The Morphologic Responses of the Lymphoid System to Homografts. I. First and Second Set Responses in Normal Rabbits

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A LARGE BODY of evidence has firmly established the rejection of grafts as a manifestation of immunity.[1] The purpose of the experiments described in this paper was to examine by morphologic technics the cellular basis of transplantation immunity. Although many studies have been made of the lymphoid responses to cellular[2,3] and highly purified protein[4,5] antigens, relatively few have dealt with the responses to solid, orthotopic homografts.[6-9] These have shown that the cells which arise in lymphoid centers in response to homografts are morphologically similar, if not identical, to those that appear after stimulation by bacterial or protein antigens. However, the previously available data indicated that the morphologic response to homografts was localized mainly to contiguous lymph nodes, in contrast to the commonly accepted idea that homograft immunity is a generalized reaction. This report of a systematic, long term study of the lymphoid responses to first and second set skin homografts demonstrates that transplantation immunity is accompanied by a proliferative response which, although it begins locally, gradually spreads to involve distant lymph nodes and the spleen. The cellular reaction, which begins before rejection of the graft becomes apparent, continues long after the transplant has been reduced to an eschar. The morphologic reaction of lymph nodes and spleen following a second set graft is consistent with the hypothesis that this reaction represents a true anamnestic response.

MATERIALS AND METHODS

Animals. One hundred ninety-two outbred rabbits were used in these experiments. They weighed between 2.5 to 3.5 Kg. No selection by sex was made. The animals were housed in an air-conditioned room and were fed water and Purina rabbit chow *ad lib.*

Technic of skin grafting. Pairs of rabbits were selected at random and, wherever possible, had contrasting fur colors. The rabbits were anesthetized by intravenous Nembutal, 20 mg. per Kg. After shaving the dorsal surface of the ear, a 2-cm. full thickness square of skin was excised and, after removing the subcutaneous fat, the graft was transplanted to a corresponding graft bed of the opposite member of the pair. The graft was held in place by #4-0 silk sutures. Each graft was inspected daily for signs of rejection.

Examination of lymph nodes and spleen. One hour prior to sacrifice of the animal, 0.5 ml. of Evan's blue dye was injected subcutaneously at the base of each ear in order to
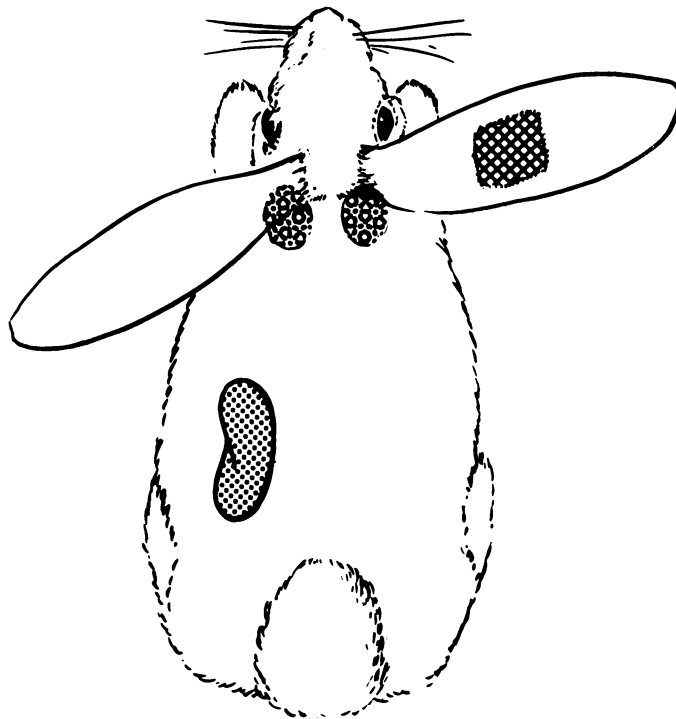
Fig. 1.—Responses of the lymphoid system to homografts: Experimental design. The lymph node draining the grafted ear is termed the "contiguous" or "draining" node; the corresponding node draining the ungrafted ear is the "contralateral" or "distant" lymph node.

facilitate identification of the cervical lymph nodes. The animal was killed with intravenous Nembutal and the lymph node most proximal to the base of the ear was identified and removed. At the same time, the corresponding lymph node draining the non-grafted ear (the contralateral lymph node), the spleen and the graft were excised (fig. 1). Imprints of the spleen and lymph nodes were made and the remainder of these tissues, along with the skin graft, were fixed in formol-saline. The following groups of rabbits were studied in this manner: 15 control animals which were not grafted, 25 animals in which autografting was performed, 8 rabbits which received an injection of 0.1 ml. of xylene into the subcutaneous tissue of one ear, 70 animals which had received a first set homograft, 70 animals in which a second set homograft was applied, and 4 rabbits which received multiple homografts. Animals with autografts were killed either on the second, fourth, sixth, eighth or tenth days after grafting. Animals with homografts were killed every other day until the tenth day after application of the graft, and then every five days until the fiftieth day. In group C, D, and E, five animals were killed on each of the days listed (table 1).

Processing of tissue. The imprints of the lymph nodes and spleens were stained with: Wright-Giemsa, methyl green pyronin, periodic acid Schiff, acridine orange, Feulgen and acid and alkaline phosphatase stains. The fixed tissues were sectioned in the usual manner and were stained with hematoxylyn-eosin, P.A.S., methyl green pyronin and silver stains. A differential count of 1,000 cells was made from the Giemsa stained imprints of the lymph nodes and spleens of each animal. The nomenclature of the various cell types followed the recommendations proposed at the Prague Meeting on Mechanisms of Antibody Formation.[10] This nomenclature includes the use of the term "hemocytoblast" for the large, probably immunologically competent, cell which appears during the immune proliferative reaction.

Table 1

Group	Number of Animals	Type of Graft	Postoperative Day of Sacrifice
A	15	none	——
B	8	none (xylene injections)	7,11
C	25	autograft	2,4,6,8,10
D	70	1st set homograft	1,2,4,6,9,12,14,19,25, 30,35,40,45,50
E	70	2nd set homograft	2,4,6,8,10,15,20,25, 30,35,40,45,50
F	4	repeated homografts	15,25

RESULTS

The differential formula cf imprints cf the normal rabbit lymph node and spleen are shown in table 2 (listed as Day 0). The histologic sections of tissues obtained from these normal animals showed the expected patterns, with well-defined lymphoid follicles, small germinal centers, easily seen medullary cords and scattered reticulum cells.

Nonspecific irritation, as produced by the subcutaneous injection of 0.1 ml. of xylene (Group B), resulted in minimal hyperplasia of the follicles of the draining lymph node cf animals killed cn the seventh day. Imprints of these nodes had 1.5–2.0 per cent hemocytoblasts,* but no other changes were found in the differential formula. The architectural pattern of the nodes remained undisturbed, and no changes were seen in the contralateral lymph nodes and the spleen. Animals sacrificed 11 days after the xylene injection had normal lymph nodes and spleens.

The histologic sections of the lymph nodes of the autografted rabbits showed slight enlargement of the germinal centers which appeared on the fourth day after application of the graft, persisted for two to three days and disappeared by the eighth day. There were no changes in the spleen sections. In the imprints of the lymph nodes, hemocytoblasts increased to about 2.5 per cent within six days and then rapidly returned to the baseline level of 1.0 per cent; by the tenth day the differential formula was normal. No changes were seen in the imprints of the spleen.

First Set

In the animals with homografts, there was mild edema and infiltration of the graft by lymphocytes on the fourth postoperative day, but the epithelium was well preserved. On that day the first distinct changes in the lymphoid tissues were localized to the lymph node draining the graft (fig. 2). Sections of these nodes showed marked enlargement of the germinal centers due to the proliferation of cells which, as seen in the imprints, were hemocytoblasts (a primitive cell having the capacity to develop into lymphocytes and/or plasma cells, fig. 3). The contralateral lymph node and the spleen showed no apparent changes at this time. On the sixth postoperative day the graft showed a marked lymphocytic infiltration, further edema and beginning disruption of

*Described below.

