

THE ROLE OF THE LYMPHATIC SYSTEM IN THE REJECTION OF
HOMOGRAFTS: A STUDY OF LYMPH FROM RENAL
TRANSPLANTS

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Studies on transplantation immunity have usually involved various model systems, based on either skin or renal homografts. While it is probable that a common immunological mechanism underlies the rejection of all foreign grafts, the manifestations of the rejection process may differ according to the nature and function of the transplanted organ. There is, in fact, some evidence in the literature which suggests that solid organ grafts are rejected in a different way from skin grafts.

In the case of skin homografts, it seems certain that an intact lymphatic drainage from the graft is crucial for sensitization of the host to occur. In areas such as the anterior chamber of the eye, the central nervous system, and the hamster cheek pouch, where there is no conventional lymphatic drainage, homografts can be installed and will grow with impunity (1). Barker and Billingham (2) showed in experiments with guinea pigs, that for host sensitization to occur against orthotopically placed skin grafts, an intact lymphatic drainage had to be present. These findings added further weight to the results of experiments which showed that the temporary disruption of the lymphatic drainage from skin homografts prolonged their survival (3-5).

A finding that seemed at variance with the idea that fixed lymphoid tissue must become involved in graft rejection came from experiments in which lymph draining areas bearing skin homografts was monitored throughout the life of the graft. Hall (6) showed that there was no change in the cell population of the afferent lymph draining from a skin homograft on the leg of a sheep during the period the graft was being rejected. Furthermore, there was no cellular response in the regional lymph node until late in the life of the graft, when the rejection process was already well advanced. This finding supported the proposition that sensitization of the host against the skin graft occurred centrally within the graft itself.

In the case of renal homografts it seems likely that intact lymphatic pathways between the graft and host are *not* necessary for the initiation of rejection (7-9). In experiments with isolated, perfused rat kidneys, Strober and Gowans (10) showed that when allogeneic lymphocytes were perfused through an isolated kidney for varying periods of time, they were capable, when transferred to syngeneic animals, of sensitizing them so that they gave second-set reactions against skin grafts from the same strain of animals as supplied the perfused kidney. In these studies, it appeared that sensitization was induced by intravascular contacts between host lymphoid cells and graft tissue without the involvement of the lymphatic system.

The migration of lymphocytes through normal tissues is occurring continually and, during a variety of pathological changes involving inflammatory and immunological responses, large increases can occur in this cellular traffic (11). The failure by Hall (6) to detect any significant changes in the cell population of afferent lymph draining from a skin graft cannot be taken as conclusive evidence that no such changes occur, for the amount of lymph produced in a small skin graft would be miniscule, and any cellular changes occurring in the lymph from this area could easily be masked by the large amount of lymph formed in the surrounding tissues of the host.

In order to study the cellular traffic through a homograft, a method was required whereby quantitative collections could be made of all the lymph formed in a grafted organ. Additionally, the fate of the cells passing through the graft and their subsequent interaction with fixed lymphoid tissues required investigation. If the events occurring within the graft tissue, lymph nodes, and within the blood vascular system could be monitored continuously throughout the life of the graft, it should be possible to obtain a more complete account of the history of graft rejection. An immune response in an antigenically stimulated lymph node can be followed closely by studying the changes that occur in the efferent lymph draining from the node (12, 13), and it was thought that by monitoring lymph coming exclusively from a homograft, the sequence of immunological events occurring within the graft itself would be revealed.

McIntosh and Morris¹ have defined the distribution of the lymphatic drainage in the sheep's kidney and shown that it occurs entirely by way of hilar lymphatics. Because of this anatomical advantage we decided to examine, in detail, the role that the lymphatic system and lymphoid cells played in the rejection of kidney homografts. What we set out to do was to effect kidney transplants with their renal lymphatics intact and, as soon as the vascular connections were established, to prepare chronic renal lymphatic and ureteral fistulae to drain the renal lymph and urine outside the body. At the same time, by establishing chronic lymphatic fistulae in other appropriate lymph nodes, reactions occurring in the regional lymph node where the graft was located could be studied, as well as any reactions in nodes remote from the transplant site.

By transferring cells that have migrated through the transplanted kidney into other lymph nodes, the subsequent fate of these cells could be followed. These studies were combined with an examination of the histopathology of the homograft to give an integrated picture of the process of renal graft rejection as it occurs in the graft and in the lymphatic system of the host.

Materials and Methods

Animals and Surgical Procedure

Animals.—Merino ewes between 2½ and 6 years of age were used for the experiments. They were housed indoors and fed lucerne chaff and grain oats ad lib.

The Lymphatic Drainage of the Kidney.—The lymph formed in the sheep's kidney is drained

¹ McIntosh, G. H., and B. Morris. The lymphatics of the kidney and the formation of renal lymph. Manuscript submitted for publication.

entirely by 2-5 hilar lymphatic ducts varying in diameter from 0.5-1.5 mm. There are abundant anastomoses between these ducts, and quantitative collections of renal lymph can be obtained by cannulating one vessel and tying off the others. The lymph thus collected, is afferent lymph, lymph that has not passed through any area of fixed lymphoid tissue.

Preparation of Donor Kidney.—The left kidney of the donor ewe was mobilized to allow access to the renal vein and artery. The ureter was cut approximately 5 cm from the renal pelvis and cannulated with polyethylene tubing of appropriate diameter. The renal vessels were freed from the surrounding tissue by blunt dissection, and any small tributaries of the renal vein were ligated and cut. The renal node was identified near the lateral aspect of the aorta and a ligature passed around it to tie off the lymphatics where they entered the capsular sinus. The ducts were dissected off the node and a careful search made for any anomalous lymphatics that might be carrying renal lymph. If any were found, they were ligated. Care was taken to leave the renal lymphatics adherent to the renal blood vessels, as this minimized trauma to them during transplantation. In some cases, the renal node was left attached to the afferent ducts and removed after transplantation. The renal artery was transected at or above the level of the renal node and cannulated with polyethylene tubing; the renal vein was transected as close to the vena cava as possible. As soon as the renal artery was cannulated, the kidney was perfused with 100-200 ml of a 0.9% NaCl solution to which heparin (Pularin, Evans Medical Ltd., Liverpool, England) had been added.

A site in the left lateral aspect in the neck of the recipient sheep was prepared to receive the transplanted kidney. The kidney was transplanted by joining the renal artery and the carotid artery with stainless steel rings (Nakayama, 4 mm, animal, Senko Medical Instruments Manufacturing Co., Tokyo, Japan), and the renal vein and the jugular vein with continuous 6-0 silk sutures. Both anastomoses were end to end. As soon as the blood flow through the transplanted kidney was established, one of the renal lymphatics was cannulated with polyethylene or polyvinyl tubing; the remaining lymphatics were left tied off. The cannula was tied in at several points with at least one ligature positioned near its tip. The renal lymphatic and the ureteral cannulae were then led out through small stab wounds in the skin. The kidney was next positioned under the brachiocephalic muscle and sutures placed through the margin of the muscle to immobilize the kidney and prevent it from being displaced. The cannulae were secured at the skin with purse string sutures, and plastic tabs were sutured to the ewe to hold the bottles for collecting lymph and urine (Fig. 1). The recipient's own kidneys were left intact. The animals were given antibiotics for 3-5 days after surgery, and were kept heparinized for the first 18 hr.

Cannulation of Other Lymphatics.—In most experiments lymphatics draining other lymph nodes were also cannulated. The lymphatic drainage from the area where the graft was settled was directed to the left prescapular lymph node. The prescapular efferent lymphatic was cannulated to follow any changes that may occur in this node in response to material escaping through the capsule of the transplanted kidney. The efferent lymphatics of the prefemoral and the popliteal lymph nodes were also cannulated in various experiments. Except for the prescapular node, the surgical techniques used for these operations have been described previously (12). The prescapular node of the sheep is situated anterior to and slightly above the level of the shoulder joint. It is covered by the omotransverse and brachiocephalic muscles and is enclosed in a palpable fat pad. Usually one or two large efferent ducts leave the node and run ventrally in the fatty tissue under the brachiocephalic muscle close to the blood vessels supplying the node. The efferent duct was cannulated about 2 cm from the node and the cannula led out ventrally through the skin (Fig. 1).

FIG. 1. A merino ewe with a renal autograft in the neck and a chronic fistula in the efferent lymphatic of the prescapular lymph node. The two smaller bottles are collecting the lymph, the larger bottle contains urine. Photograph taken 2 wk after operation.



The timing of these several operations varied, sometimes the efferent lymphatics of the several lymph nodes were cannulated a few days before the kidney was transplanted, while on other occasions these lymphatics were cannulated at the same time as the transplant operation.

Analytical Procedures

Collection of Lymph and Cell Counting.—Lymph and urine specimens were collected continuously in polyethylene bottles containing a small amount of powdered heparin (Evans) and penicillin (Crystapen, Glaxo-Allenbury's Proprietary Ltd., Melbourne and Sydney, Australia). Cell counts were done on an electronic cell counter (Coulter model B) and differential cell counts were made on Leishman stained smears. When hourly lymph samples were collected, a fraction collector was used.

Antibody Determinations.—Lymphocyte-agglutinating antibody titers were determined by a modification of the capillary tube migration test (14). Lymphocytes were obtained for the test from fistulae in the efferent lymphatics of the popliteal nodes of the kidney donor. The lymphocytes were suspended in homologous lymph at a concentration of 1×10^7 /ml, and serial two-fold dilutions of serum or lymph were incubated with an equal volume of the suspension of donor lymphocytes for 10 min. Samples were then drawn up in 25 microliter microcapillary tubes, and one end of the tube was sealed in a flame. The microcapillary tubes were centrifuged at 3000 rpm for 1 min, and then inverted at an angle of 45 degrees for 30 min. The end point of the titration was designated at the last tube in which there was significant inhibition of migration of the lymphocytes.

Lymphocyte toxicity tests were done by trypan blue dye exclusion tests (15).

Protein Determinations.—Protein concentrations were measured in samples of lymph and blood plasma by micro-Kjeldahl digestion and titration or by the biuret reaction. Globulins were fractionated on a column of Sephadex G-200.

Radioautography.—Cells were incubated for 1 hr in Eagle's medium containing $1.0 \mu\text{Ci/ml}$ of ^3H -thymidine. The cells were washed 3 times in Hanks' solution, resuspended in sheep plasma, and smears prepared in the conventional way. The cell smears were coated with Kodak AR-10 stripping film, exposed for 5 days and then developed. After the development, radioautographs were stained with Azure-A.

Electron Microscopy.—Freshly collected cells were fixed in 1.25% glutaraldehyde in Tyrode's solution for 30 min at 4°C . Sections of kidney were fixed overnight in 1.25% glutaraldehyde in Tyrode's solution. Tissues and cells were washed in cold Tyrode's solution, and postfixed in 1% osmium tetroxide in 50% Tyrode's for 2 hr, and in 1% formaldehyde in Ca^{++} -free Tyrode's solution for 30 min. The specimens were stained in 1% aqueous uranyl acetate for 2 hr, dehydrated in acetone and embedded in Durcupan (Fluka AG, Basel, Switzerland). Grids were cut on an LKB microtome with a diamond knife and stained with lead citrate (16). Sections were examined in a Philips model EMU 200 electron microscope.

Lymphocyte Cultures.—Lymphocytes were cultured in modified Millipore chambers (17) placed on the chorioallantoic membrane of 11-day chicken embryos. A suspension of 5×10^6 cells in 0.1 ml homologous lymph was placed in each chamber, and the cultures were maintained for 3 days at 37°C .

