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INVASION AND DESTRUCTION OF HOMOLOGOUS KIDNEY BY LOCALLY INOCULATED LYMPHOID CELLS*

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PLATES 30 TO 35

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There exists convincing evidence that vascularized homografts of solid tissues are destroyed by an immune reaction of the delayed hypersensitive type, and that the lymphocyte plays some crucial role in the genesis of such reactions (1–4). Infiltration by mononuclear inflammatory cells (lymphocytes and histiocytes) is a fundamental characteristic of primary homografts undergoing rejection (5–8), but the question remains as to the precise role which these mononuclear cells play in the destruction of the tissue they have infiltrated. Are they the agents which perpetrate the destruction or are they secondary invaders which have congregated in tissue already injured by some other agency?

Recently a number of elegant experiments have shown that specifically sensitized lymphocytes cluster upon and destroy their intended target cells *in vitro* (9–11), so it does seem that lymphocytes are capable of killing antigenic cells with which they are in contact.

The experiments described here and in a preliminary report (12) show that lymphoid cells possess a similar destructive capacity *in vivo*. The evidence consists of the demonstration that local inoculation of lymphoid cells from inbred parental strain donors is followed by the infiltration and destruction of renal parenchyma in F_1 hybrid rats. It will be shown that this reaction develops when the immunogenetic conditions for unidirectional graft *vs.* host reactions (GVHR) have been met (13), so that humoral antibody and lymphoid cells of host origin cannot play a primary, immunologically specific role in the reaction. Additional experiments which confirm the nature of the observed reaction and which demonstrate the role of lymphocytes of *donor* origin in it are presented.

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Materials and Methods

Animals.—Inbred rats of the BN and Lewis strains and their F_1 hybrids were utilized as donors and hosts. These animals were produced in the breeding colony of Dr. W. K. Silvers. These two isogenic parental strains have been shown to differ with respect to at least four major histocompatibility loci (14). The recipient rats ranged in age from 2 to 12 months.

Sensitization of Donors.—Some of the experimental hosts received inocula of lymphoid cells or serum from donors of one parental strain which had been previously immunized to the other parental strain. Three immunization schedules were employed:

For sensitized cells: The donor was first grafted orthotopically with a skin graft from a donor of the other strain (15). Soon after the homograft had been rejected, the donor received an intraperitoneal injection of ¼ to ⅛ splenic equivalent, as a saline suspension of viable cells from the donor strain. The lymphoid cells from these "sensitized donors" were harvested within 1 to 4 weeks after the booster injection of splenic cells.

For immune serum: Each prospective BN donor received a Lewis skin homograft as described above. On the day following rejection, the animal was exsanguinated and the serum obtained was utilized within 24 hours. Steinmuller has shown that BN anti-Lewis serum obtained in this fashion is capable of conferring passive immunity against Lewis skin homografts upon normal BN hosts (16).

For hyperimmune serum: Two young adult Lewis rats were injected intraperitoneally every other week with 0.5 to 1 kidney equivalent as a saline suspension of cells obtained by mincing kidneys from BN and F_1 donors. After four such inoculations, serum was obtained and utilized within 24 hours.

Tolerant Donors.—A portion of the parental strain rats utilized as donors was rendered immunologically tolerant of the foreign transplantation antigens of the other parental strain by neonatal injection of high doses (40 to 80 million) of F_1 hybrid lymphoid cells, according to the technique described by Billingham (15). When 2 months old, the prospective tolerant donors were test grafted with an orthotopic skin homograft from the other parental strain. If, after 50 days, the test graft remained in good condition and bore a good hair crop, the rat received a second test graft from the donor strain. If both skin grafts appeared well tolerated during the next 30 days, it was assumed that the prospective donor was in fact completely tolerant. The spleen, lymph nodes, or leucocytes from 3 such tolerant donors were prepared as described below and inoculated in doses of 50 and 100 million cells.

Preparation of Lymphoid Cell Suspensions.—The donors of lymphoid cell suspensions were 1 to 10 months old, and those for thymocytes were 1 to 2 months old (preinvolutional).