Table 2.—Cellular Constituents of the Lymphoid Tissues in the Rabbit During the First Set Homograft Reaction

| Day	Hemocytoblasts			Proplasma Cells			Plasma Cells			Eosinophils			Reticulum Cells		
	Draining node	Contra-lateral node	Spleen	Draining node	Contra-lateral node	Spleen	Draining node	Contra-lateral node	Spleen	Draining node	Contra-lateral node	Spleen	Draining node	Contra-lateral node	Spleen
0	1.0 ± 0.7	1.0 ± 0.7	0.5 ± 0.4	0.1 ± 0.3	0.1 ± 0.3	0.1 ± 0.4	0.5 ± 0.6	0.5 ± 0.6	0.3 ± 0.7	0.7 ± 0.8	0.7 ± 0.8	3.0 ± 0.8	4.7 ± 1.1	4.7 ± 1.1	6.1 ± 1.1
2	1.3 ± 1.0	1.2 ± 1.4		0.1 ± 0.1	0.1 ± 0.1		0.7 ± 0.7	0.1 ± 0.1		0.9 ± 1.1	2.5 ± 3.7		5.3 ± 1.3	4.2 ± 2.3	
4	4.5 ± 3.8	3.0 ± 1.9	0.6 ± 0.6	0.1 ± 0.1	0.1 ± 0.1	0.0 ± 0.0	1.2 ± 1.4	0.6 ± 0.7	0.7 ± 0.7	1.1 ± 1.3	1.3 ± 2.0	7.8 ± 1.3	5.0 ± 1.1	5.3 ± 1.0	6.5 ± 1.5
6	4.2 ± 1.6	0.7 ± 0.6	0.3 ± 0.2	0.6 ± 0.5	0.2 ± 0.2	0.1 ± 0.1	0.5 ± 0.5	0.5 ± 0.9	0.6 ± 0.0	0.9 ± 0.8	0.9 ± 0.7	5.6 ± 1.4	5.0 ± 0.8	5.5 ± 0.2	6.1 ± 0.2
9	4.0 ± 2.3	1.0 ± 0.7	0.3 ± 0.2	0.3 ± 0.5	0.2 ± 0.2	0.2 ± 0.1	0.5 ± 0.5	0.5 ± 0.9	0.2 ± 0.1	1.0 ± 1.1	0.9 ± 0.3	7.8 ± 1.2	5.5 ± 2.6	6.1 ± 0.4	6.1 ± 0.3
12	2.0 ± 1.2	0.8 ± 0.9	0.1 ± 0.1	0.6 ± 0.9	0.1 ± 0.3	0.2 ± 0.1	0.6 ± 0.9	0.6 ± 0.5	0.4 ± 1.0	1.0 ± 1.0	1.4 ± 1.0	9.0 ± 1.6	5.5 ± 1.8	7.0 ± 1.1	5.9 ± 0.8
14	1.0 ± 1.0	1.2 ± 0.8	0.3 ± 1.5	0.1 ± 0.1	0.1 ± 0.2	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.6	0.2 ± 0.5	2.0 ± 0.8	2.0 ± 1.1	8.0 ± 1.8	5.0 ± 1.7	5.2 ± 1.0	6.1 ± 0.7
19	1.2 ± 1.2	0.8 ± 0.6	0.3 ± 0.2	0.1 ± 0.2	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.5	0.3 ± 1.3	0.4 ± 0.8	0.6 ± 1.4	1.6 ± 1.3	6.7 ± 2.8	6.5 ± 0.9	5.5 ± 0.7	5.6 ± 0.7
25	1.5 ± 1.3	0.7 ± 0.7	0.3 ± 1.4	0.1 ± 0.2	0.1 ± 0.1	0.1 ± 0.1	0.5 ± 0.9	0.2 ± 0.5	0.3 ± 0.6	1.7 ± 1.3	1.8 ± 1.0	9.5 ± 3.1	5.4 ± 0.6	4.9 ± 0.7	5.7 ± 1.5
30	1.2 ± 0.5	1.5 ± 1.7	0.3 ± 1.0	0.2 ± 0.2	0.2 ± 0.5	0.4 ± 1.0	0.5 ± 1.0	0.1 ± 0.1	0.4 ± 1.0	0.4 ± 0.5	0.8 ± 0.8	6.0 ± 1.1	5.0 ± 1.1	4.7 ± 1.0	5.5 ± 1.8
35	1.2 ± 1.0	0.8 ± 0.8	0.3 ± 0.4	0.1 ± 0.1	0.1 ± 0.3	0.1 ± 0.1	0.4 ± 1.2	0.1 ± 1.0	0.4 ± 0.7	1.9 ± 1.2	1.7 ± 0.8	6.5 ± 2.2	5.5 ± 1.8	5.5 ± 1.8	5.6 ± 2.4
40	1.4 ± 1.4	1.1 ± 0.8	0.4 ± 0.6	0.1 ± 0.3	0.1 ± 0.2	0.2 ± 0.3	0.4 ± 1.0	0.5 ± 0.8	0.4 ± 0.6	1.0 ± 0.9	1.5 ± 0.8	6.4 ± 3.3	5.0 ± 0.9	5.3 ± 0.8	5.5 ± 1.0
45	1.5 ± 1.4	1.5 ± 1.0	0.4 ± 1.2	0.4 ± 1.0	0.1 ± 0.3	0.3 ± 0.4	0.7 ± 1.0	0.4 ± 0.7	0.7 ± 0.7	1.4 ± 1.0	0.9 ± 0.9	6.0 ± 2.3	4.8 ± 1.0	4.2 ± 0.8	5.6 ± 0.8
50	1.2 ± 0.9	0.6 ± 1.7	0.5 ± 0.7	0.3 ± 0.8	0.4 ± 0.4	0.3 ± 0.5	0.8 ± 0.9	0.5 ± 0.5	0.5 ± 0.7	0.8 ± 0.5	0.1 ± 0.4	5.1 ± 2.4	4.7 ± 0.6	4.6 ± 0.6	5.7 ± 1.0
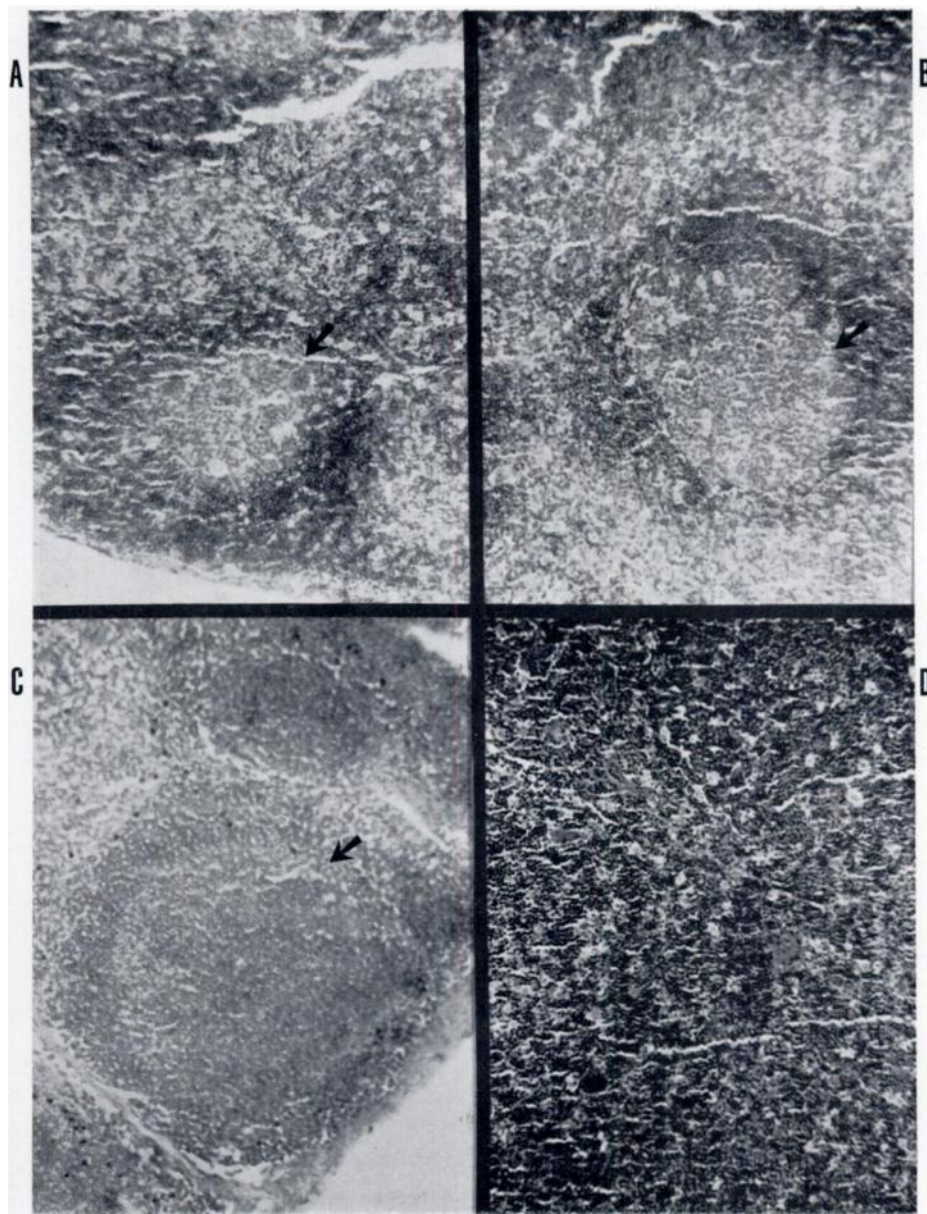
Fig. 2.—Morphologic changes in lymph nodes contiguous to the homograft during the development of transplantation immunity. Note particularly the progressive enlargement of the germinal center (arrows) and finally the disruption of normal architecture (d) which occurs at the height of the graft rejection. (a) 4th post-operative day; (b) 6th post-operative day; (c) 8th post-operative day; (d) 10th post-operative day. Reduced from 430 x.