RESULTS

The Renal Autograft.—Preliminary experiments were done to study the changes that occurred in renal lymph as a result of transferring the animal's own kidney from its normal site into the neck, where the renal homografts were subsequently to be established. Four animals were studied in this way, and pro-

vided that the surgical technique was adequate, the autografts presented no problems and functioned in an essentially normal manner (Fig. 2). There was a temporary rise in the red cell and the protein content of the renal lymph and a transient influx of polymorphonuclear neutrophils for the first 1-2 days after

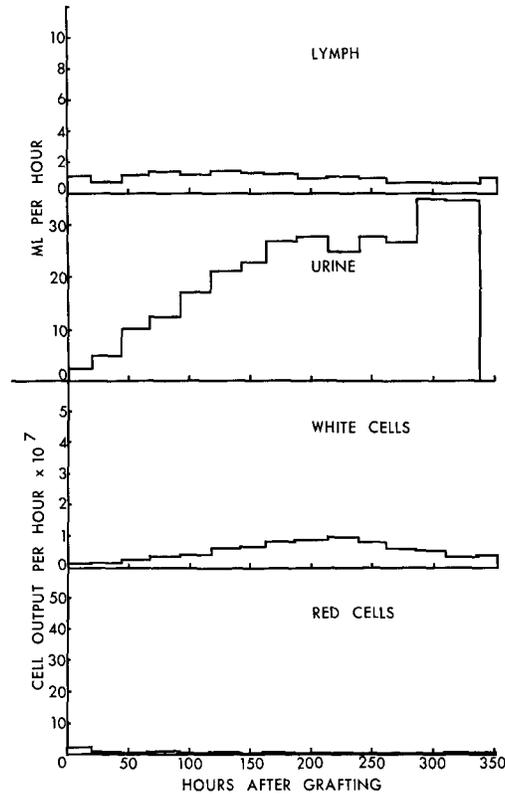


FIG. 2. The rate of lymph flow, the output of white cells and red cells in the lymph, and the urine production of an uncompromised renal autograft. Urine production reached normal levels soon after grafting, while the rate of lymph flow, and the white cell and the red cell content of the renal lymph, remained fairly constant during the period of observation.

the operation. Once these initial effects had passed, the only significant change from normal in the renal lymph of the autografted kidney, was in increase in the total number of lymphocytes (Table I). Never at any stage in the history of the autograft did these cells show any evidence of transformation and blast cells were rarely seen.

In contrast to the three autografted kidneys that were transplanted efficiently, one kidney underwent a prolonged period of anoxia due to a poor surgi-

cal performance, and the subsequent events showed the effects of this incompetence (Fig. 3). Initially, there was a rapid rise in the renal lymph flow rate and in the cell output in the lymph. Red cells were present in large numbers in the lymph while urine production was scanty for the first 5 days. The flow rate, total cell output, and numbers of red cells in the renal lymph decreased to the levels found in uncomplicated autografts by 225–250 hr after the operation and, from this time onwards, the kidney functioned normally. The large volume of lymph draining from this transplanted kidney during the early postoperative

TABLE I
*Characteristics of Lymph from Renal Autografts Transplanted into the Necks of Sheep**

Lymph determinations	Kidney (in situ)	Kidney (autograft)
Lymph flow rate (ml/hr)	0.5–3.0	0.9–3.2
Lymph total protein (g/100 ml)	2.80	3.82 mean‡
Lymph, albumin: globulin	1.35	1.39
Ratio of lymph protein to plasma protein	0.43	0.60
White cells/ml $\times 10^6$	0.2–0.5	1.95–5.73
Differential:		
Lymphocytes	90%	90–99%
Macrophages	10%	1–10%
Polymorphonuclear cells	rare	rare§
Red cells/ml $\times 10^6$	variable	2.08–10.6

* The results are compared with renal lymph collected from kidney lymphatics cannulated *in situ*.

‡ Postsurgical levels approach blood protein concentrations. Levels fall to near mean levels within several days.

§ There is a large transient influx of neutrophils for the first 6–18 hr after grafting.

|| Red cells are present in larger numbers immediately after surgery, but decrease rapidly. These figures relate to the situation after the kidney is functioning normally.

period made it evident that adequate lymphatic drainage was important in reducing the edema of the kidney that is an inevitable consequence of transplantation.

The Renal Homograft.—For the first 24–48 hr the homografted kidney behaved in the same way as the autograft. After this time, however, significant changes occurred in the renal lymph and urine production became scanty. The cell output in the renal lymph usually began to increase by 24 hr and lymph formation began to increase by 48 hr (Fig. 4). The extent of the subsequent changes varied from graft to graft (Table II). The total production of renal lymph over the periods during which the grafts survived was between 5 and 10 liters and the maximum flow rates recorded were up to 60 ml/hr. This copious

volume of lymph formed in the capillary bed of the grafted kidney and was drained off through the tiny renal hilar lymphatics.

The Protein Content of Lymph from Homografts.—In all cases, the protein content of the renal lymph was high immediately after the graft was established.

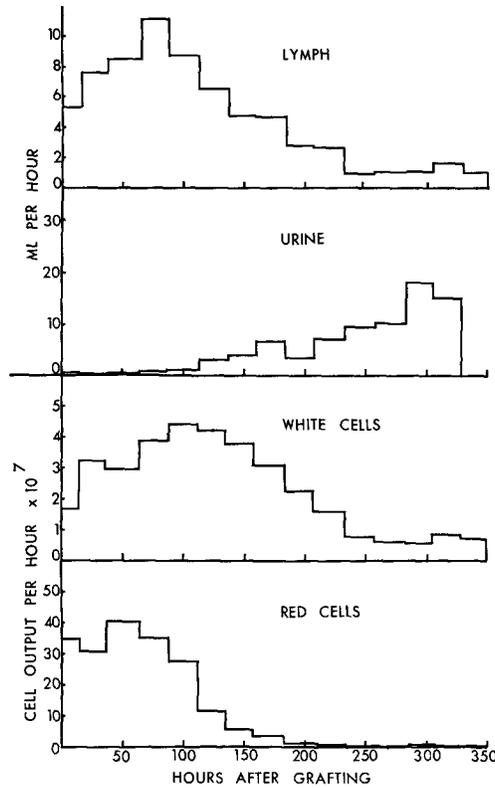


FIG. 3. The rate of lymph flow, the output of white cells and red cells in the lymph, and the urine production of a renal autograft which underwent a period of prolonged anoxia before revascularization. Little urine was produced for the first 100 hr after grafting, whereas the production of lymph, and the output of cells in the lymph were greatly increased.

This high protein content together with the large numbers of red cells in the lymph was probably due to an increased permeability of the renal blood capillaries as a result of the anoxia incurred during the transplant. After the first 1-2 days, the levels of protein in the lymph fell progressively (Table III). The protein content fell as the rate of lymph formation increased, indicating that alterations in renal hemodynamics (increased capillary filtration pressure, increased filtration area, reduced reabsorption of water) rather than drastic increases in capillary permeability were responsible for the high rates of lymph production.

This interpretation of the mechanisms involved in the increased rate of lymph formation in the kidney will be considered further when the histopathological changes occurring within the graft are described.

The Cell Content of Lymph from Homografts.— The changes in cell content of the renal lymph were most striking. Cell outputs reached levels of $1-4.8 \times 10^8$

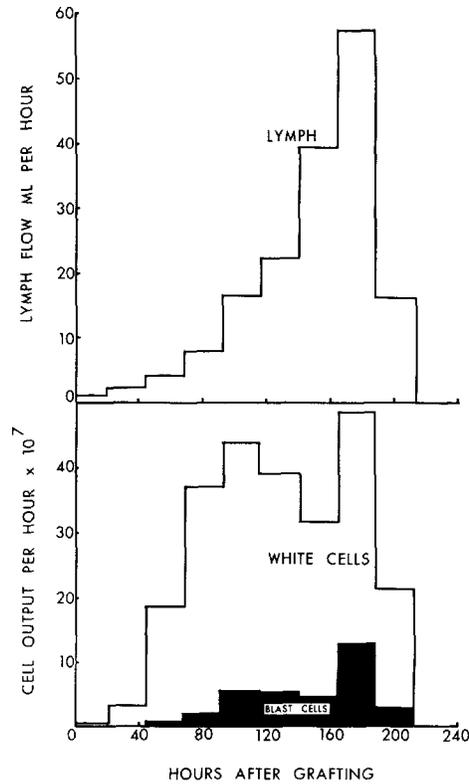


FIG. 4. The rate of lymph flow and the output of white cells from a renal homograft. The final rejection of the graft was taken to occur at 215 hr when lymph flow ceased abruptly.

cells/hr and, over the life of the grafts, the traffic of white cells through the homografted kidney into the renal lymph amounted to between 15 and 60 g wet weight of cells. This compared with a traffic of 100–300 mg wet weight of white cells from the renal autografts during a similar period of time. Thus, the mass of free-floating lymphoid cells leaving the graft via the lymph was of the same order as the weight of lymphoid cells in the spleen; when the cells accumulating in the graft itself were taken into account, the magnitude of the cellular onslaught against the graft became evident.

Most of the cells in the lymph were lymphocytes and transforming lymphoid blast cells. There was usually about 3-10% of macrophages in the lymph throughout the response; this figure indicated that at the peak of the response about 20 million macrophages were passing through the kidney and

TABLE II
*History of the Renal Homografts Transplanted with Their Lymphatic Drainage Intact**

Graft No.	Rejection time†	Maximal cell output	Maximum lymph flow rate
	<i>hr</i>	<i>per × 10⁸</i>	<i>ml/hr</i>
1	212	4.84	57.20
2	188	0.90	9.70
3	192	1.30	7.14
4	113	2.05	12.50
5	183.5	2.56	5.25
6	150.5	2.02	60.00
7	205	2.14	31.50
8	154	2.45	25.00

* The rejection time is judged from the time lymph flow from the kidney ceased. Cell outputs and flow rates are for the lymph from the graft.

† As indicated by a cessation of lymph flow. Urine production often increased abruptly at this time and continued for an additional 24-72 hr.

TABLE III
The Flow Rate and Protein Concentration of Lymph Coming from a Renal Homograft Throughout its Lifetime

Time after grafting	Lymph flow	Plasma protein	Lymph protein		
			Total protein	Albumin	Globulin
<i>hr</i>	<i>ml/hr</i>	<i>g/100 ml</i>	<i>g/100 ml</i>	<i>g/100 ml</i>	<i>g/100 ml</i>
0-20	1.28	6.47	6.12	4.04	2.08
20-44	2.19	—	5.77	2.86	2.91
44-68	4.02	—	5.01	3.72	1.29
68-92	7.80	—	4.89	2.32	2.57
92-116	16.46	—	5.91	2.88	3.03
116-140	22.16	—	5.34	1.78	3.56
140-164	39.38	—	4.77	3.00	1.77
164-188	57.20	—	3.51	1.54	1.97
188-212	16.00	—	3.22	1.12	2.10

entering the lymph each hour. These cells had exuberant cytoplasmic veils and, within their cytoplasm, there were numerous vacuoles containing cell detritus and, on occasions, whole red cells and living lymphocytes. Only rarely were granulocytes seen in the lymph. Between 48 and 72 hr after the graft was established, basophilic lymphoid cells first appeared in the lymph. After this time,