1. Thymus, spleen, and axillary and cervical lymph nodes were excised and prepared in Hanks' solution as suspensions according to the technique of Billingham (15). After a cell count was obtained in 2 per cent acetic acid diluent, the suspension was centrifuged (1000 RPM) for 10 minutes. The cells were resuspended in a volume of fresh Hanks' solution so as to yield the desired cell doses in aliquots of 0.1 to 0.2 cc.

2. Thoracic duct lymphocytes were collected for 12 to 14 hours following catheterization of the duct (17). The lymph was drained into Hanks' solution with heparin (20 u./ml) maintained at 4°C, and further preparation of the cell suspension was as described above.

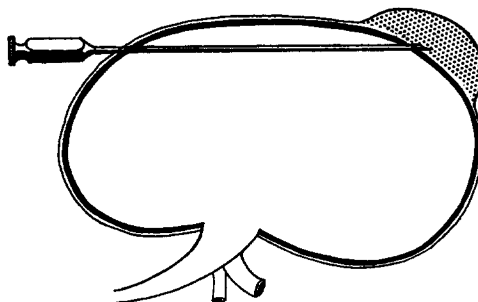
3. Blood leucocytes were concentrated from heparinized or citrated fresh blood by sedimentation of erythrocytes in 6 per cent dextran (molecular weight, 240,000). Further preparation of the cell suspension was as described above.

4. Neoplastic lymphoid cells were obtained from the peripheral blood of young Lewis donors bearing the transplantable lymphoma, L8, generously supplied by Dr. Ira Klein of Microbiological Associates, Bethesda. This tumor originated in a Lewis rat and grows progressively only in rats of this strain and appropriate hybrids thereof. Circulating L8 cells were harvested by the same technique employed for normal leucocytes 10 to 14 days after subcutaneous inocu-

lation of the donor with tumor. Doses of 10 and 25 million cells were employed for subcapsular transfer.

Inoculation of Lymphoid Cells and Sera.—The intended hosts were anesthetized with chloral hydrate (15). The left kidney was delivered through a paravertebral incision in the lumbar region. Dosages of 10 to 100 million lymphoid cells in 0.1 to 0.2 cc aliquots or similar volumes of fresh serum were injected into the subcapsular space *via* a 30 gauge needle as illustrated in Text-fig. 1. This produced a transient bleb on the surface of the kidney. Application of gentle pressure with a cotton-tipped applicator over the needle tract for a few seconds minimized back leakage of the suspension in most instances.

Autopsies.—The animals were sacrificed at 3, 7, 14, and 42 days after the subcapsular injection. The kidneys and the spleen were removed from each rat, bisected, blotted gently to remove excess urine and blood, and weighed to the nearest milligram. Cervical, axillary, and intra-abdominal lymph nodes, thymus, and liver were visualized and in some cases samples



TEXT-FIG. 1. Illustration of technique of subcapsular injection.

taken for histologic study. These tissues were fixed in Tellyesniczky's fluid, embedded in paraffin, sectioned, and stained with hematoxylin-eosin and pyronin–methyl green.

Chromosome Preparations.—In order to discover the origin of the infiltrating cells in kidneys with GVHRs, a karyotype analysis with respect to the sex chromosomes was carried out on dividing cells from lesions which developed after transfers in which host and donor were of opposite sex.

Thirty minutes prior to sacrifice, 0.75 mg colchicine in 0.1 cc normal saline was injected intraperitoneally into each rat. Tissue from the reaction zone visible in the gross was excised and minced with fine scissors. Similar suspensions were made from the spleen and cervical lymph nodes of the host. The resulting saline suspensions were then processed according to the technique of Nowell *et al.* (18).

The total number of technically adequate metaphases from each specimen, up to a maximum of fifty, was analyzed microscopically with respect to sex, and the relative proportions of host and donor type were recorded.

