to skin homografts, *Ann. New York Acad. Sc.*, 1958, **64,** 1028.
39. Billingham, R. E., Krohn, P. L., and Medawar, P. B., Effect of cortisone on survival of skin homografts in rabbits, *Brit. Med. J.*, 1951, **2,** 1049.

40. Lawrence, H. S., Rapapport, F. T., Converse, J. M., and Tillet, W. S., Transfer of delayed hypersensitivity to skin homografts with leukocyte extracts in man, *J. Clin. Inv.*, 1960, **39,** 185.
41. Dammin, G. J., and Murray, J. E., Criteria for acceptance of skin grafts, *Transplant. Bull.*, 1959, **6,** 429.
42. Billingham, R. E., Brent, L., and Medawar, P. B., Actively acquired tolerance of foreign cells, *Nature*, 1953, **172,** 603.
43. Harris, S., Harris, T. N., and Farber, M. B., Studies on the transfer of lymph node cells. I. Appearance of antibody in recipients of cells from donor rabbits infected with antigen, *J. Immunol.*, 1954, **72,** 148.
44. Roberts, J. C., Dixon, F. J., and Weigle, W. O., Antibody-producing lymph node cells and peritoneal exudate cells, *Arch. Path.*, 1957, **64,** 324.
45. Holub, M., Antibody production by lymphocytes after *in vitro* contact with bacterial antigen and transfer to new-born rabbits, *Nature*, 1958, **181,** 122.
46. Chase, M. W., The cellular transfer of cutaneous hypersensitivity to tuberculin, *Proc. Soc. Exp. Biol. and Med.*, 1945, **59,** 134.
47. Mitchison, N. A., Studies on the immunological response to foreign tumor transplants in the mouse. I. The role of lymph node cells in conferring immunity by adoptive transfer, *J. Exp. Med.*, 1955, **102,** 157.

EXPLANATION OF PLATES

PLATE 48

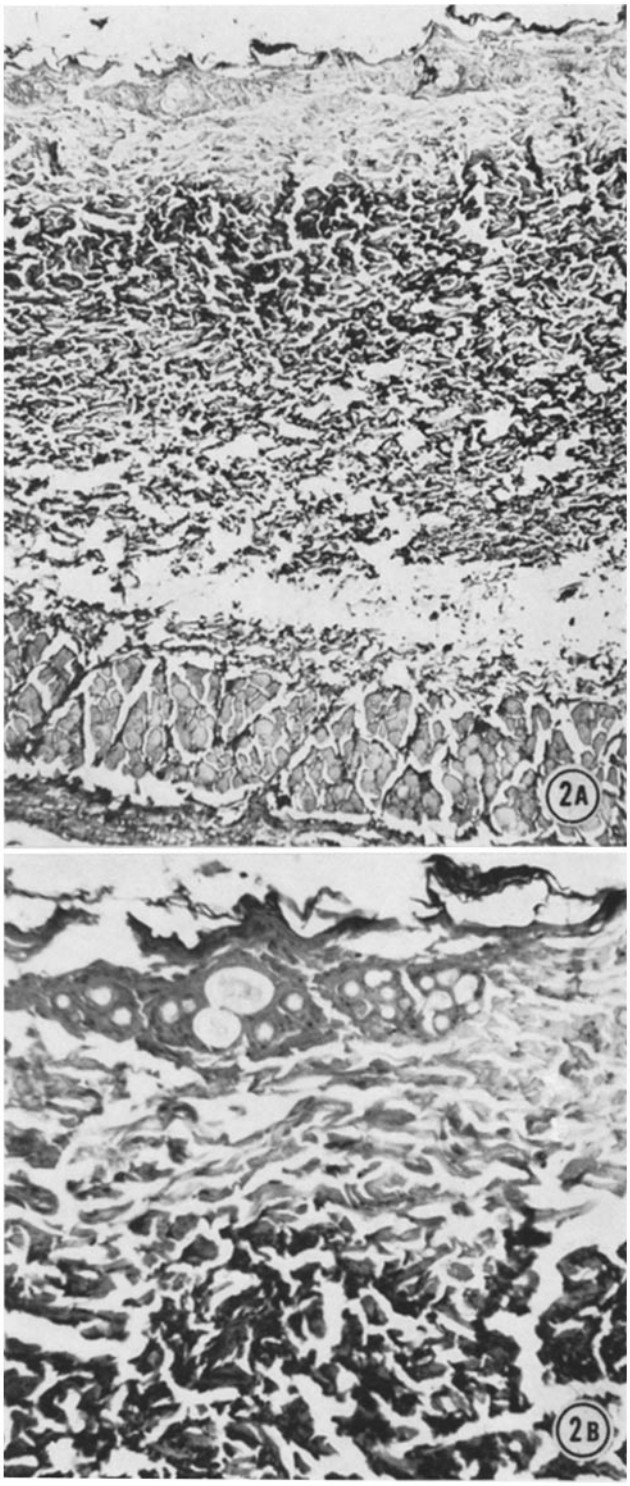
FIG. 1 *A*. General aspect of control-skin homograft in cortisone-conditioned rabbits given immune serum locally and followed for 5 days after grafting. There is slight disruption of dermal collagen fibers and minimal inflammatory infiltration near the smooth muscle. A blood vessel without thrombosis can be seen in the lower portion of the photograph. The epithelium is clearly discernible. × 120. Hematoxylin and eosin. *B*. A closer view of the upper dermis and epithelium of the same skin graft as in *A*. Smooth muscle fibers and individual epithelial cells can be easily identified, as well as the nuclei of connective tissue cells. × 240. Hematoxylin and eosin.



(Kretschmer and Pérez-Tamayo: Humoral antibodies)

PLATE 49

FIG. 2 *A*. Problem-skin homograft in cortisone-conditioned rabbits given immune serum locally and followed for 5 days after grafting. This is the same animal used for Fig. 1. Dermal collagen bundles are fragmented and basophilic. No nuclei are visible. In the deep dermis there are polymorphonuclear leukocytes infiltrating the muscle. The epithelium is almost entirely erased. × 120. Hematoxylin and eosin. *B*. Higher magnification of *A*. Absence of connective tissue nuclei, fragmentation and basophilia of collagen, and necrosis of epithelium are readily apparent. × 240. Hematoxylin and eosin.



(Kretschmer and Pérez-Tamayo: Humoral antibodies)