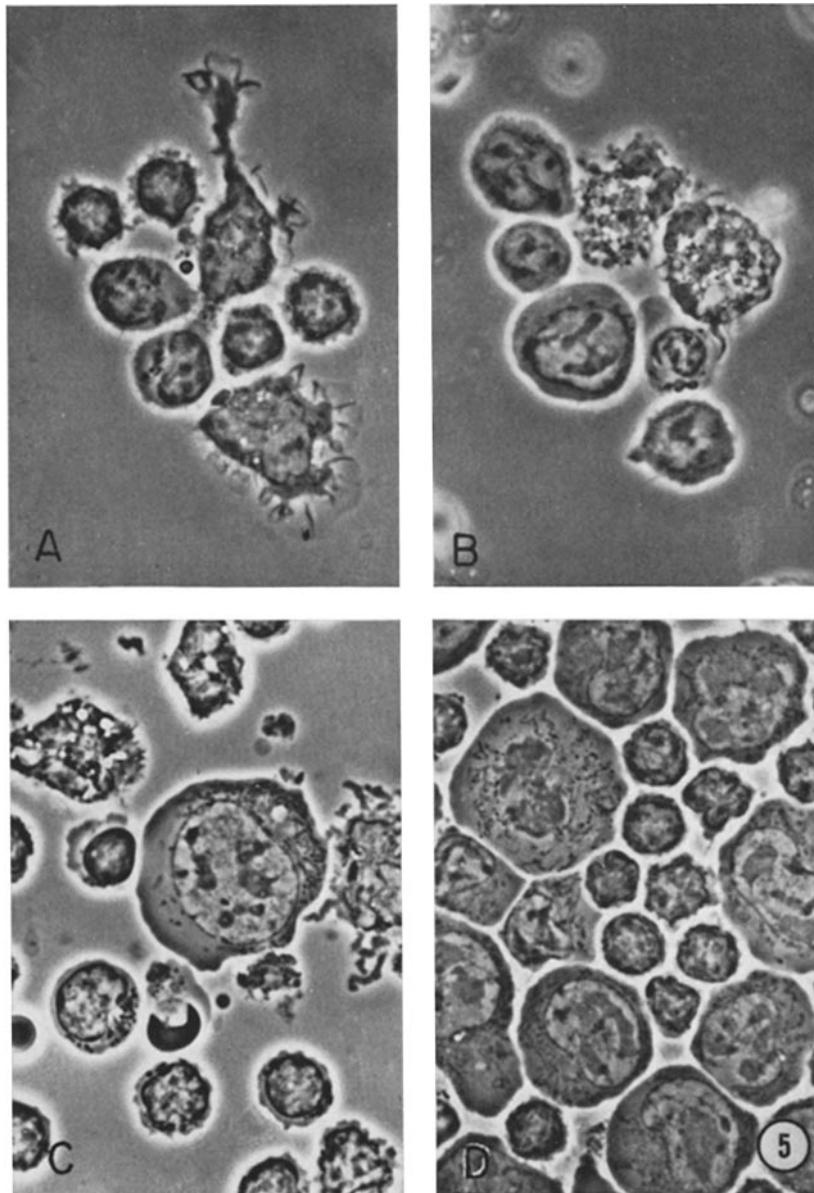


FIG. 5. Phase microscope pictures of the cells in lymph coming from a renal homograft. A. 24 hr after grafting; B. 48 hr after grafting; C. 72 hr after grafting; D. 120 hr after grafting. $\times 1000$.

the proportion of these cells increased rapidly until, at the height of the response, they represented as many as 60% of the total cell population. Examples of the cell population in lymph coming from a grafted kidney are shown in Fig. 5.

Samples of lymph cells examined under the electron microscope at this time showed the proportion of transforming cells was underestimated in the stained smears, for in some samples on which an electron microscope differential count was made, they accounted for 90% of the total cell population. In some samples of lymph collected 24 hr after grafting, lymphocytes with an increased content of polyribosomes could be seen in the electron microscope. This was taken as the first evidence of transformation, and as time progressed, there appeared in the lymph a wide range of cell types, varying from small lymphocytes with mature nuclear structure and some polyribosomes in their cytoplasm, to large, primitive blast cells with immature nuclear chromatin and cytoplasm replete with polyribosomes in a variety of forms. The larger blast cells had enlarged, dilated Golgi vesicles and on occasions, short profiles of endoplasmic reticulum (Fig. 6).

Electron micrographic studies gave the impression that the cells in the lymph were undergoing a progressive differentiation from small lymphocytes to large blast cells. When lymph cells were incubated with ^3H -thymidine, it was found that a portion of the small lymphocytes leaving the kidney between 48 and 72 hr after grafting, was incorporating the label (Fig. 7 D). Shortly after this time, label began to appear in the larger blast cells, so that by 96 hr, the predominant labeling was in these large basophilic cells (Figs. 8 and 7 E).

The labeling pattern differed somewhat from that found with cells coming from antigenically stimulated lymph nodes. Almost all antigen-stimulated blast cells will label when incubated with ^3H -thymidine (13); in the case of cells stimulated within the graft, the proportion of blast cells incorporating label was much smaller (Fig. 8). This indicated either that for many of the cells, differentiation was not necessarily a prelude to cell division, or that the generation time was much slower for the homograft-stimulated cells than for antigen-stimulated cells coming from a lymph node.

An aspect of the life history of the lymphocytes leaving the homograft was studied by cultivating them *in vitro*. During the initial 18–48 hr period after the graft was established, practically no blast cells were present in the lymph. Samples of these cells collected from the lymph early after transplantation were taken and cultivated for 72 hr in Millipore culture chambers. At the end of this time many of the cells were found to have transformed into large basophilic cells and were undergoing active proliferation (Fig. 7 A, B, and C). It seemed fairly certain that these blast cells originated from small lymphocytes initially present in the culture chambers.

Changes Occurring within Renal Homograft.—To correlate the findings in the

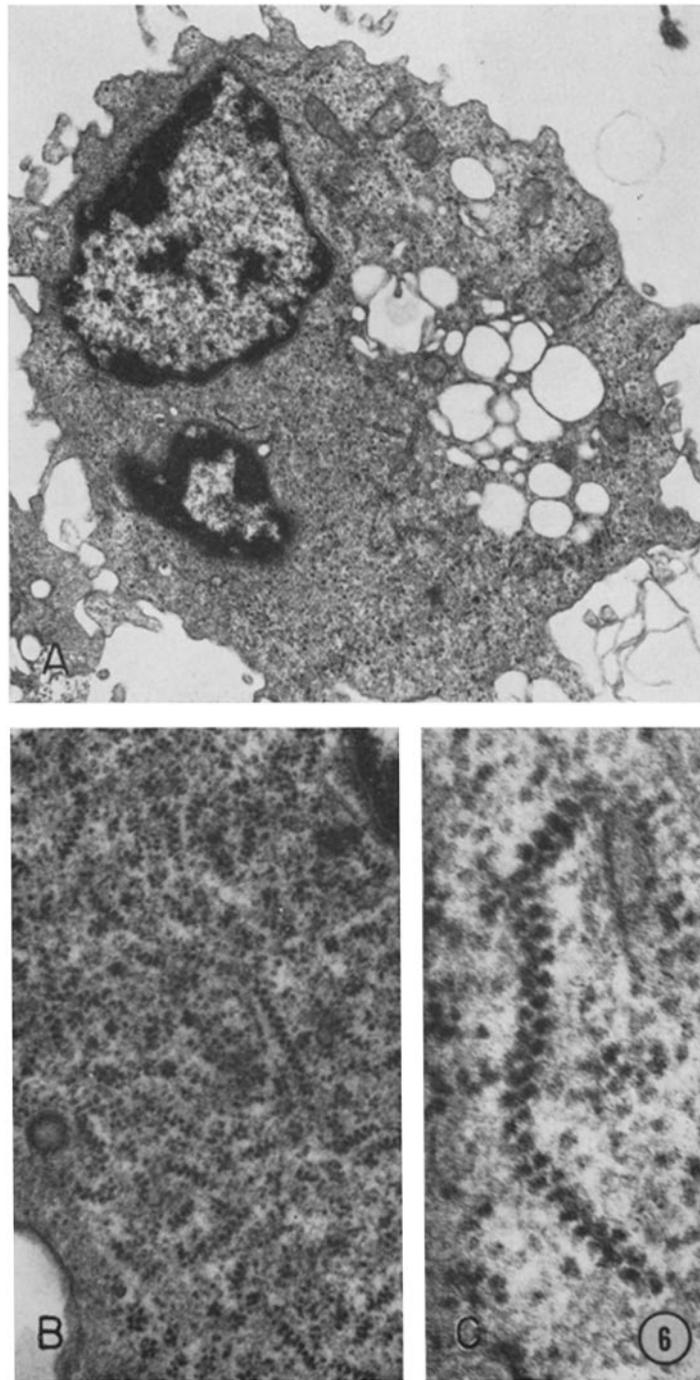


FIG. 6. A. A typical blast cell seen in lymph draining from a renal homograft. The nucleus is primitive in appearance, the Golgi highly dilated, and the cytoplasm is replete with polyribosomes. There is little development of the endoplasmic reticulum. $\times 13,000$. B. The cytoplasm of a blast cell in renal lymph filled with polyribosomes in chains, clusters and rosettes. $\times 44,500$. C. A spiral chain with about 50 ribosomes in the cytoplasm of the blast cell shown in A. $\times 118,000$.

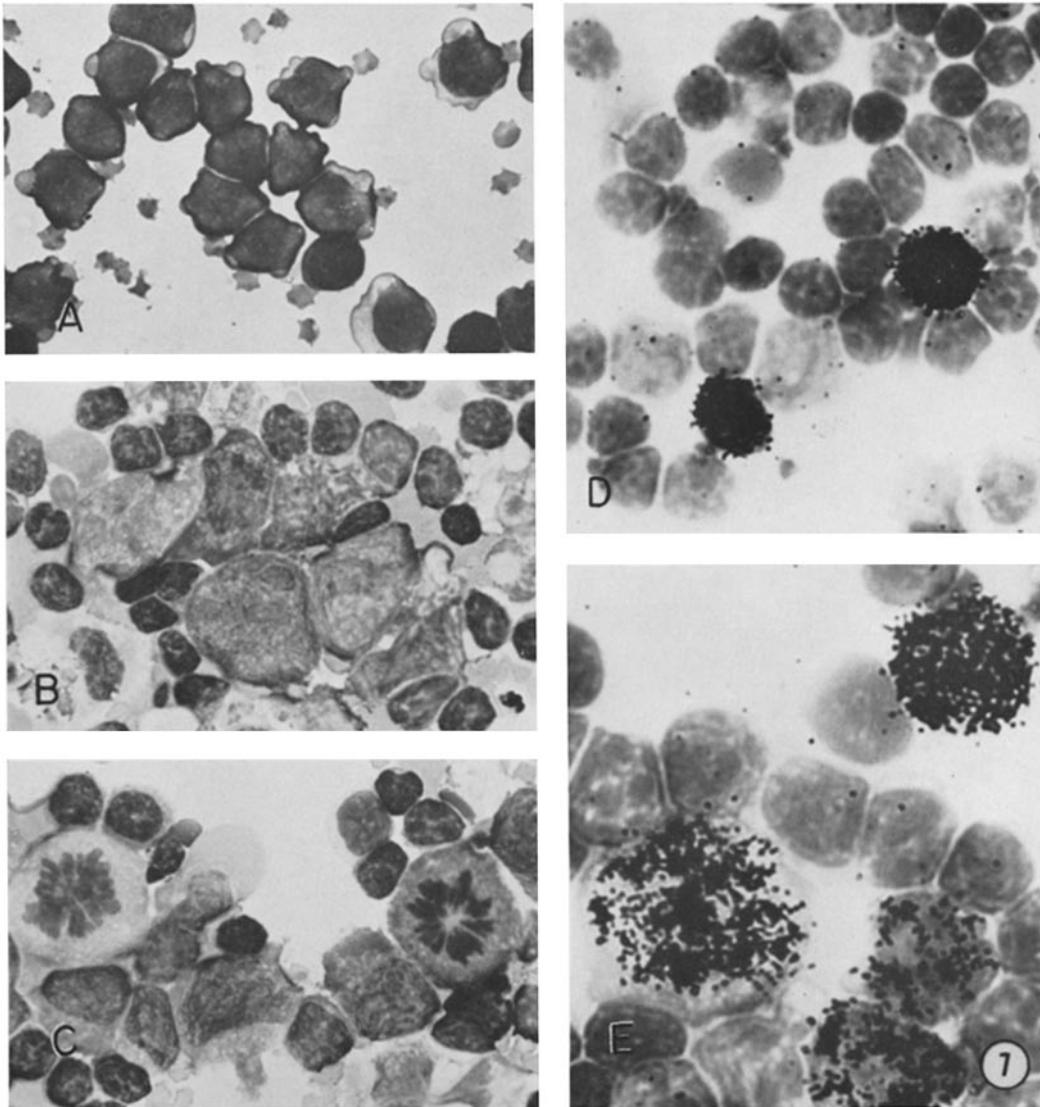


FIG. 7. A. Small lymphocytes in lymph from renal homograft lymph collected between 18-24 hr after grafting. There are no blast cells in the lymph at this time. B and C. The appearance of lymphocytes collected from a renal homograft 18-24 hr after grafting and cultured in vitro for 72 hr. $\times 1100$. D. ^3H -Thymidine labeled cells which were collected from renal homograft lymph 48-72 hr after grafting. Most of the labeled cells are small basophilic lymphocytes. $\times 1100$. E. Cells collected from renal homograft lymph 78-96 hr after grafting. They have been incubated in vitro for 1 hr with ^3H -thymidine. Most of the labeled cells are large basophilic cells. $\times 1400$.