The normal karyotypes of cells derived from BN and Lewis rats have recently been described by Hungerford and Nowell (19). Although the Y chromosome is not discernible in BN males, it is readily picked out in the Lewis. For this reason, when male F_1 hybrids were utilized as recipients, care was taken to use only progeny from Lewis male x BN female matings. Moreover, when F_1 females were utilized as recipients, only Lewis males were used as donors for the lymphoid inocula. Thus a visible Y chromosome was always present in metaphases derived from whichever member of the donor-host combination was male. Metaphases from BN, Lewis, or F_1 hybrids may also be sexed by the number of large acrocentrics corresponding

to the X chromosomes present. Both criteria were utilized in classifying the sex of each mitotic figure (Figs. 1 and 2).

RESULTS

I. Histopathologic Observations

A. Uninjected Control Kidneys.—Occasional lesions of relatively minor extent were seen in the contralateral, uninjected kidneys in rats of each experimental group. These abnormalities were taken to be representative of the renal disease endemic in the rat colony, and consisted of (*a*) discrete perivascular foci of mononuclear cells, (*b*) focal atrophy of cortical tubules with associated fine

TABLE I

Correlation of Histologic Evidence of GVHR with Immunogenetic Relationship of Donor and Host

Type of transfer	Fraction of injected kidneys displaying evidence of GVHR following injection of competent* cells			
	Day 3	Day 7	Day 14	Day 42
$P \rightarrow F_1$	7/7	25/25	15/15	17/37‡
$F_1 \rightarrow F_1$	0/2	0/4	0/5	0/6
$P \rightarrow P$	0/2	0/10	0/8	0/20
$F_1 \rightarrow P$	0/2	0/4	0/5	0/4

* Sources of competent lymphoid cells are spleen, lymph node, leucocytes, and thoracic duct of normal or senstitized adult donors.

‡ A weight differential falling outside the upper 99 per cent confidence limits of the controls and histologic signs of GVHR are requisites for positive diagnosis of a GVHR in 42-day specimens.

mononuclear infiltration, (*c*) cortical retention cysts, (*d*) infarction (1 case), and (*e*) colloidal exudation in Bowman's space.

B. Injected Control Kidneys.—The following lesions were noted in all kidneys subjected to injection irrespective of the nature of the inoculum, and are considered to represent the non-specific sequellae of inoculation, (*a*) hematoma (Fig. 3), (*b*) capsular thickening, and (*c*) lymphocytic infiltration in the subcapsular space. The latter infiltrates possessed neither mitotic nor invasive activity and were of microscopic dimensions.

C. Histopathology of the GVHR.—A consistently observed phenomenon following $P \rightarrow F_1$ transfers was the invasion and destruction of the underlying renal cortex by the injected lymphoid cells and/or their progeny. The outermost layer of the cortex in the rat consists of a prominent rim of tubules which overlies the outermost glomeruli (Figs. 3 and 5). This cortical rim was the first to undergo invasion and destruction following $P \rightarrow F_1$ transfer but remained intact in all the controls examined except the few with cortical infarcts. Erosion of this

mantle of tubules provided an objective sign that some minimal amount of parenchymal destruction had occurred. The histologic nature and abundance of the infiltrate as described below permitted further discrimination between local GVHRs and non-specific control lesions. Table I gives the results of such histologic evaluations for each experimental group at each stage of development.

In *3rd day* reactions lymphocytes were seen lying in a thin layer upon the renal surface and infiltrating down into the interstitium of the outer cortex (Fig. 4). The infiltrate was relatively sparse, and there was little evidence of parenchymal damage. At the forefront of the more advanced reactions many mononuclear cells clustered around Bowman's capsules and along cortical blood vessels. Some of the peritubular capillaries within the reaction zone contained large numbers of mononuclear cells similar to those of the infiltrate.

Seventh day reactions contained more prominent and cytologically distinctive cellular infiltrations, which consisted of lymphocytes of various sizes and degrees of maturity, histiocytes, and primitive-looking large cells containing a large, rounded, pale-staining nucleus, one to three prominent pyroninophilic nucleoli, and scanty, irregularly disposed, pyroninophilic cytoplasm (Fig. 10). Mitoses occurred frequently in the latter cells, thus confirming the interpretation that they are blast forms. Polymorphonuclear and eosinophilic granulocytes were noted only occasionally.