the epithelium. The draining lymph nodes showed even more prominent germinal centers and beginning obliteration of the margins of the lymph follicle. A marked increase in primitive reticulum cells in both the germinal centers and medullary cords was also seen. Similar changes were beginning to appear in
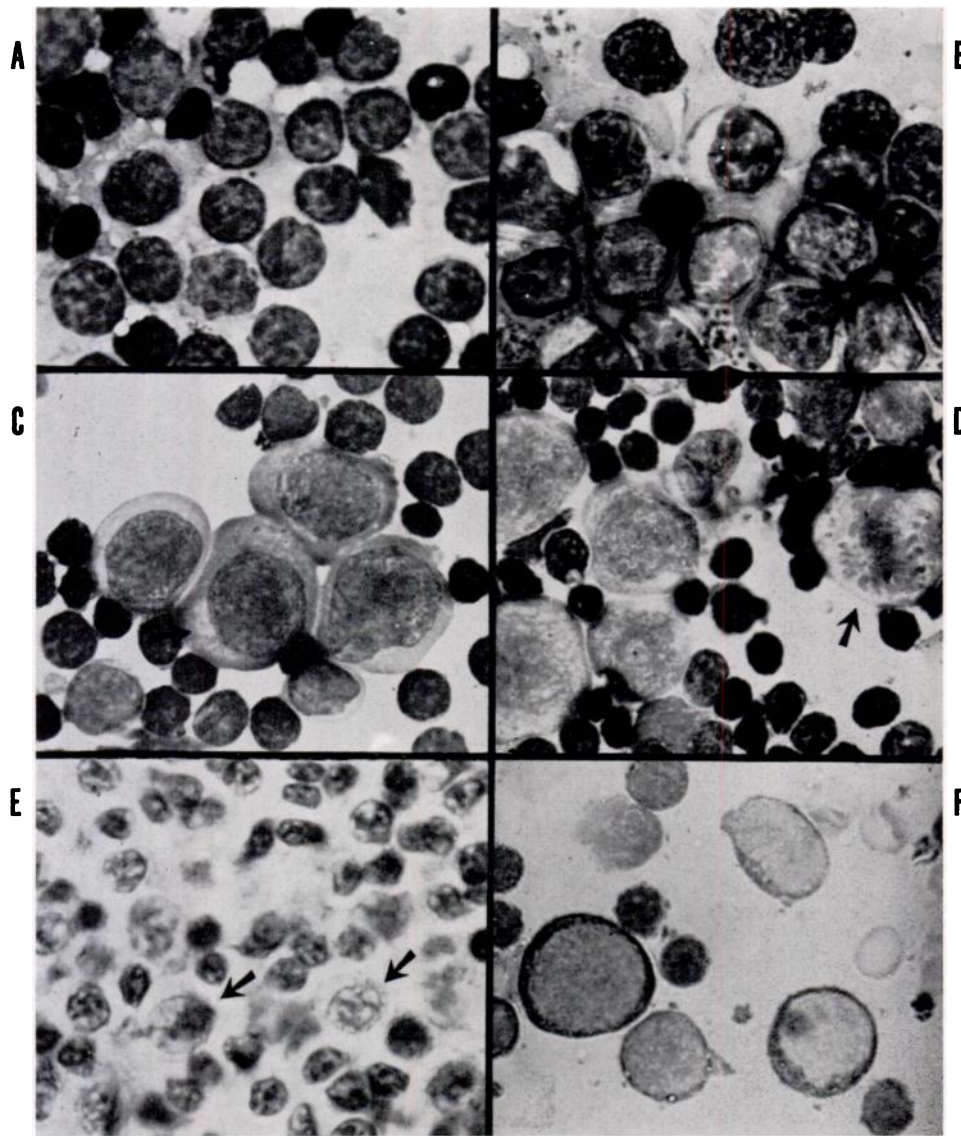
Fig. 3.—(a) Normal lymph node imprint. (b) Normal splenic imprint. (c) Hemocytoblasts; note the perinuclear arcoplasm (arrow). (d) Hemocytoblasts; note mitotic figure (arrow). (e) Appearance of hemocytoblasts (arrows) in tissue section, H & E stain. (f) Hemocytoblasts (pyronin stain), imprint, showing pyroninophilia.

the contralateral lymph node. The spleen was still unchanged. These changes were reflected in the imprints of the draining lymph node by an increasing number of hemocytoblasts (4.2 per cent) (fig. 4). It is probable that a far higher *absolute* concentration of these cells was present due to the marked hyperplasia of the entire node.