lymph with the events occurring within the grafted kidney, sections were taken from grafts and examined histologically, using both light and electron microscopy. It was apparent that after 4 days, the kidney was invaded throughout the cortex with lymphoid cells; many of these were blast cells, identical with those

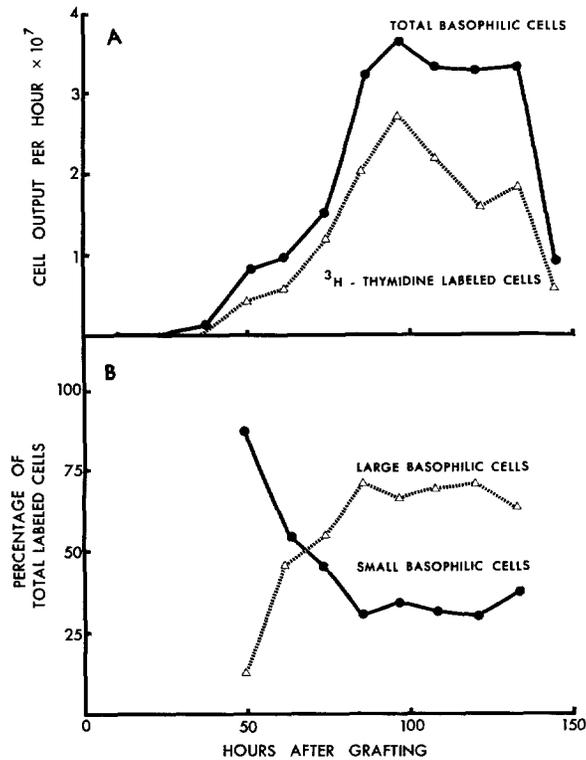


FIG. 8. A. The output of basophilic cells and the output of cells incorporating ^3H -thymidine in the lymph draining from a renal homograft. B. The percentage of small and large basophilic cells in the total ^3H -thymidine labeled cell population in the lymph from a renal homograft. Initially the label was only taken up by small basophilic cells; later most of the labeled cells were large basophilic cells.

cells found in the lymph. Blast cells were present in the interstitial spaces of the kidney and within blood vessels, particularly the peritubular and glomerular blood capillaries. Lymphoid cells were found transforming into blast cells while still within the blood vessels, and on occasions, cells inside blood vessels were found in mitosis (Figs. 9 and 10). These blast cells and lymphocytes often blocked the small blood capillaries partially or completely (Fig. 11). They were frequently attached closely to the vessel walls so that the membranes of the

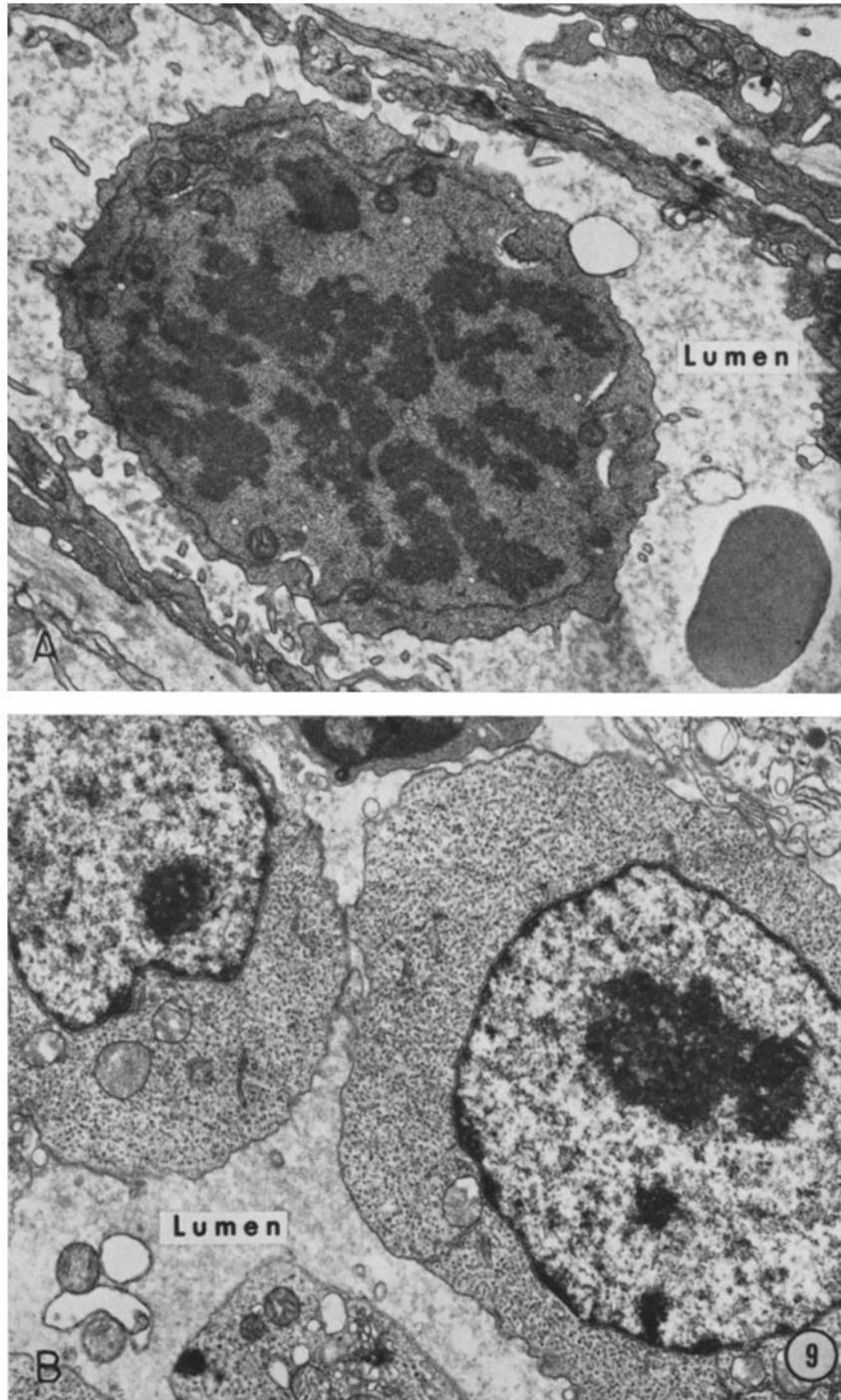


FIG. 9. Renal homograft. 4 days. A. A blast cell in mitosis within the lumen of a peritubular blood capillary. $\times 10,600$. B. Several large primitive blast cells within the lumen of a peritubular blood capillary. The cytoplasm of these cells is full of polyribosomes. $\times 9200$.

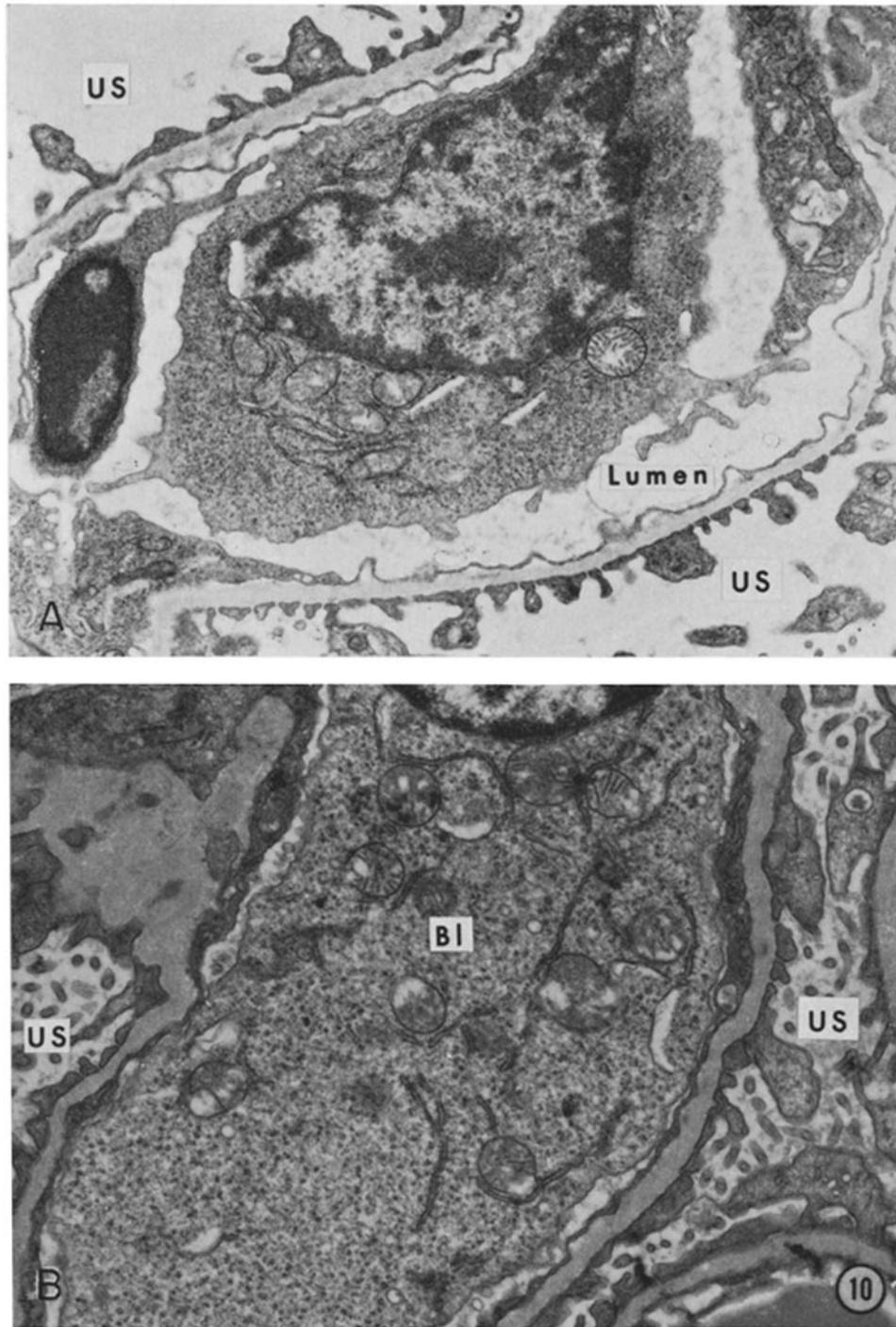


FIG. 10. Renal homograft. 4 days. A. A blast cell and a lymphocyte within the lumen of a glomerular capillary. $\times 12,000$. B. A large blast cell in the lumen of a glomerular capillary. The cell is closely adherent to the endothelium at several points. Bl, blast cell. US, urinary space. $\times 13,000$.