The 7th day reactions may be conveniently divided into 3 groups according to the degree of invasive activity exhibited by the infiltrate.

Grade I, non-invasive lymphoid grafts: In the least virulent reactions, a subcapsular cap of mononuclear cells was seen overlying the surface of the kidney but showed little propensity to invade it (Fig. 5). The same cell types seen in the more virulent 7th day GVHRs are to be found in these "grafts," but the proportion of mitotic pyroninophilic elements was lower. The distinctive nature of the cellular constituents and the thickness of the graft visible in the gross distinguishes this reaction from the microscopic subcapsular foci of lymphoid cells seen in kidneys injected with control (isologous) cell suspensions. Although a few tubules at the graft-kidney interface which had been "dissected" free by infiltrating mononuclear cells showed degenerative changes, the rim of convoluted tubules overlying the outermost glomeruli remained intact.

Grade II, lymphoid graft with invasive tongues: The reactions comprising this group were characterized by a limited but definite invasive tendency. Grafts of pleiomorphic mononuclear cells were seen overlying the renal surface, and from these discrete tongues of cells penetrated into the cortex (Fig. 7).

For the most part the tubules directly underlying the graft and those along the borders of the invading tongues showed no significant degenerative changes. Those tubules, however, that had been completely isolated in the midst of the infiltrate often demonstrated degenerative changes (Fig. 8) such as are described below for grade III reactions.

Grade III, extensive invasive-destructive reactions: The reaction zone was

visible in the gross as a large whitish mass which protruded from and replaced the cortex in the region of deposition of the inoculum. Sections through these zones showed that 20 to 60 per cent of the cross-sectional area of the kidney was infiltrated (Fig. 9). Renal parenchyma was almost completely obliterated in some areas by the massive cellular infiltrate, and only scattered and necrotic renal structures remained (Figs. 6 and 11). In every case the convoluted tubules, especially those in the peripheral rim, were the elements most susceptible to destruction; moreover, *degenerative changes occurred only in those tubules which were invested with infiltrating mononuclears.*

Pathologic change in the besieged tubules took several forms. In many cases the tubule became fragmented so that disaggregated epithelial cells were present in the interstitial infiltrate (Figs. 11 and 13). In some places mononuclears appeared to have penetrated into the walls of tubules (Fig. 13), thereby contributing to the disruption. Many other tubules had been reduced to shrunken cords of featureless cells, and these atrophic remnants often showed a thickened hyaline basement membrane.

A third manifestation of the destructive activity of the invading mononuclears consisted of random, individualized changes in cells of the convoluted tubules. Some cells appeared necrotic with cytoplasmic vacuolation and karyolysis, others were atrophic, and still others were in mitosis. Centripetal displacement of nuclei within cells and also of whole cells into the tubular lumen were other indications of insult. The variously altered epithelial cells coexisted with normal ones in different combinations and permutations in given sections of tubules, and conferred a bizarre appearance upon them (Figs. 11 and 13).

Although the glomeruli mostly retained their integrity, the glomerular capillaries were characteristically devoid of blood (Figs. 11 and 15). The tufts, therefore, appeared collapsed and their component capillary loops often could not be made out. This ischemic change was in sharp contrast to the relative congestion noted in glomeruli beyond the margins of the reaction zone (Fig. 16). Consistent with the glomerular ischemia was the observation that many peritubular capillaries, small veins, and interlobular arteries within the reaction zone were dilated and filled with mononuclear cells (Fig. 12), often to the apparent exclusion of erythrocytes. Some of the "plugged" capillaries which were devoid of erythrocytes could well have been cortical lymphatics. At the advance borders of the reaction, the mononuclears tended to collect around the glomerular capsule and along blood vessels, and these cells were commonly observed traversing the walls of patent capillaries and veins.

Interstitial hemorrhage and pathologic changes in the walls of blood vessels in the invaded zone were extremely rare and unimpressive. Moreover, the degree of edema even in these most acute and virulent reactions must be termed moderate. These negative findings are perhaps best interpreted as the results of early and complete cessation of blood flow within the region of the local GVHR.