By the ninth postoperative day the histologic changes in the graft were at

their peak; there was massive lymphocytic infiltration, complete disruption of the epithelium, pronounced edema and no evidence of blood flow. The draining lymph nodes now showed many areas in which the normal architecture was replaced by a structureless mass of lymphocytic cells (fig. 2d). In many areas lymphoid follicles could not be seen and in others there was no clear demarcation between the cortical area of the follicle and the medullary cords. These changes were accompanied by a marked increase in the number of primitive reticulum cells, which were seen in all areas of the lymph node. Touch preparations showed 4 per cent hemocytoblasts. In the distant node, the germinal centers were enlarged and primitive reticulum cells were more prominent, but the follicular pattern was well preserved. A slight increase in hemocytoblasts was present. There was a very discrete enlargement of the splenic follicles at this stage, but no changes in the differential formula of the splenic imprints were observed. By the 14th postoperative day the changes in the draining lymph node were beginning to subside. Lymph follicles became distinct again in many areas of the nodes and the germinal centers were once again easily seen. Reticulum cells, however, were still numerous. The contralateral lymph node was almost normal in structure, except for a slight increase in reticulum cells. Slight follicular enlargement persisted in the spleen. The changes in the draining lymph node gradually subsided, the germinal centers returning to their normal appearance first, followed by a gradual reduction in the size of the lymph follicles until the 25th day, when the lymph node was normal. The distant lymph nodes were completely normal by the 15th postoperative day. By the 20th day the splenic follicles were normal in size.

The fundamental response in the lymphoid system appeared to occur within the follicles and was characterized by a cellular proliferation resulting in the rapid enlargement of pre-existing germinal centers. Although coalescence with adjacent follicles took place at times, it was usually possible to see borderlines between them. New (secondary) follicles of lymphoid tissue appeared in the medullary cords and eventually the entire lymph node was filled with lymphocytes. In this rather uniform appearing lymph node, nodules of more primitive cells were occasionally found. These cells were strongly pyroninophilic and represented proliferating germinal centers "lost" in the apparent (but not real) effacement of the architecture.

The observation that the major area of immunologic response is the follicle of the lymph node and spleen, and particularly its germinal center, has also been made following stimulation with defined antigens in the rabbit[2,11] and mouse[12] and in a study of rheumatoid arthritis in man.[13] However, the primary seat of reaction to antigenic stimuli in the rat spleen is the red pulp.[14] The reasons for this species difference in response are not clear. However, it is apparent from these studies that the first cell responding to transplantation antigen present in the graft, whatever its nature, arises within the germinal center.

The characteristic cell in the lymphoid response to homografts is the "hemocytoblast." The earliest cell of this series is from 12–15 μ in diameter and has a large nucleus surrounded by a thin ring of moderately basophilic cytoplasm. The nuclear chromatin is arranged in a fine, delicately stippled pattern which
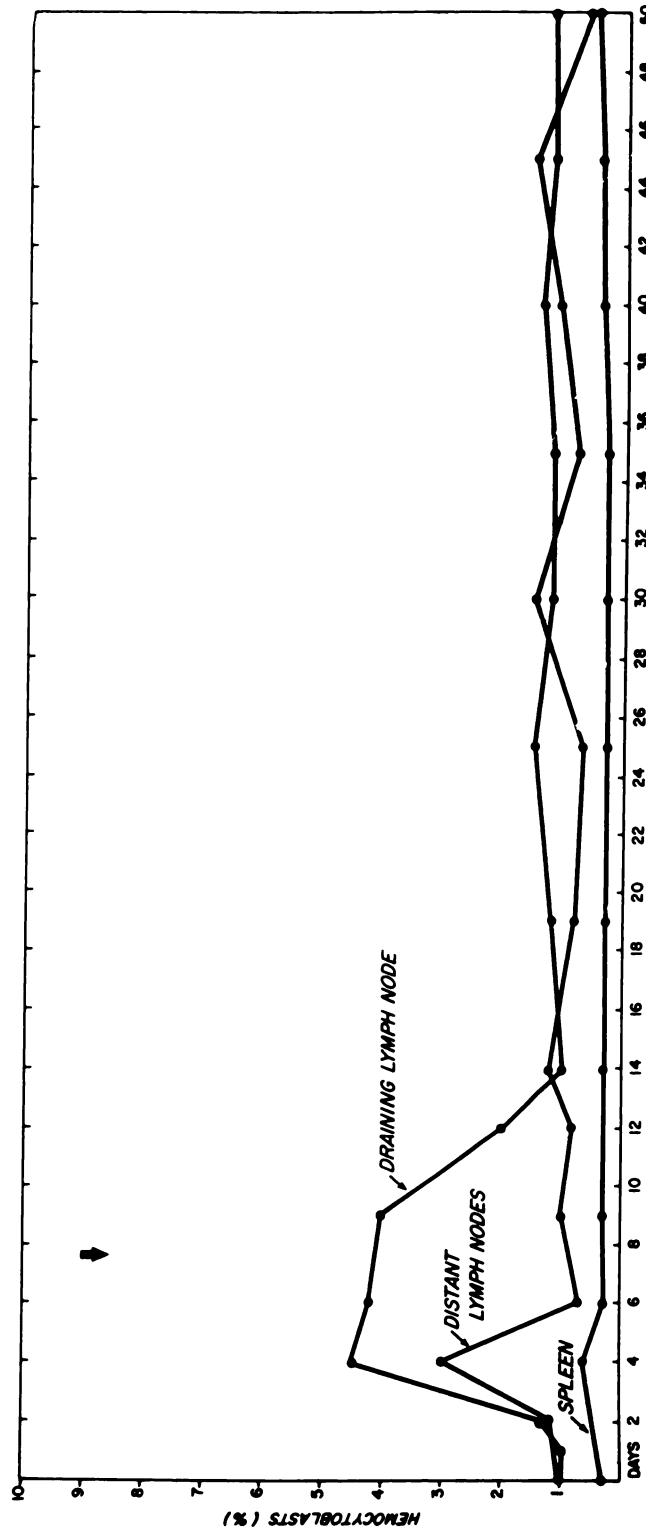
Fig. 4.—Hemocytoblast responses to first set skin homografts. Arrow indicates average time of skin graft rejection as determined by macroscopic examination.

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Table 3.—*Cytochemistry of the Homograft Response*

CELL TYPE	PAS	PAS/DIAST	METHYL GREEN PYRONINE	ACRIDINE ORANGE	ACID-PHOSPHA TASES	ALKALINE PHOSPHATASES
HEMOCYTOBLASTS	0	0	++++	++	0	0
PRO-PLASMA CELLS	0-+	0-+	++++	++	0	0
PLASMA CELLS	±-+	±	+++	++	Occ +	0
LYMPHOCYTES	+⁻++	0	++	0	0	0
RETICULUM CELLS	±	±	±	0	+⁻++	0

is similar to that seen in primitive reticulum cells. Nucleoli are not prominent. As it matures, the diameter of this cell enlarges and the nuclear:cytoplasmic ratio diminishes. The cytoplasm is now intensely basophilic, and the beginnings of a perinuclear clear zone can be discerned. Nucleoli now appear, and at times the nucleolar membrane can be distinctly seen. The next cell of the series is smaller in diameter, its cytoplasm is still very basophilic and it now contains an obvious arcoplasm. The centrally placed nucleus no longer contains nucleoli and the chromatin network, although still reticulated, is finer than before. A small number of these cells appear to mature into large oval cells with eccentric nuclei, perinuclear clear zones and deeply basophilic cytoplasm (proplasmocytes?).

The cytochemical characteristics of the cells arising during the homograft reaction are seen in table 3. They indicate the presence of large amounts of RNA and little or no glycogen or acid mucopolysaccharides in hemocytoblasts. In contrast, the mature lymphocytes were weakly pyroninophilic and contained numerous glycogen granules. Mature plasma cells not only gave strong reactions with pyronin, but were positive with the P.A.S. stain and occasionally showed the presence of acid phosphatase as well. Reticulum cells were weakly pyroninophilic, moderately positive in the P.A.S. reaction and usually positive for acid phosphatase.

Hemocytoblasts, also called by others transitional cells,[2] lymphoblasts,[15] acute splenic tumor cells[3] and reticular cells[16] are most probably immunologically competent cells. These cells arise primarily within the germinal centers of the lymphoid follicles, but, as the homograft reaction proceeds, they may also be seen in the perifollicular area of the red pulp. They are not conspicuous within the medullary cords. These cells are strongly pyroninophilic and their cytoplasm fluoresces brilliantly when stained with acridine orange, thus indicating that they contain RNA and are at least morphologically suited for the production of antibody. Increases in plasma cells are rarely seen during or after the destruction of a first set homograft.