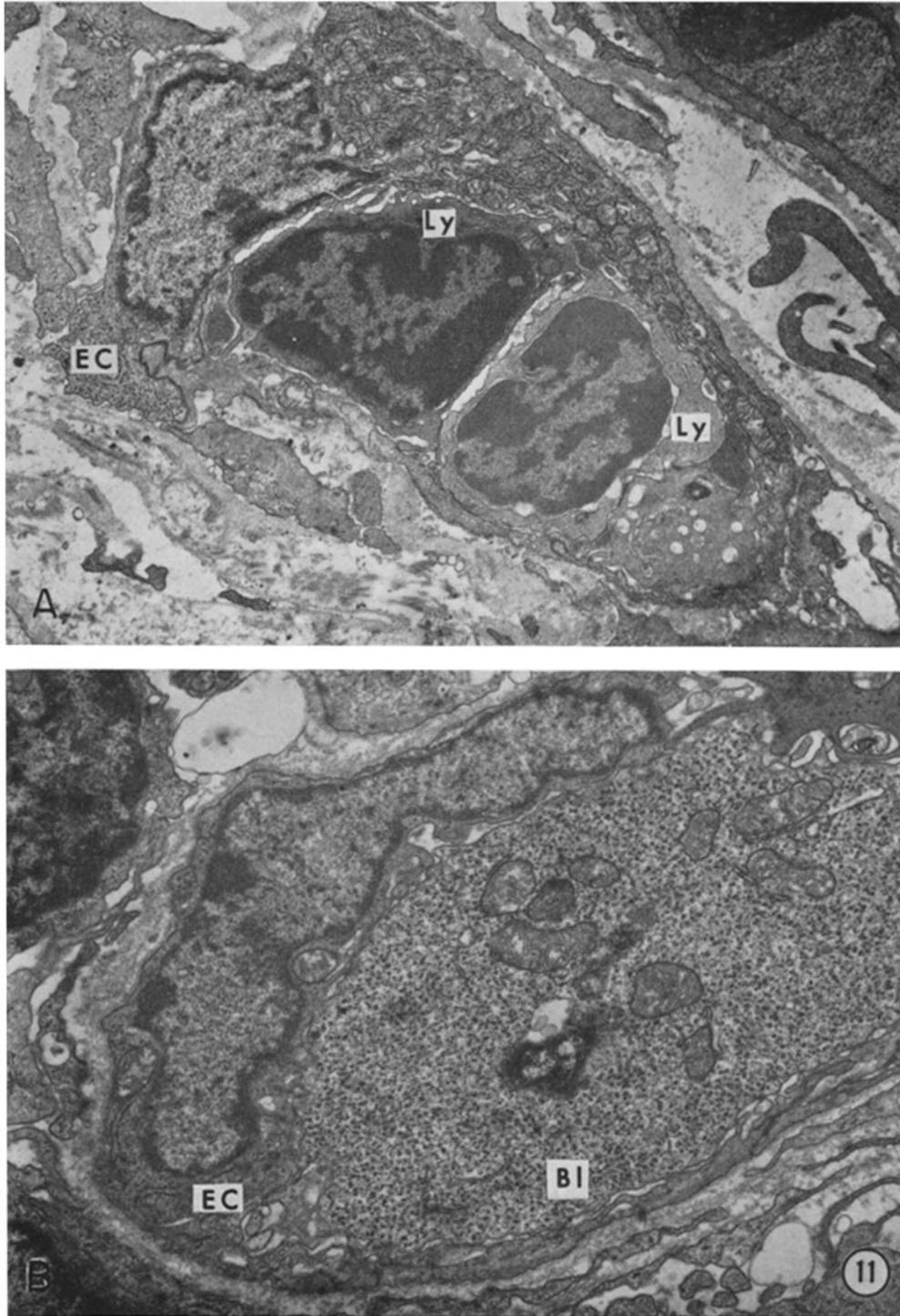


FIG. 11. Renal homografth. 4 days. A. An embolus of lymphocytes (Ly) plugging the lumen of a peritubular capillary. The peritubular endothelium (EC) is showing reactive changes with extensive development of ergastoplasm and polyribosomes. $\times 10,000$. B. A large blast cell (Bl) which is completely filling the lumen of a blood capillary in the cortex of the homografth. The blast cell and the endothelial cell (EC) membranes are closely adherent to one another. $\times 12,800$.

blast cells and the endothelial cells appeared to be fused; on occasions there were interdigitating processes between lymphoid and endothelial cells (Fig. 12). Lymphocytes and blast cells were also found in the process of migrating across the endothelium of the peritubular capillaries and were seen beneath the endothelium and immediately adjacent to it (Fig. 13).

The emboli comprised of transforming blast cells and lymphocytes were not in any way similar to thrombi; there were no polymorphonuclear cells, platelets, or fibrin in these emboli. Throughout the cortex of the kidney they appeared to be, for the most part, plugging capillaries in which the endothelium was still intact. This observation could explain the lack of any massive exudation of protein and red cells in the lymph. Only in areas of advanced tissue damage were emboli found inside vessels with grossly damaged endothelium (Fig. 14). This progressive plugging of the renal capillaries with blast cells would lead to profound changes in the hemodynamics in the kidney and could well have been responsible for an increased filtration pressure in the patent capillaries which would account for the increased lymph production. It appeared that blood flow through the capillaries ceased abruptly with the formation of blast cell emboli; lymph flow in that area would consequently cease before any pronounced changes occurred in capillary permeability.

One of the most striking features of the reaction in the graft was that the endothelial cells of the peritubular capillaries and the glomeruli showed a considerable degree of reactive change. Many endothelial cells appeared with large, rounded, primitive nuclei and there were large numbers of polyribosomes in their cytoplasm. In many cases, they had a considerable development of ergastoplasm which was dilated and filled with secretory products (Fig. 15). Some endothelial cells were also found in mitosis. The interaction between the host lymphoid cells and the donor endothelium appeared to be one of the crucial changes in the homograft. Both these cell lines showed evidence of pathology, as though the interaction was a mutually destructive one.

As well as the extensive infiltration of the kidney tissue with lymphoid blast cells, there were plasma cells and macrophages scattered throughout the interstitium; many of these cells were close to the walls of blood vessels (Fig. 16). In general the renal tubules showed only slight degrees of damage, when compared with the renal blood vessels, and there was little evidence that the host's lymphoid cells were invading these structures. On occasions though, blast cells could be found beneath the basement membrane of the tubular epithelium (Fig. 17), and lymphoid blast cells and macrophages were sometimes found in the urinary space of the Bowman's capsule and in the urine.

Participation of Fixed Lymphoid Tissue of Host in Rejection Process.—Evidence obtained up to this point indicated that sensitization of the host's lymphoid cells occurred in the graft itself; in particular, within the blood vessels of the graft. The final thing to decide was the role of the lymph nodes of the host in

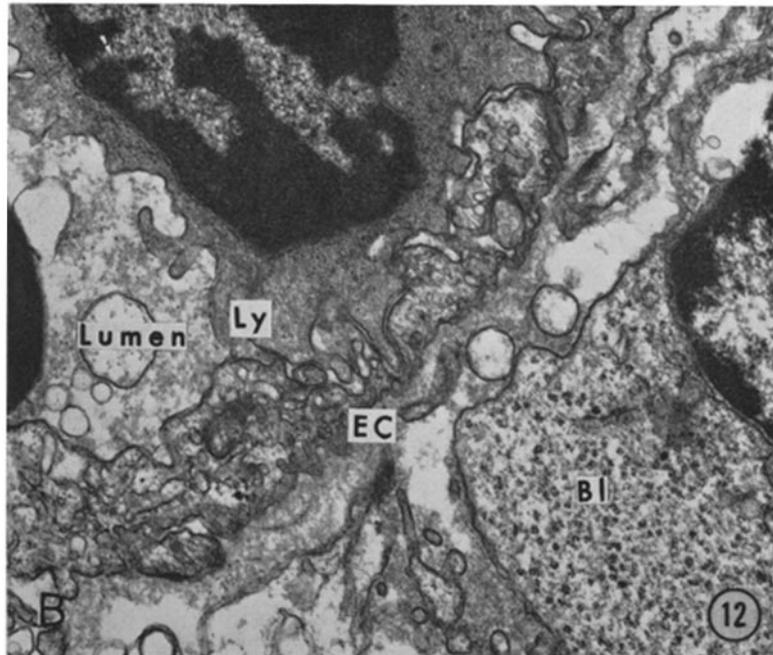
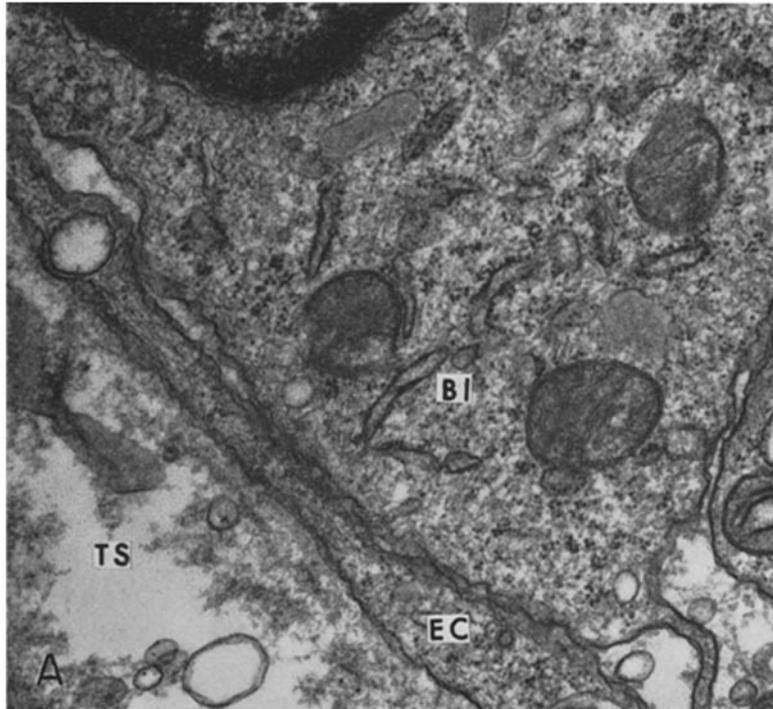
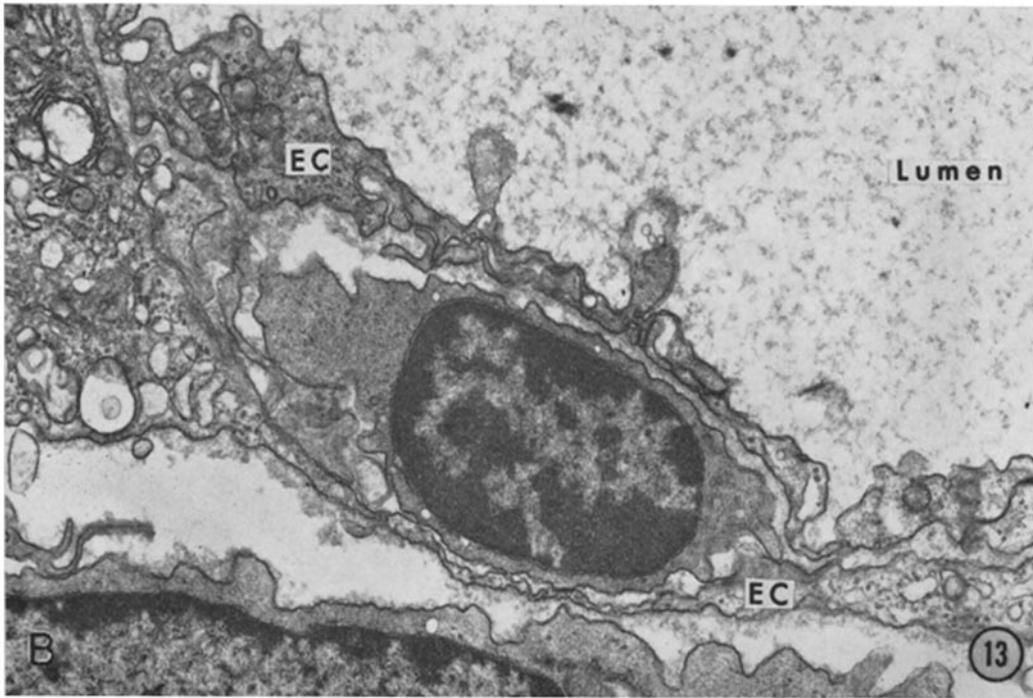
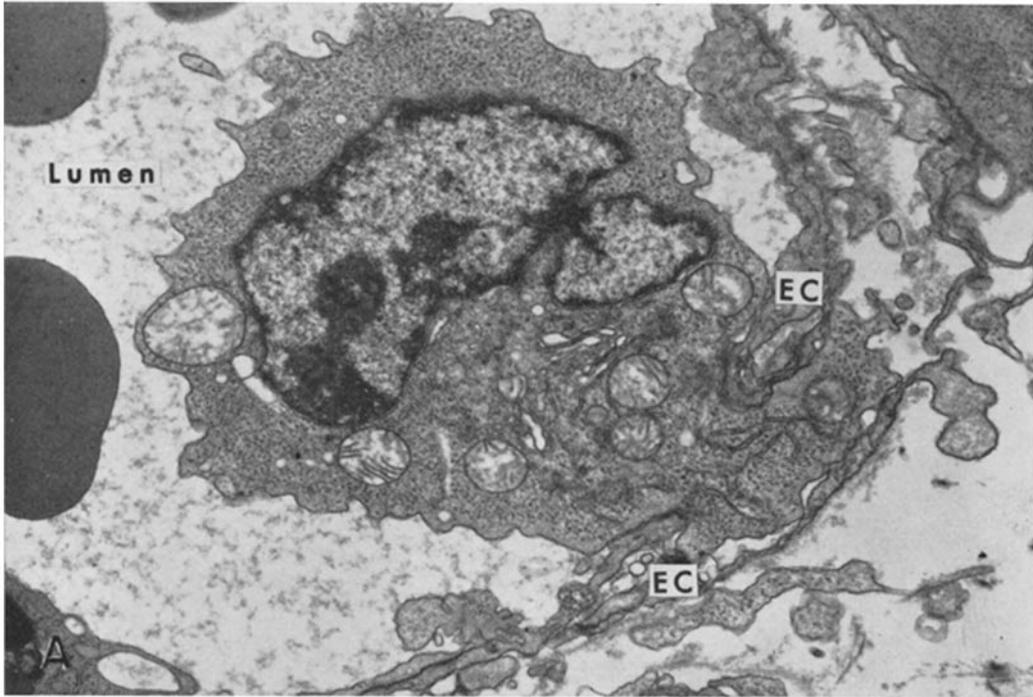