In contrast to the dramatic events occurring in the cortex, the renal medulla showed little of interest. The arcuate vessels at the corticomedullary junction always remained patent and there was usually no infiltration deep to these.

Most of the *14th day GVHRs* showed signs of diminished vigor. In these the cellular infiltrate had thinned (Figs. 14 and 15), the frequency of blast cells and mitotic figures decreased, and pyroninophilic cells, resembling mature and immature plasma cells, had become prominent in the infiltrate (Figs. 17 and 18). A few reactions appeared to be still active, as judged by the presence of blast cells and paucity of plasma cells.

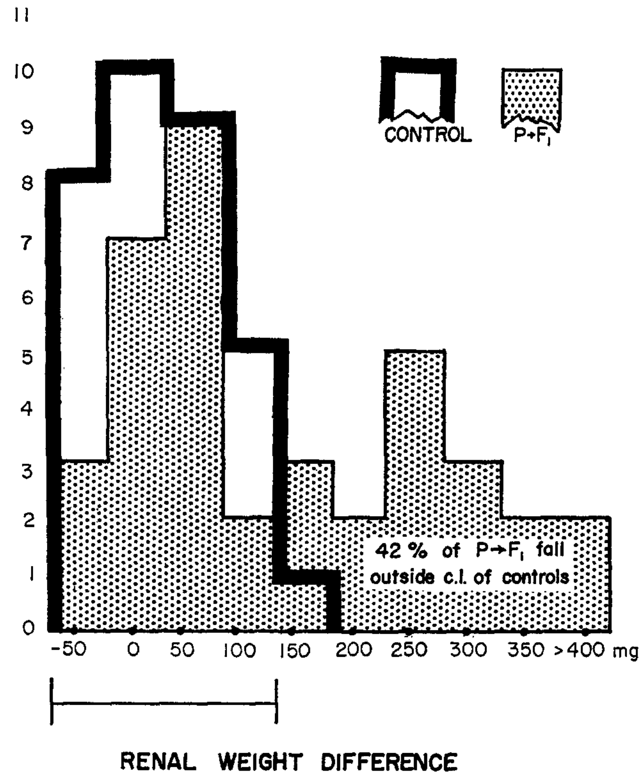
As a result of the outright destruction of the majority of the convoluted tubules and the reduced amount of interstitial infiltrate, there developed "bunching" of ischemic but otherwise resistant glomeruli in the reaction zone (Figs. 14 and 15). Intravascular plugs of mononuclear cells were present only in the more active reactions, but most of the small vessels in the reaction zones remained devoid of blood even in involuting GVHRs.

By *the 42nd day* none of the injected kidneys was enlarged by the lymphoid tissue masses seen in 7th and 14th day GVHRs. Often there was little macroscopic evidence that any significant reaction had transpired, but in a good number there existed a saucer-like cortical depression with a shaggy, whitish surface which involved up to about ⅓ of the renal surface. Microscopically these areas revealed extensive loss of cortical tubules. Bunched ischemic glomeruli resided in granulation tissue or were surrounded by severely atrophic tubular remnants. The interstitium usually showed many capillary loops filled with blood, but the arterioles associated with glomeruli and the capillaries of the tufts remained empty. Lymphocytes and plasma cells were usually present in the reaction zone, but there was little evidence of continued invasion.

It must be pointed out that the smaller of these "healed" lesions mimic those of healed infarction in control kidneys, and for this reason the diagnosis of GVHR at this late stage depended not only on histologic evidence but also upon demonstration of a significant weight deficit in the inoculated kidneys compared to the 99 per cent confidence limits of that in the controls (Table I, Text-fig. 2).