Second Set

In contrast to the changes seen during a first set homograft response, those which occurred after the application of a second set skin graft appeared

Table 4.—Cellular Constituents of the Lymphoid Tissues in the Rabbit During the Second Set Homograft Reaction

Day	Hemocytoblasts			Proplasma Cells			Plasma Cells			Eosinophils			Reticulum Cells		
	Draining node	Contra-lateral node	Spleen	Draining node	Contra-lateral node	Spleen	Draining node	Contra-lateral node	Spleen	Draining node	Contra-lateral node	Spleen	Draining node	Contra-lateral node	Spleen
0	1.0 ± 0.7	1.0 ± 0.7	0.5 ± 0.4	0.1 ± 0.3	0.1 ± 0.3	0.1 ± 0.4	0.5 ± 0.6	0.5 ± 0.6	0.3 ± 0.7	0.7 ± 0.8	0.7 ± 0.8	3.0 ± 0.8	4.7 ± 1.1	4.7 ± 1.1	6.1 ± 1.1
2	3.5 ± 2.1	0.9 ± 0.3	1.4 ± 0.4	3.5 ± 0.8	0.9 ± 0.3	0.1 ± 0.4	2.9 ± 1.2	1.8 ± 3.9	2.2 ± 2.4	2.0 ± 1.9	0.7 ± 2.0	9.0 ± 2.6	3.1 ± 0.8	3.1 ± 0.6	6.1 ± 0.9
4	4.7 ± 3.8	2.4 ± 1.6	2.9 ± 1.4	1.5 ± 1.7	0.3 ± 0.3	0.1 ± 0.1	1.0 ± 1.5	1.8 ± 1.0	1.8 ± 1.4	0.7 ± 0.7	1.2 ± 0.8	9.0 ± 3.8	2.7 ± 0.5	2.1 ± 0.5	6.8 ± 0.8
6	6.8 ± 3.3	2.4 ± 1.1	1.9 ± 1.0	1.6 ± 1.8	0.7 ± 0.7	1.5 ± 1.9	1.3 ± 1.8	1.5 ± 0.9	1.6 ± 1.4	1.8 ± 1.9	0.8 ± 0.7	7.5 ± 1.7	5.6 ± 1.2	6.4 ± 0.7	6.5 ± 1.5
8	7.7 ± 4.3	2.8 ± 2.4	2.2 ± 1.2	1.0 ± 1.2	1.8 ± 1.5	1.7 ± 1.5	1.3 ± 1.6	1.5 ± 1.2	1.2 ± 1.8	0.9 ± 1.3	1.6 ± 1.1	7.8 ± 2.2	5.8 ± 1.1	4.9 ± 0.8	6.6 ± 1.8
10	3.1 ± 1.3	1.8 ± 1.3	1.7 ± 1.0	0.9 ± 1.0	0.5 ± 0.2	1.0 ± 1.0	0.9 ± 1.0	1.5 ± 0.9	1.5 ± 0.8	2.1 ± 1.7	1.3 ± 1.4	11.0 ± 6.2	5.8 ± 0.3	5.7 ± 0.7	6.8 ± 0.7
15	3.2 ± 2.2	1.7 ± 1.1	1.7 ± 0.7	0.9 ± 0.4	0.8 ± 1.0	0.9 ± 1.0	0.9 ± 0.4	1.4 ± 1.7	1.9 ± 0.8	2.3 ± 1.7	1.8 ± 2.5	7.0 ± 1.9	6.3 ± 1.1	5.6 ± 0.9	6.9 ± 0.8
20	2.5 ± 1.2	2.0 ± 1.2	1.5 ± 0.8	0.2 ± 0.1	0.8 ± 0.2	1.0 ± 1.2	1.1 ± 0.2	0.4 ± 0.5	1.0 ± 0.8	1.4 ± 0.8	1.2 ± 0.3	8.5 ± 1.3	5.8 ± 0.1	6.5 ± 0.3	6.5 ± 0.7
25	2.4 ± 1.7	1.6 ± 1.3	1.7 ± 1.3	0.1 ± 0.1	0.4 ± 0.2	1.0 ± 0.9	1.1 ± 0.5	1.3 ± 1.2	1.6 ± 1.5	2.2 ± 1.4	1.2 ± 1.1	8.8 ± 1.2	6.4 ± 0.0	6.3 ± 0.2	6.4 ± 2.0
30	1.6 ± 0.4	1.4 ± 0.3	1.5 ± 0.0	0.6 ± 0.3	1.0 ± 0.1	0.4 ± 0.2	1.5 ± 1.1	0.5 ± 0.4	1.4 ± 1.4	1.4 ± 1.0	0.4 ± 0.4	8.2 ± 3.7	6.1 ± 1.0	4.0 ± 1.2	6.7 ± 0.6
35	1.7 ± 0.7	1.8 ± 0.2	1.3 ± 0.5	0.5 ± 1.5	0.2 ± 0.4	0.1 ± 0.1	1.2 ± 1.5	0.7 ± 0.2	0.4 ± 0.5	1.1 ± 1.2	0.4 ± 0.6	5.9 ± 2.5	6.4 ± 0.5	6.5 ± 0.6	6.4 ± 0.3
40	1.8 ± 0.6	1.6 ± 0.7	1.3 ± 1.0	0.1 ± 0.4	0.9 ± 0.9	0.1 ± 0.1	0.6 ± 0.7	0.7 ± 0.4	0.7 ± 1.6	1.8 ± 1.7	1.7 ± 1.7	8.7 ± 3.1	6.1 ± 0.5	6.1 ± 0.5	6.5 ± 1.8
45	2.0 ± 1.2	1.8 ± 1.0	1.3 ± 0.9	0.6 ± 1.1	1.0 ± 1.2	0.2 ± 1.0	1.3 ± 2.5	1.0 ± 1.2	0.8 ± 1.7	0.3 ± 0.7	0.8 ± 0.9	7.1 ± 2.2	6.1 ± 0.6	6.1 ± 0.7	6.2 ± 1.4
50	1.5 ± 0.9	1.1 ± 1.0	1.5 ± 0.5	0.6 ± 0.8	0.4 ± 0.5	0.1 ± 0.2	0.5 ± 0.9	0.4 ± 0.5	0.9 ± 0.6	0.8 ± 0.7	0.9 ± 0.9	5.6 ± 0.6	6.0 ± 0.7	6.2 ± 0.6	6.4 ± 0.2