FIG. 12. Renal homograft. 4 days. A. A blast cell (Bl) closely adherent to an endothelial cell (EC) in a renal blood capillary. TS, tissue space. $\times 33,500$. B. A lymphocyte (Ly) in a renal blood capillary with interdigitating processes attaching it to the endothelial cell. A large blast cell (Bl) lies outside the blood capillary. $\times 21,000$.



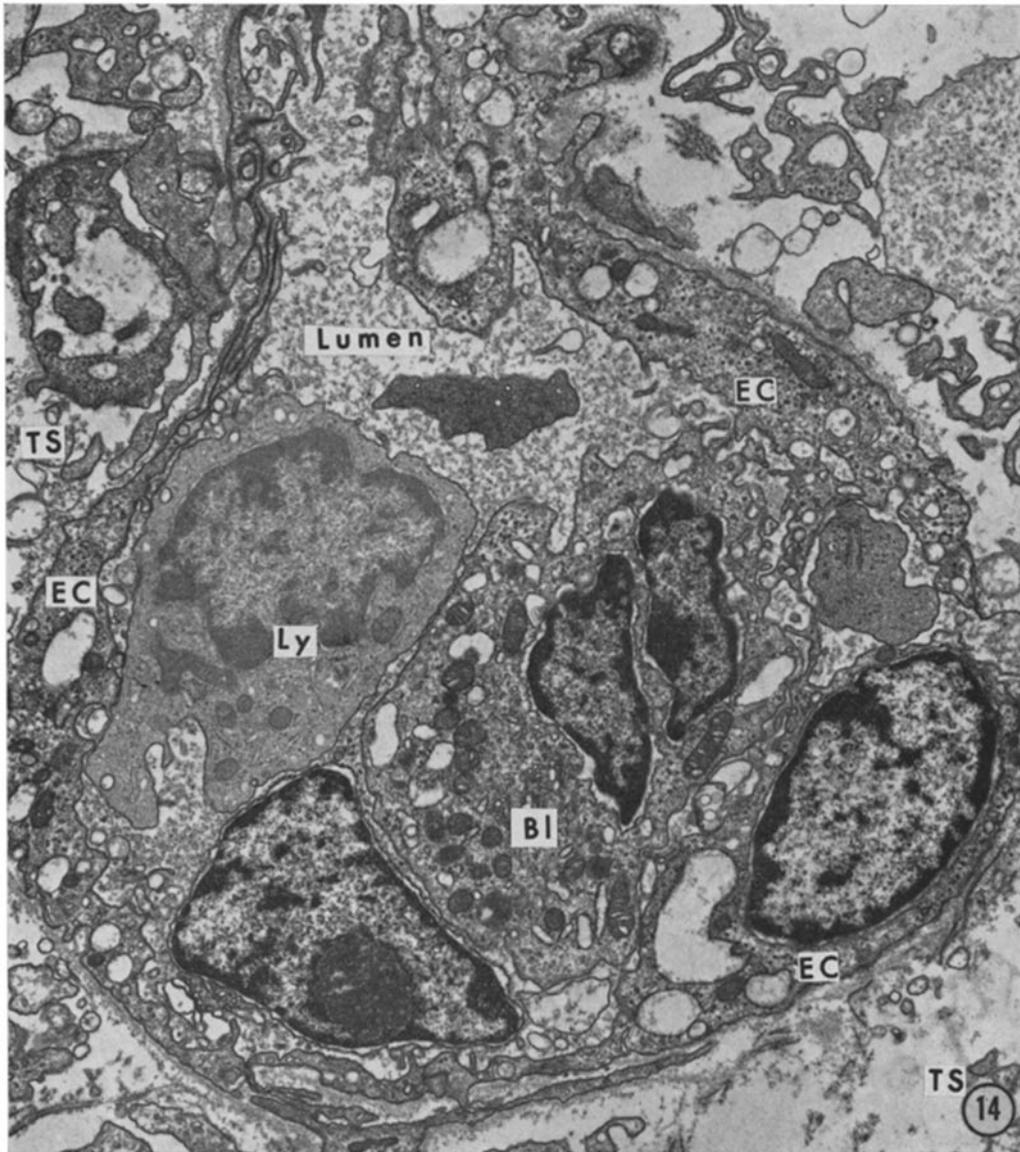


FIG. 13. Renal homograft. 4 days. A. A blast cell seen migrating through a gap in the endothelium (EC) of a renal blood capillary. $\times 12,400$. B. A lymphocyte lying immediately beneath the endothelium (EC) of a renal peritubular blood capillary. $\times 14,500$.

FIG. 14. Renal homograft. 4 days. A group of blast cells (Bl) and lymphocytes (Ly) forming an embolus in the lumen of a renal blood capillary. The cytoplasm of the endothelial cells (EC) and the blast lymphoid cells (Bl) shows degenerative changes with extensive vacuolation. TS, tissue space. $\times 11,500$.

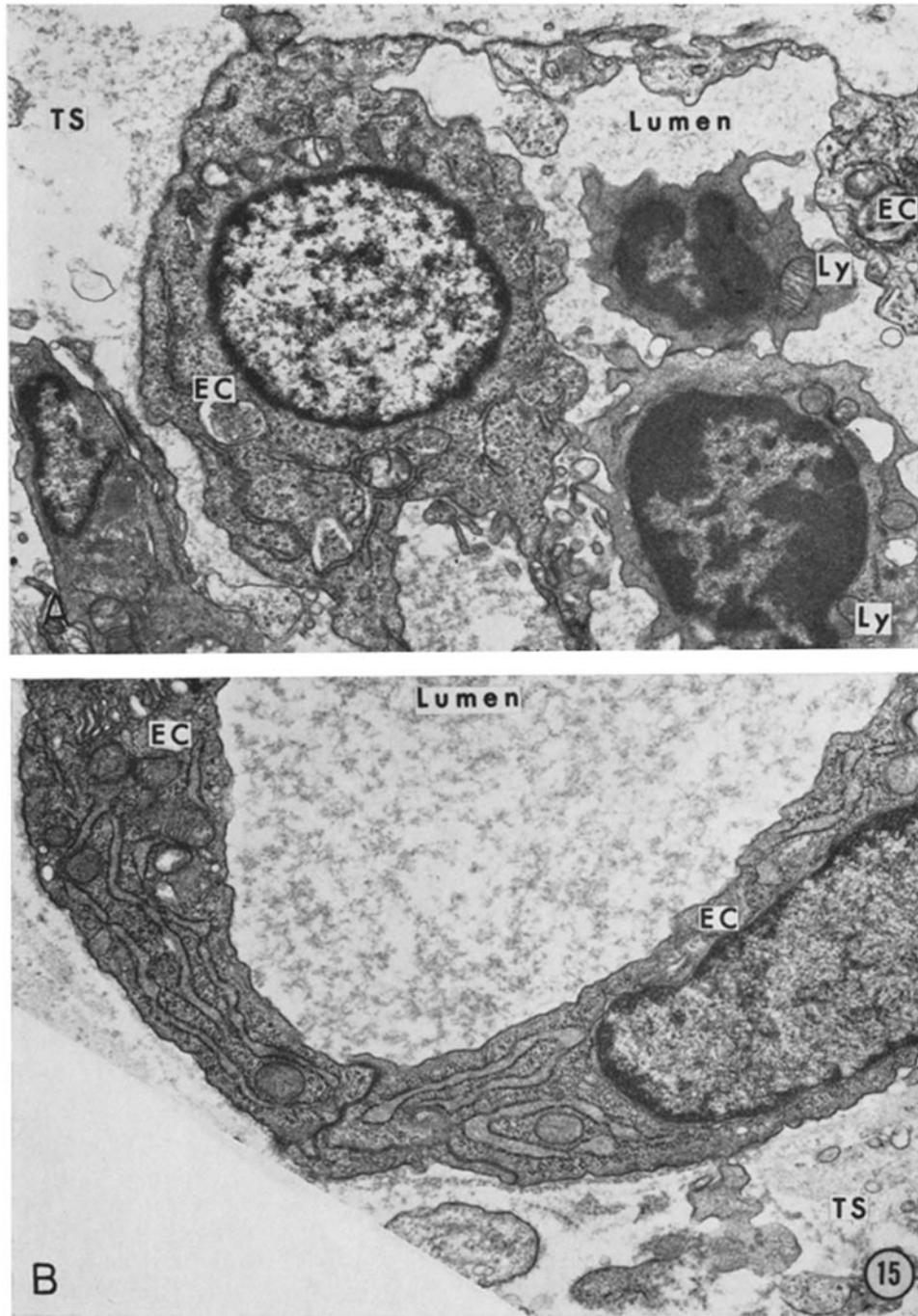


FIG. 15. Renal homograft. 4 days. Reactive changes in endothelial cells in the blood capillaries of a renal homograft. A. The endothelial cell nucleus appears primitive and the cytoplasm is full of ribosomes. $\times 12,300$. B. Extensive ergastoplasmic development in endothelial cells. The cisternae are dilated and contain secretory material. EC, endothelial cell. Ly, lymphocyte. TS, tissue space. $\times 20,800$.