II. Loss of Renal Mass as a Consequence of Local GVHR

As noted above, many kidneys which had borne a reaction for 42 days presented a defect in the cortex and weighed less than their contralateral counterparts. This apparent decrease in weight was taken as an approximate index of the amount of parenchymal destruction which had transpired during the course of the local GVHR, and was calculated by subtracting the weight of the injected kidney from that of the contralateral kidney. The distribution of the weight differentials in the 42-day reactions is shown in Text-fig. 2, where those for the $P \rightarrow F_1$ group may be compared to those of the pooled $F_1 \rightarrow F_1$, $P \rightarrow P$,



TEXT-FIG. 2. Distribution of renal weight differences (milligram) in rats with and without local GVHRs prior to autopsy on 42nd day. ⊢⊣, 99 per cent confidence limits (c.l.) of controls.

and $F_1 \rightarrow P$ controls. It will be noted that the distribution of renal weight differentials in the $P \rightarrow F_1$ group is bimodal, and that 42 per cent fell outside the 99 per cent confidence limits of the control series. The two series differ significantly ($P = < 0.01$) by the Mann-Whitney U test (20).

It is unlikely that compensatory hyperplasia in the uninjected kidneys contributes to the magnitude of the weight differentials, for a comparison of the weights of non-injected contralateral kidneys, corrected for body weight, revealed no significant difference between the control rats and those in which virulent GVHRs had occurred.

The percentage by weight of renal destruction consequent to a GVHR can thus be estimated by the expression:

$$\frac{\text{weight differential} - \text{mean weight differential of controls}}{\text{weight of contralateral kidney}}$$

By this means it has been calculated that up to 25 per cent by weight of an F_1 kidney may undergo destruction following inoculation of parental strain lymphoid cells. Were it possible to inject the cell concentrates so that the bleb covered more of the renal surface without leaking out through the injection site, it would have been possible to have increased this figure.

III. On the Origin of the Infiltrating Cells

Although the immunogenetic aspects of these GVHRs indicated that the renal destruction had been initiated by the immunologic activity of the injected cells, the possibility remained that much of the cellular infiltrate consisted of inflammatory cells of host origin which had mobilized in response to the local renal injury. For this reason experiments were performed in an attempt to define the source of the infiltrate.

By utilizing inocula of lymphoid cells from donors opposite in sex from the recipients, and by karyotype analysis of the dividing cells subsequently obtained from the reaction zone, it was possible to show that the mitotic component of the infiltrate on the 7th day was almost entirely of donor origin, and that donor type cells may be present as late as the 14th day (Table II).

Among those 7- and 14-day reactions which provided sufficient mitoses for analysis, all contained large numbers of donor type cells, and all showed primitive pyroninophilic blast cells among the infiltrating mononuclears in routine histologic preparations. Most of the mitoses appeared to occur in these primitive forms; a few were seen also in renal tubule cells. On the other hand, those reactions which yielded only occasional mitoses contained no blast forms in their infiltrates. Thus it seems likely that the donor component which is detected by the sex chromosome markers consists of these pyroninophilic blasts.

The source of the non-dividing cells in the infiltrate is, of course, not revealed by this technique; however, by transferring lymphoid cells directly between the two parental strains, BN $\rightleftarrows$ Lewis ($P \rightarrow P'$), one can make some further inferences on the origin of the infiltrate. During the early stages of the reaction one might expect a GVHR to develop as the transferred cells are stimulated by the antigenic renal cortex. But meanwhile, the host will be developing immunity to the foreign strain lymphoid cells, so that probably between the 4th and 10th days, the donor type infiltrating cells will be destroyed. From the 14th day on, any infiltrate within the kidney may reasonably be presumed to represent those host elements which are responding to the presence of foreign strain cells and/or damaged parenchyma within the kidney. Four such $P \rightarrow P'$ reactions were studied histologically on the 3rd, seven on the 7th, six on the 14th, and six on the 42nd day after injection. In each of the 3-day specimens mononuclear cells were seen infiltrating through the superficial cortical interstitium, just as occurred in the $P \rightarrow F_1$ reactions at this stage (Fig. 19). However, the massive infiltrate characteristic of $P \rightarrow F_1$ reactions at later stages never developed. The