earlier, were greater in intensity and persisted for a longer period of time (table 4). Thus, by the second postoperative day the skin graft was heavily invaded by lymphocytes and edema was beginning to appear. Simultaneously, the draining lymph node showed enlargement of its follicles and, for the first time, plasma cells were seen, especially in the perifollicular areas (fig. 5). Reticulum cells did not appear increased. In the imprints, hemocytoblasts were easily seen and already constituted 3.5 per cent in the differential formula (fig. 6). The contralateral node and the spleen showed no changes. By the fourth postoperative day the follicles were even more prominent, with strikingly enlarged germinal centers, whereas the contralateral lymph node and spleen was still normal. In the imprints, the hemocytoblasts increased to 4.7 per cent in the draining node and to 2.4 per cent in the contralateral node. A slight increase in plasma cells was seen on both sides, but was more marked on the grafted side. On the sixth postoperative day the histologic changes indicative of rejection in the graft were at their peak. The draining lymph node now showed distinct signs of architectural disruption, similar to those seen in the first set reaction on the eighth-tenth days. During this period of follicular and germinal center enlargement, masses of pyroninophilic cells were again seen in the germinal centers. However, in contrast to what was seen in the first set response, the medullary cords showed nodular formations of plasma cells, particularly in relation to blood vessels. Some effacement was also seen in the contralateral lymph node. The spleen showed pronounced enlargement of the lymphoid follicles (fig. 7). A marked proliferation of hemocytoblasts was seen in the imprints at this stage, particularly in the draining lymph node. For the first time, increased numbers of hemocytoblasts and plasma cells were seen in the splenic imprints. The effacement of the lymphoid follicles reached its peak by the eighth postoperative day, and by the tenth day follicles began reappearing. By the 15th day the contralateral nodes were histologically normal, but enlargement of the follicles and germinal centers in the draining lymph node persisted until the 25th postoperative day; by the 30th postoperative day the draining lymph nodes were normal. Follicular enlargement in the spleen gradually diminished until the 35th–40th postoperative day, when the spleen sections were normal. In the imprints of the lymph nodes and spleen, the percentage of hemocytoblasts reached a peak on the eighth day and thereafter slowly diminished in number; however, slightly increased numbers of these cells persisted in the draining node until the fiftieth postoperative day. Plasma cells showed a slight but persistent increase throughout the reaction. An interesting feature of the second set response was the finding in the perivascular fibrous tissue of cells which appear to be fibroblasts, with elongated vesicular nuclei, but which, in contradistinction to fibroblasts, give strongly positive pyronine reactions. This finding lends support to previous work indicating the formation of plasma cells from perivascular fibrous tissue,[17] which is known to contain multipotent primitive cells. However, the character of the cellular infiltrate in the graft remained unchanged, lymphocytes predominating. Neither hemocytoblasts nor plasma cells were ever seen in the graft. Further evidence that plasma cells are not a prominent feature of the lymphoid response to skin homografts was obtained from a study of multigrafted rabbits (Group F). These animals, which had received from three to five homografts
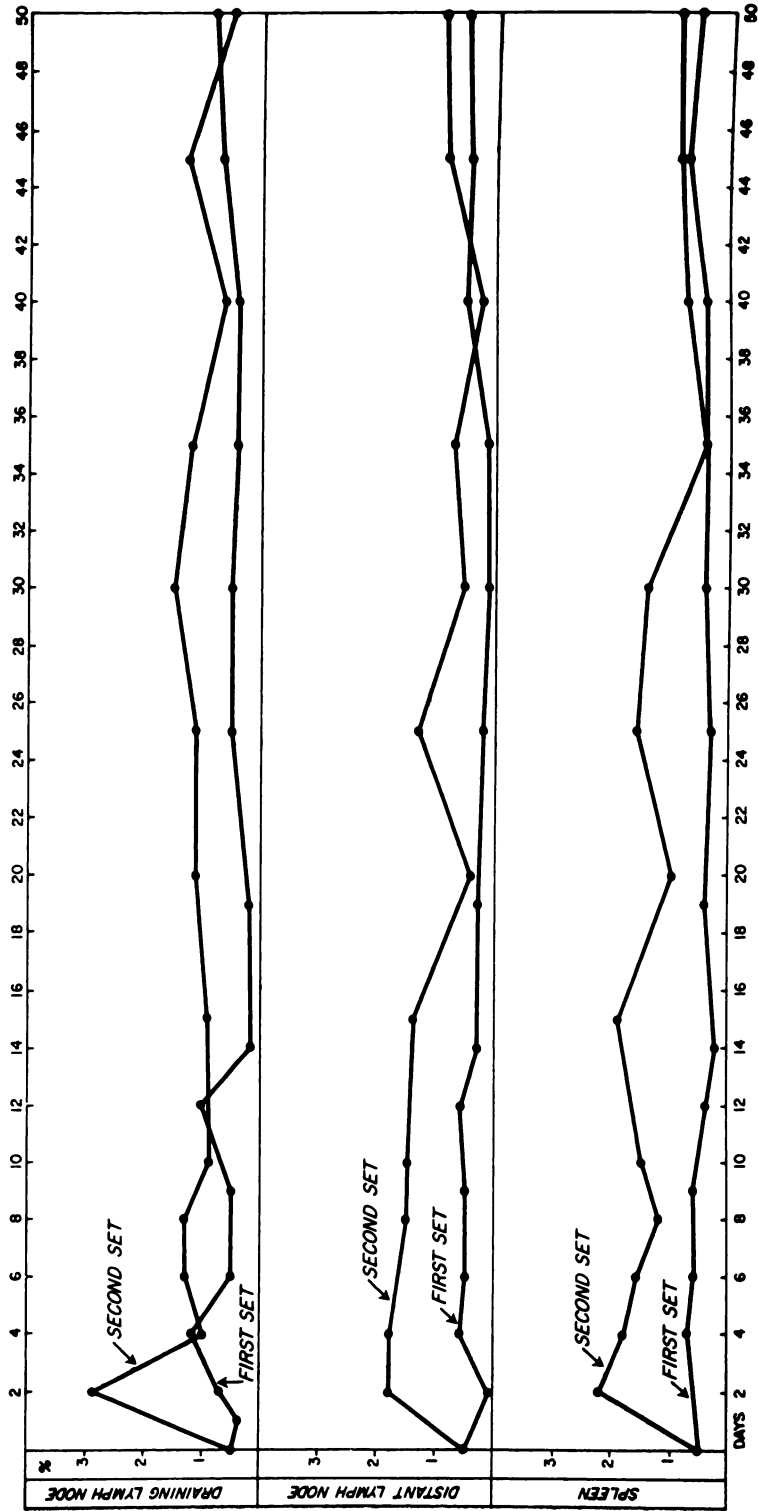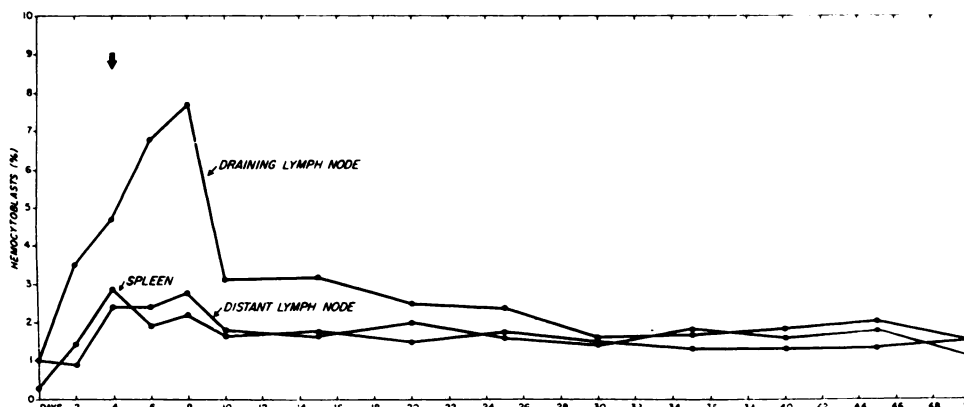
Fig. 5.—Plasmocytic responses to skin homografts.

Fig. 6.—Hemocytoblast responses to second set skin homografts. Arrow indicates average time of skin graft rejection as determined by macroscopic examination.

cn the same ear, showed striking hyperplasia not only in the draining lymph node, but in the contralateral node and in the spleen. Triple zoning in the spleen was pronounced. Hemocytoblasts constituted 6–7 per cent of the differential formula, but plasma cells never exceeded 1 per cent.