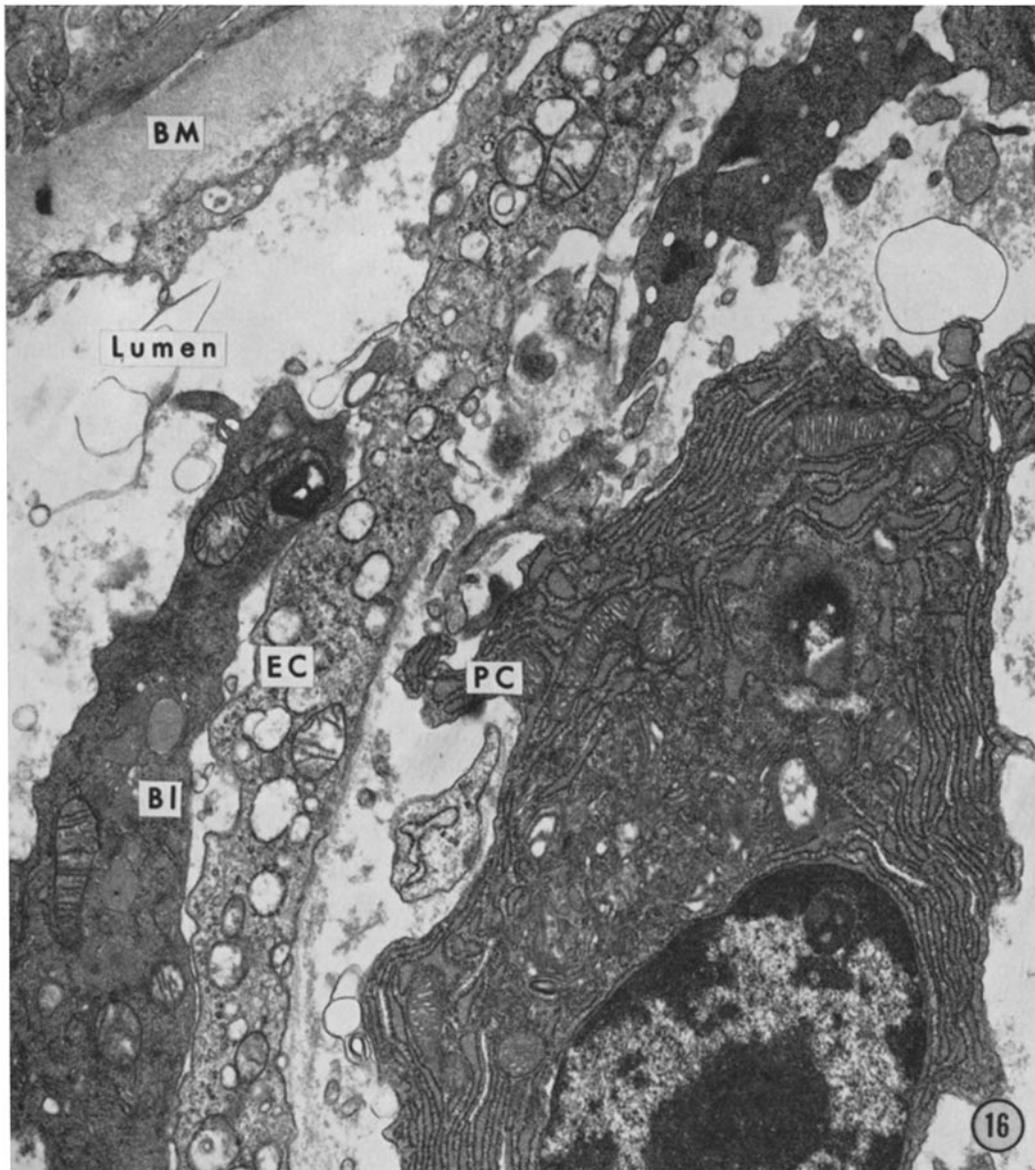


FIG. 16. Renal homograft. 4 days. A plasma cell (PC) immediately outside the endothelium (EC) of a renal peritubular capillary. A blast lymphoid cell (BL) is inside the capillary and adherent to the endothelium. BM, basement membrane of the renal tubule. $\times 15,800$.

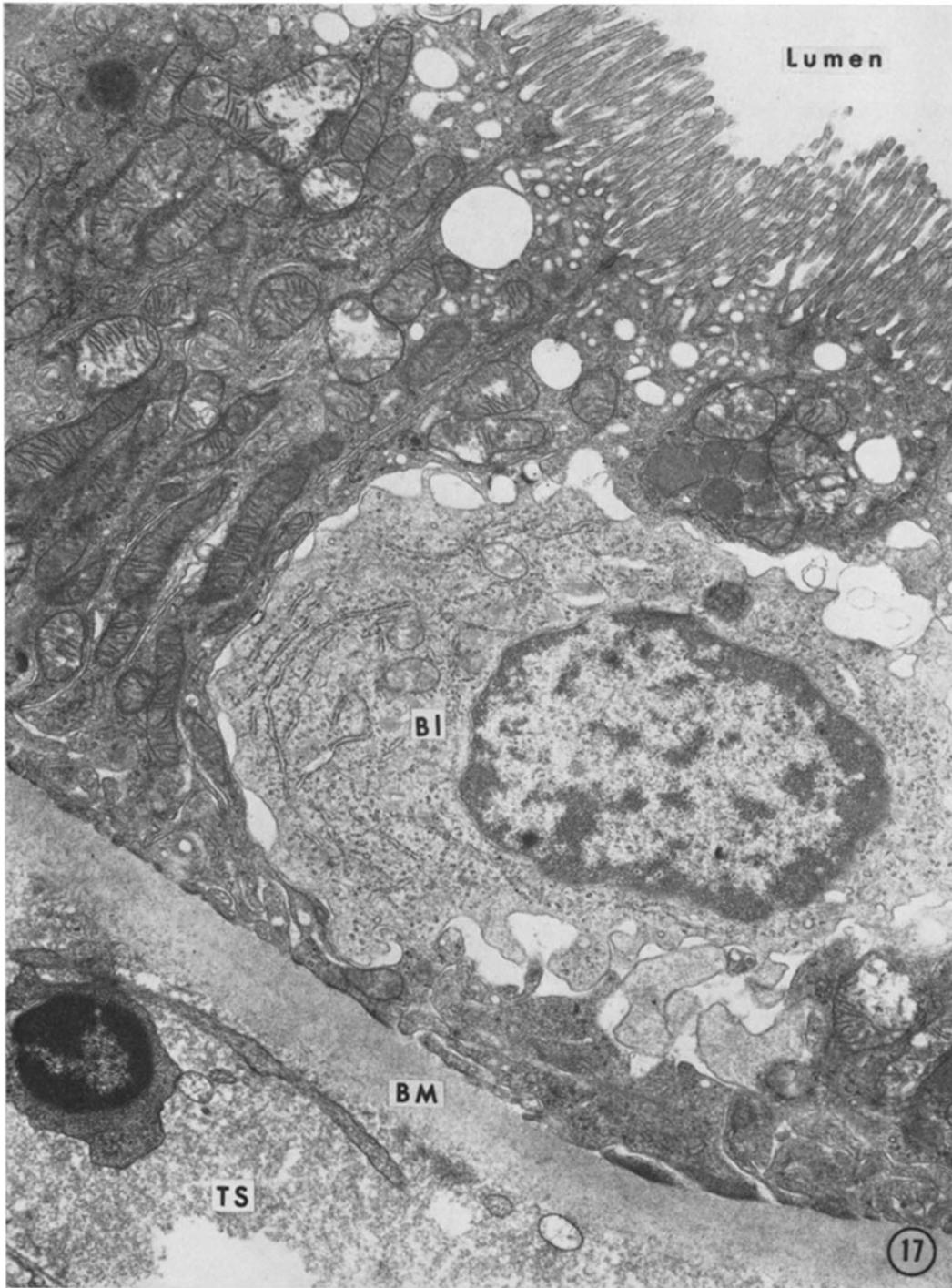


FIG. 17. Renal homograft. 4 days. A blast lymphoid cell (Bl) within the epithelium of a renal tubule. BM, basement membrane of tubule. TS, tissue space. $\times 14,000$.

the rejection process. Studies on the significance of lymph-borne cells in the propagation of immune responses to *Salmonella* and red cell antigens had demonstrated that an immune response could be confined to an antigenically stimulated node by diverting the efferent lymph and its contained cells from the

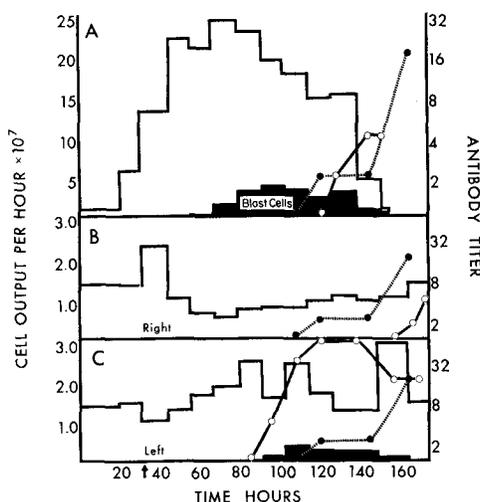


FIG. 18. A. The hourly cell output, the blast cell and the antibody response of a renal homograft. Antibody appeared in blood and renal lymph at 114–126 hr after grafting, and lymph and blood titers coincided closely with each other. ●-----● antibody titers in blood, ○—○ antibody titers in the renal lymph. B. The hourly cell output and antibody titers in the lymph from the popliteal lymph node of a sheep with a homograft in the neck. No blast cell response occurred in the lymph and the level of antibody in the popliteal lymph closely reflected the levels of antibody in the blood, ●-----● antibody titers in blood; ○—○ antibody titers in popliteal lymph. C. The hourly cell output and antibody titers in the lymph from a popliteal lymph node which received 5.5×10^8 washed cells collected from the afferent lymph of a renal homograft during the 20–31 hr period after grafting. The injection of these cells into the drainage area of this node produced a vigorous immune response with active blast cell and antibody production. The antibody titers in the popliteal lymph coincided with the appearance of blast cells in the lymph, and were not related to antibody titers in the blood which appeared later. ●-----● antibody titers in blood; ○—○ antibody titers in popliteal lymph.

body (18). To test the hypothesis that the sensitization of the regional lymph node was unimportant in the rejection of renal grafts, the reactions to the graft were followed in the efferent lymph of the prescapular lymph node. In addition the response of the popliteal lymph node, distant from the graft, was also studied. Anatomical studies in which T-1824 dye was injected into the region where the renal homograft was placed showed that the prescapular lymph node was the regional node to which lymph from this area drained.

The first experiments were done to study the effects of the surgical operation on the regional node. To this end, a renal autograft was placed in the neck and the lymph from the prescapular lymph node monitored over the next 14 days. Apart from a transient change in cell output, the node did not give an immune response after the surgical manipulations in the drainage area.

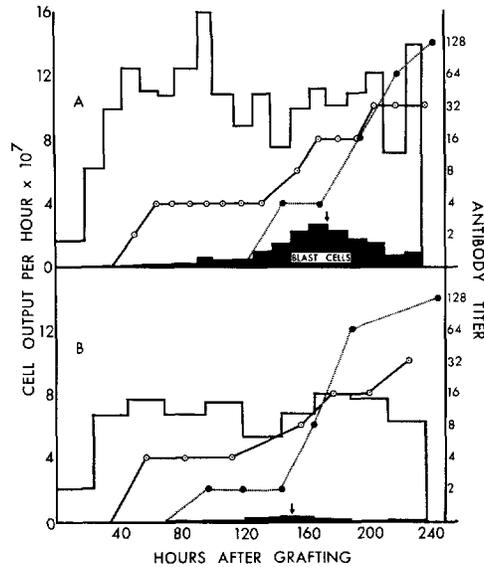


FIG. 19 A. The changes in the efferent lymph from the prescapular lymph node induced by the presence of a renal homograft in its drainage area. The renal lymphatics were not ligated or cannulated, and the lymph formed in the homograft was allowed to drain to the prescapular node. The blast cell response continued for some time after the kidney was rejected (shown by arrow). ●—● antibody titers in blood; ○—○ antibody titers in prescapular lymph. B. The changes in the lymph from the regional prescapular lymph node due to the presence of a renal homograft in its drainage area. The renal lymphatics were cannulated and graft lymph diverted from the body. Although the blast cell response in the prescapular lymph was feeble, antibody production still continued. ●—● antibody titers in blood; ○—○ antibody titers in prescapular lymph.

To establish whether the lymph-borne cells leaving the graft were capable of propagating an immune response in a lymph node, washed cells from the lymph of a homograft were injected into the drainage area of the popliteal lymph node, and the subsequent changes in the efferent lymph were monitored over the ensuing 10 days. The injection of 5.5×10^8 cells collected from the kidney 20–31 hr after grafting, produced a vigorous cellular response in the node with the production of large numbers of blast cells (Fig. 18 C).

To mimic the clinical homograft situation and to demonstrate the reaction that occurs in the regional lymph node when all the cells and breakdown products from the homograft are being carried to the node, a kidney was trans-

planted, leaving the renal lymphatics cut, and allowing the lymph to drain into the area adjacent to the graft. The lymph and cells that left the kidney were conveyed to the regional prescapular node by way of the host's lymphatics in that area and produced a vigorous immune response (Fig. 19 A). In experiments in which the renal lymphatics were cannulated and where the afferent graft lymph was diverted from the body, the cellular response of the prescapular node was usually much reduced and delayed in appearance (Fig. 19 B). However, even when the graft lymph and regional node lymph were diverted from the body, there was no alteration in the pathology of the rejection process or any delay in the time in which it was completed. These findings showed that sensitization of the host by way of the lymphatic system is not important in renal homograft rejection.

There was no change in the efferent lymph from lymph nodes distantly situated to the graft and no detectable cellular or humoral antibody response occurred in these sites during the life of the graft (Fig. 18 B).

The Humoral Antibody Response.—Although the rejection of first-set renal grafts has been attributed primarily to cellular mechanisms, the possible role of humoral antibody in homograft rejection requires further investigation. It has been assumed that the production of humoral antibody is not related to the onset of the rejection process and therefore is not specifically involved in first-set graft rejection, but more recent evidence has suggested that humoral antibody may be detected as early as 48–72 hr after grafting (19–21), and that antibody may be important in effecting graft rejection (22, 23).

In the present experiments with kidney homografts, lymphocyte-agglutinating antibody rapidly appeared after the graft was established. This antibody was primarily of the IgM class, and had cytotoxic activity at the higher agglutinating titers. It appeared initially between 48 and 60 hr in efferent lymph draining from the regional prescapular lymph node. Antibody was not detected in the blood until 96–120 hr after grafting, and blood titers remained below those of the prescapular lymph until near the time of rejection (Fig. 19 A, B). Because the antibody-laden lymph from the prescapular node was diverted from the body, the antibody in the blood must have originated from some other source. Nodes situated distant to the graft did not produce any antibody and the low titers that did appear in the lymph from these nodes appeared late in the response and reflected blood levels rather than active synthesis within the node. Porter's (24) description of alterations in host lymphoid tissue after renal transplants indicated that the spleen undergoes pronounced cellular changes analogous to those occurring in the regional nodes draining the graft, while distant lymph nodes were much less affected. Since the present studies showed no active synthesis in distant lymph nodes, it was thought likely that the regional prescapular node and, probably the spleen, accounted for most of the antibody formed during the primary rejection of the renal graft.

Antibody appeared in the lymph draining from the kidney homograft at ap-

proximately the same time as antibody was first detected in the blood (Fig. 20). After this time, the titers in renal lymph coincided so closely with the blood titers that it was impossible to decide whether active antibody synthesis occurred within the graft or not. The numerous plasma cells and large blast cells seen in the kidney indicated that the necessary essentials for local antibody pro-

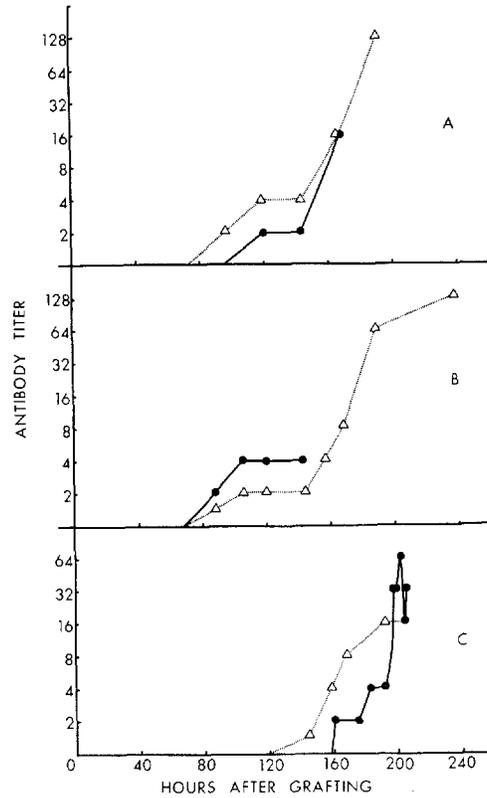


FIG. 20 A-C. The levels of lymphocyte agglutinating antibody in the lymph from a renal homograft compared with the levels of antibody in the blood. The antibody titers were measured in the renal lymph up to the time when the flow ceased. Δ --- Δ antibody titers in blood; \bullet — \bullet antibody titers in renal lymph.

duction were present. As it was found that lymphoid cells leaving the graft were capable of stimulating antibody production when transferred to another node, it seemed likely that similar types of cells present in the graft were also capable of synthesizing antibody. This indirect evidence, combined with the abrupt rise in the titers in both blood and renal lymph near the time of rejection supported the idea that the graft tissue was avidly binding antibody and at the same time cells within the graft were also producing it.

DISCUSSION

Remarkable changes occurred in the lymph draining from renal homografts. These changes indicated that, by monitoring the lymph continuously, immunological events occurring within the graft could be followed in a precise and sensitive way. The substantial rise in numbers of lymphocytes traversing the graft tissue after the first 18–24 hr unequivocally demonstrated the specific attraction of host lymphoid cells to the foreign graft tissue. Because this increased migration of lymphoid cells did not occur to anything like the same extent in isogenic renal grafts, it seemed that the recognition of “antigenic” difference must be the essential stimulus initiating the migration. This may not, however, be the whole story, as lymphocyte migration can occur on an extensive scale through isogenic kidneys when the ureter is partially or completely occluded, and in these circumstances, the only possible antigenic stimulus for this migration would be an autoantigen (25). The increased migration of lymphocytes occurred within the first 18 hr, indicating that the stimulus to the host’s lymphoid cells to migrate was transmitted early after the transplant was established. This can be considered the inductive phase of the rejection process.

Electron microscopic examination of the cells in the lymph demonstrated that cells leaving the graft during the first 24 hr were already showing signs of transformation to blast cell types. These cells continued to dedifferentiate and divide *in vitro* when transferred into cultures. This meant that not only did the recognition of the homograft occur within the graft itself, but so, too, did the dedifferentiation and proliferation of the lymphoid cells involved in the rejection process. Because of the magnitude of the cell traffic through the kidney and because large numbers of stimulated blast cells were present throughout the kidney parenchyma, it seemed unnecessary to postulate that cells sensitized remote from the graft, were travelling to it, and effecting its rejection.

Electron micrographs established that a good deal of cellular transformation occurred within the blood vessels of the graft. The first contacts of host and graft tissue occur between the graft vascular endothelium and blood-borne host cells; it can be inferred that this primary interaction is likely to be the first step in initiating the processes of selective lymphocyte migration and cell differentiation and proliferation.

In addition to lymphocytes, macrophages were also a feature of the cell population in lymph draining from the renal homograft. This cell is not present in the blood and its origin is obscure. It seemed most likely that the lymph macrophages were derived from blood monocytes, although there was no evidence for this. As seen in the lymph, the morphology of these cells was identical with the macrophages that characterize afferent lymph coming from a variety of normal tissues. During the rejection of the homograft, a large proportion of these macrophages contained material scavenged from the graft.

The present experiments have also established the role which the host’s lymph

nodes play in the rejection of renal homografts. It has been shown that diversion of the afferent lymph from the body will greatly delay and reduce the cellular response in the regional lymph node without affecting the survival of the graft. When lymph from the graft was allowed to drain to the regional node, the events occurring within the node followed closely the events taking place within the graft itself. The reactions of the regional lymph node certainly did not precede the reactions in the homograft. Finally, the diversion of lymph and cells coming from both the graft and the regional lymph node in no way altered the course of rejection. All these facts indicated that the regional node was not important in deciding the fate of the graft, but as in an immune response to a conventional antigen, the lymphatic system and the lymph nodes functioned to amplify the homograft response and establish widespread secondary reactivity (26). This amplification was seen from the results of experiments which traced the fate of the lymphoid cells leaving the renal homograft. Lymph-borne cells collected during the first few days after the graft was established were found to initiate the production of both blast cells and humoral antibody when introduced into a lymph node remote from the graft. This indicated the way in which the rejection process would be amplified in a clinical kidney graft situation. While the mass of cells leaving the kidney via the lymph was extremely large, there was almost certainly a big additional component of similar cells leaving the graft via the blood vascular routes and also a big component remaining within the graft itself.

Studies on humoral antibody production during the rejection of renal homografts suggested that the significance of this aspect of graft rejection may be undervalued. The present experiments have shown that detectable antibody was produced in the regional node as early as 48–60 hr after grafting. This is the same time that antibody first appears in lymph after stimulation of a lymph node with a conventional antigen (12) and is well within the time-scale of the rejection process. Although antibody titers were not detectable in the blood or in the lymph from the graft until later, indirect evidence suggested that this failure to detect antibodies was probably due to their being bound by the tissues of the graft. Further experimental data supported the idea that antibody was also produced in locations other than the regional node, in all probability, in the spleen.

The changes that occur in the lymph draining from autografted and homografted kidneys gave a good index of disturbances in the grafted organ due to pathological as well as immunopathological changes. When there was a period of prolonged anoxia before the graft was established, proteins and red cells leaked profusely from the kidney and the rate of lymph formation was high. These changes were interpreted as being due to damage of the endothelium, unassociated with any immunological reaction. High rates of lymph flow occurred in homografted kidneys, and this was associated with a reduction in the concentra-

tions of protein, and in red cell numbers in the lymph. In these circumstances, the increased lymph formation was thought to be due primarily to changes in hemodynamics within the graft rather than the result of increased capillary permeability.

SUMMARY

The rejection of renal homografts has been studied in sheep by transplanting kidneys into the neck and preserving the renal lymphatic drainage intact. Chronic fistulae were established in the transplanted renal lymphatics and lymph collected throughout the life of the graft.

The changes that occurred in homografts during the process of rejection were reflected in changes in the lymph. Large numbers of basophilic, blast, lymphoid cells appeared in the lymph, and lymph production in the grafted kidney increased 20–50 fold. Over a period of about 10 days, up to 60 g wet weight of lymphoid cells and up to 10 liters of lymph were collected from the graft.

Within 24 hr of grafting, the host cells present in the renal lymph had become sensitized to the graft and transformed into blast cells when cultivated in Millipore chambers in vitro. When the cells leaving the graft during the first 18–48 hr were injected into distant nonstimulated lymph nodes of the host sheep, they evoked significant cellular and antibody responses in the nodes.

Within the graft, the main pathological changes were found in the vascular endothelium and many of the peritubular capillaries become plugged with emboli comprised of blast cells. There was extensive infiltration of the renal parenchyma with lymphoid cells and evidence of their transformation and proliferation within the renal blood capillaries.

When all the lymph and cells leaving the homograft were diverted from the body, there was a greatly decreased reaction in the regional prescapular lymph node, and no reaction in lymph nodes distant from the graft. In these circumstances, the survival of the graft was not prolonged, and it was rejected without involvement of the lymph nodes of the host.

Humoral antibody was produced in the lymph node regional to the homograft within 48–60 hr of grafting. Antibody was not detected in the blood or in the renal lymph until near to the time the graft was rejected. It was thought that this was due to the binding of antibody by the kidney graft tissue.

We conclude that all the events which lead to the recognition and rejection of renal homografts can occur centrally within the graft itself.

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