DISCUSSION

Perhaps the first hint of the part played by immunity in the destruction of grafts was recorded in 1897 by Born[18] who attempted to unite two different amphibian embryos into one organism. These embryonic grafts failed within 14–16 days; grafts between members of different orders of amphibia (i.e., heterografts) broke down within one or two days. Six years later, Jensen[19] solidly established the importance of immunization in transplantation reactions in his study of transplanted mammary carcinoma in mice and thereby created the atmosphere of understanding in which the fundamental observations of Tyzzer,[20] Little,[21] Snell[22] and Medawar[23] were made. Within a few years of Jensen's studies, the central and cbligatory role of the lymphoid apparatus in graft destruction had begun to be appreciated. These early reports[24-27] clearly demonstrated the presence of lymphocytes in rejected tumor and cartilage grafts. The majority of these studies were interpreted as showing the local manifestations of a generalized immune reaction against the graft. Particular emphasis was placed on the lymphocyte as an active participant in the transplantation reaction by Da Fano[28] and Rous and Murphy;[29] a series of brilliant experiments published between 1912 and 1926 led Murphy to conclude that the lymphocyte not only interacted with foreign grafts but was in fact the cell responsible for the development of transplantation immunity.[30-32]

The decisive function of the lymphoid system in transplantation immunity has been established not only by morphologic studies but also by other lines of evidence. The most convincing experiments dealt with the transfer of transplantation immunity from a sensitized animal to a second, "neutral" recipient by means of lymphoid cells. The first demonstration of this effect was reported in 1938 by Potter, Taylor and MacDowell[33] who conferred immunity in mice to a transplantable leukemia by splenic cells. In 1954, Mitchinson[34] showed
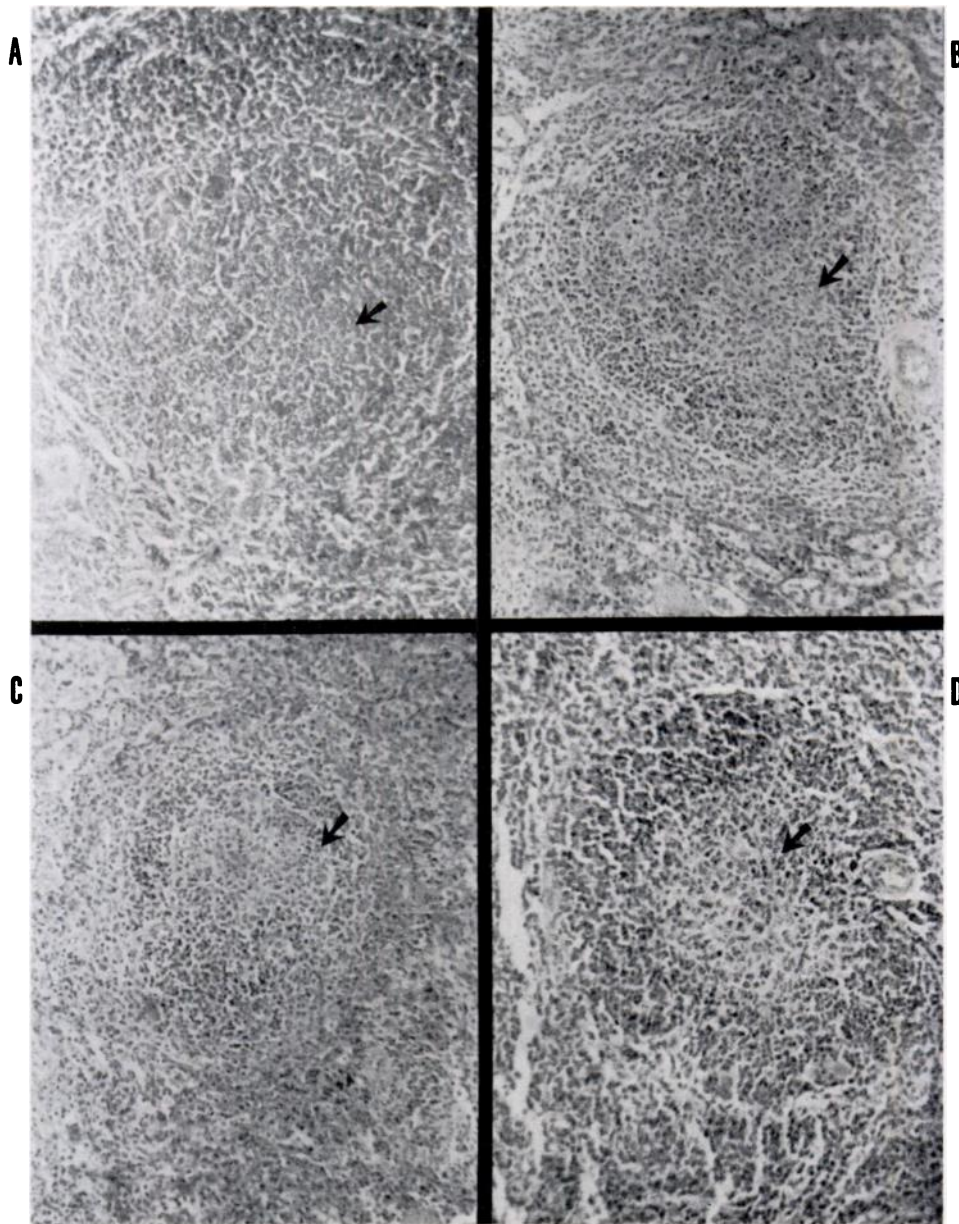
Fig. 7.—Morphologic changes in the spleen during the second set homograft response. Progressive enlargement of germinal centers (arrows) is evident, and these areas remain enlarged long after the homograft has been rejected. (a) Normal spleen. The germinal center is small and indistinct. (b) 6th post-operative day; (c) 15th post-operative day; (d) 25th post-operative day. Reduced from 430 x

that transplantation immunity in mice could be transferred by cells obtained from lymph nodes draining tumor grafts. However, cells from distant lymph nodes or the spleen failed to induce immunity in the new recipient. Billingham, Brent and Medawar[35] and Voisin and Mauer[36] repeated these experi-

ments using skin homografts and showed that living lymphocytes were necessary in order to transfer homograft immunity. The abolition of acquired immunologic tolerance by injection of the tolerant mouse with isologous lymph node cells[37] supported the hypothesis that the transferred lymphocytes function immunologically in the tolerant host and confer upon it transplantation immunity. However, the failure of Mitchison and Dube[38] to find acriflavine labeled sensitized lymph node cells in tumor grafts of secondary hosts has remained unexplained.

The effects of x-radiation,[39] cortisone[40] and antimetabolites[41] in prolonging the survival of homografts underscores the central position of the lymphoid cell system in transplantation immunity, since the most important effect of these agents is to destroy the lymphatic tissue of the recipient, or at least the ability of this tissue to produce immunologically competent cells ("hemocytoblasts").

A further demonstration of the relationships of the lymphocytic system to homograft destruction is the effect of denying lymphocytes access to the graft by natural or artificial means. Thus, the prolonged survival of foreign tissues in the anterior chamber of the eye[42] or the brain[43] reflects the limited passage of lymphocytes into these sites; only when vascularization occurs in these areas does graft breakdown ensue.[44] Enclosure of the tissue, as in bone,[45] cartilage[46] or millipore chambers[47] prolongs its life by a similar mechanism: quarantine from lymphocytes. The prolonged retention of homo- or heterografts within the cheek pouch of the hamster[48] is now known to be due to the presence of a thick fibrous tissue barrier which effectively protects the graft from the host's lymphoid tissue.[49] An antithesis to these observations is the rapid rejection of skin grafts by goldfish[50] and chickens,[51] species in which the lymphoid system is diffused throughout the integument and thereby virtually abut the graft.

In a classic study of skin grafts in rabbits, Medawar[23] showed that dying and dead homografts were heavily infiltrated by lymphocytes. Toolan and her colleagues[52] later demonstrated that the destruction of transplanted tumors was always accompanied by a lymphocytic infiltration around the transplant and that the lymph node draining the graft became hyperplastic. Similar findings in rejected kidney homografts were also reported by Dempster[53] and Simonsen.[54] Reactions within the lymphoid system following the application of skin homografts were studied by Gallone, Radici and Riquier,[6] Scothorne and MacGregor,[7] Radici and Piredda,[8] and by Masse, Chassaigne and Kermarec.[9] These authors found hypertrophic draining lymph nodes containing large pyroninophilic lymphoid cells; no changes were observed in distant lymph nodes. However, only a small number of animals were used in these studies and the second set homografts was not studied in any detail. Andreini, Drasher and Mitchison[55] demonstrated increases in the nitrogen, DNA and RNA contents of regional lymph nodes draining heterografts. Reactions within lymph nodes to cartilage, bone and tumor homografts have been studied by Craigmyle,[56] Burwell,[57] and Shepra.[58] The studies of Dempster and Simonsen on the kidney, of Dammin[59] on the spleen, and of Darcy[60] on the submandibular gland are noteworthy in that plasmocytic reactions were prominent within these grafts, whereas in most other types of homografts, lymphocytic

reactions predominate. Simonsen believed the plasma cells found in the kidney originated within the graft, a conclusion recently contested.[61,62] Darcy did not believe the plasma cells present in the salivary gland homografts he studied played an important role in their rejection by the host. The anomalous behavior of kidney, salivary gland and splenic homografts in eliciting predominantly plasmocytic reactions is not understood.

The results of the present study indicate that the cellular events leading to homograft rejection are activated days before any signs of distress appear in the graft. The reaction of a graft to its new environment has been observed to begin within minutes after its application by the formation of a cellular exudate in the graft bed.[63] During this period of "plasmatic circulation," the graft vessels are filled with fluid and cells from the host bed.[64] The rate of re-vascularization of skin grafts has long been controversial. Thiersch[65] described the direct connection of host and graft vessels as soon as 18 hours after the application of the graft; Davis and Traub[66] demonstrated that within 22 hours of its application, a skin graft unites with the recipient by capillaries formed within the graft bed which anastomose with pre-existing capillaries in the graft. More recently, however, Scothorne and MacGregor,[67] using a dye injection technic in rabbits, were unable to demonstrate functionally important vascular connections before the third to fourth postoperative day. The studies of Converse and Rapaport[68] in man gave similar results. Few studies have been made of the lymphatic connections between skin grafts and recipients. MacGregor and Conway[69] found that lymph flow begins around the sixth postoperative day in autografts, but never appears in homografts. Presumably, then, antigenic substances diffuse from the transplant via extravascular and extralymphatic routes, since by the third postoperative day cellular changes are beginning to appear in the lymph node draining the graft. This finding of a morphologic activation of lymphoid structures within three days after graft application is consistent with the observation that, when the direct hyper-sensitivity technic is used,[70] transplantation immunity can be detected in guinea pigs as early as two or three days after exposure to the antigen.

Although the draining lymph node consistently undergoes the most profound morphologic changes, it is apparently not essential for the establishment of homograft immunity. Thus, extirpation of that node may affect only slightly the rejection time of skin grafts in mice and rabbits.[37] Hume and Egdahl[71] ingeniously isolated kidney homografts from regional lymph nodes by enclosing them in plastic containers and demonstrated that the rate of rejection of these grafts was no different than that of grafts inserted in the conventional manner. To be sure, the draining lymph nodes may be said to bear the brunt of the initial antigenic assault. On the other hand, the contralateral and other lymphoid masses are soon involved. These findings thus support Medawar's[72] contention that the morphologic activation of the contralateral lymph node is due to the systemic circulation of antigen, and not to a local action secondary to the "leakage" of antigen across the midline. This was shown by the following experiment: 1.0 ml. of Evans blue dye was injected into the left ear of rabbits. Twenty-four, 48, and 72 hours later the animals were killed and both cervical areas were dissected. The dye stained only the lymphatics, lymph

nodes and subcutaneous tissues of the injected side. It was sharply limited in its distribution by the median raphe of the neck and did not diffuse to the contralateral side, even after three days. On the other hand, the possibility is present that some immunologically competent cells may circulate in the blood, be deposited in other lymphoid areas, and proliferate there as well without reference to antigen.

The controversy as to whether the second set response represents an anamnestic reaction as exemplified by animals immunized with protein antigens or whether it indicates a pre-existing, sustained type of sensitization provoked by the first graft has been cogently summarized by Hildemann,[73] Brent and Medawar[74] and Kabat.[75] Our studies would be consistent with the first concept since they demonstrated the sudden mobilization of apparently normal lymphoid structures by the advent of the second graft. The morphologic features we observed in the lymph nodes and spleen were almost identical to those which occur following a second injection of purified antigens or bacteria. Steinmuller recently showed that under certain circumstances a true secondary response to transplantation antigens can be elicited in mice.[76]

It is clear from these studies that the morphologic indications of transplantation immunity, which begin as an almost exclusively regional effect, eventually develop into a generalized response. The finding of a widespread morphologic reaction to homografts in the lymphoid centers of the recipient may at first seem at variance with Mitchison's[77] findings that only the regional lymph node draining a tumor homograft is capable of transferring immunity to other mice. The draining lymph node cells retain this competence for about two weeks; thereafter, attempts to transfer transplantation immunity with them have failed. However, as Medawar[37] has pointed out, active immunity in the donor mice persisted much longer than the ability of regional nodes to transfer immunity, and the "loss of competence of the regional nodes was (presumably) accompanied by a slow activation of the other lymphoid centers."

This study has demonstrated that the hemocytoblast, which arises following a variety of antigenic stimuli, also appears after the application of homografts. Although this cell undoubtedly plays a central role in the immune response, its exact relation to the actual mechanics of homograft rejection is unclear. In a subsequent paper[78] it will be shown that when the proliferation of this cell is blocked by antimetabolites, homograft rejection either does not occur or is considerably delayed.

Summary

The morphologic responses of the lymphoid system of the rabbit were studied. The results demonstrated: (a) the proliferation of a characteristic cell, the "hemocytoblast", within lymphoid centers; (b) architectural changes in lymph nodes and spleen, at first characterized by enlargement of follicles and germinal centers, later by effacement of normal landmarks and finally by reconstitution of lymphatic structures; (c) a barely perceptible plasmocytic response, especially in the first set reaction.

These changes, although most pronounced in the lymph node proximal to the homograft, were also found later in distant lymph nodes and in the spleen.

They arose more rapidly, were of greater intensity and were more persistent in the second set reaction than in the first set reaction. These findings indicated that transplantation immunity eventually invokes generalized morphologic changes in the lymphoid system. The events which followed a second set graft suggested that the second set phenomenon had the features of an anamnestic response.

SUMMARIO IN INTERLINGUA

Esseva studiate le responsas morphologic del systema lymphoide del conilio. Le resultatos provava le occurrentia de (a) le proliferation de un cellula characteristic, le "hemocytoblasto," intra le centros lymphoide, (b) alterationes architectural in le nodos lymphatic e in le splen, characterisate initialmente per un allargamento de folliculos e centros germinal, subsequentemente per le oblitteration del marcas normal de recognition, e finalmente per le reconstitution de structuras lymphatic, e (c) un responsa plasmocytic de intensitate difficilmente perceptibile e occurrente specialmente in le reaction a prime graffos.

Iste alterationes esseva le plus pronunciate in le nodo lymphatic proximal al homograffo, sed plus tarde illos esseva etiam trovate in distante nodos lymphatic e in le splen. Illos se formava plus rapidemente, habeva un plus alte intensitate, e esseva plus persistente in le reaction a secunde graffos que in le reaction a prime graffos. Iste constatationes indica que le immunitate de transplantation evoca in le curso del tempore un serie de generalisate alterationes morphologic in le systema lymphoid. Le evenimentos que sequeva un secunde graffo suggereva que le phenomeno del reaction a secunde graffos ha le characteristicas de un responsa anamnestic.

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