TABLE II

Donor and Host Metaphases in Renal GVHRs and Lymphoid Organs of the Host

Rat No.	Day of autopsy	Lymphoid cell transfer	Abundance of mitoses	Presence of blasts in infiltrate	Relative No. of analyzable mitoses			
					Renal GVHR		Spleen and lymph nodes of host	
					Donor type	Host type	Donor type	Host type
15	7	50 × 10^6 spleen cells, Lewis male to F_1 female	High	+	46	4	4	76
18	7	25 × 10^6 thoracic duct cells, Lewis female to F_1 male	High	+	48	2	3	25
16	7	50 × 10^6 blood leucocytes, Lewis female to F_1 male	High	+	46	4	2	36
20	14	50 × 10^6 spleen cells, Lewis female to F_1 male	Low	—	0	1	0	4
21	14	50 × 10^6 spleen cells, Lewis female to F_1 male	Low	—	1	0	0	1
19	14	25 × 10^6 thoracic duct cells, Lewis female to F_1 male	Low	—	0	0	0	8
17	14	25 × 10^6 blood leucocytes, Lewis female to F_1 male	Low	—	0	0	0	5
9	14	100 × 10^6 blood leucocytes, Lewis female to F_1 male	High	+	13	20	0	3
11	14	60 × 10^6 lymph node cells Lewis male to F_1 female	Low	—	0	0	—	—

infiltrate on the 7th day showed a distribution similar to P → F_1 reactions but was relatively sparse and contained mostly pyknotic mononuclears (Fig. 20). At 14 and 42 days few mononuclears were present (Fig. 21). Parenchymal destruction occurred in some cases but was limited to the outer cortical rim. These results provide further evidence that the majority of infiltrating mononuclears in the P → F_1 reactions are of donor origin.

IV. Potency of Cells from Various Sources

If the renal reactions represent homograft reactions on the part of the parental lymphoid cells against hybrid kidney, one would not expect to observe any reaction when the transferred cells are immunologically incompetent. Lymphoid cells from specifically tolerant parental strain donors were expected to furnish one source of incompetent cells, and thymocytes from normal and

TABLE III

Occurrence of GVHRs with Lymphoid Inocula from Various Sites of Origin ($P \rightarrow F_1$) Transfer

Histologic evaluation of 7- and 14-day reactions, origin of inocula	Normal donors					Sensitized donors					Total No. of gr. III GVHR/No. rats
	No.	Neg.	Grade*			No.	Neg.	Grade*			
			I	II	III			I	II	III	
Thymus	8	2	3	3	0	6	2	2	1	1	1/14
Blood	6	0	0	1	5	8	0	0	2	6	11/14
Lymph node	4	0	0	1	3	4	0	0	1	3	6/8
Thoracic duct	4	0	0	0	4	3	0	1	2	0	4/7
Spleen	5	0	2	0	3	6	0	0	1	5	8/11

Evaluation of 42-day reactions‡	Normal donors No. GVHRs/No. rats	Sensitized donors No. GVHRs/No. rats	Total GVHRs/No. rats
Thymus	1/2	0/3	1/5
Blood	2/8	2/6	4/14
Lymph node	0/2	2/5	2/7
Thoracic duct	2/3	2/2	4/5
Spleen	4/7	3/5	7/12

* Grade I, non-invasive graft; Grade II, discrete invasive tongues; Grade III, invasive-destructive reactions.

‡ Significant weight differential in addition to histologic evidence is necessary for diagnosis of GVHR.

TABLE IV

Occurrence of GVHRs with Tolerant, Immune, and Normal Donors ($P \rightarrow F_1$ Transfer)

Status of donor	Days 7 and 14					Day 42, No. pos/No. rats	Total No. pos/No. rats
	No.	Neg.	Grade*				
			I	II	III		
Tolerant	6	5	1	0	0	0/3	1/9
Normal	27	2	5	5	15	9/22	34/49
Immune	27	2	3	7	15	9/21	34/48

* Grade I, non-invasive graft; Grade II, discrete invasive tongues; Grade III, invasive-destructive reactions.

sensitized donors another. The degree of invasive-destructive activity as graded histologically in 7- and 14-day reactions (see Results, section I C), and also the incidence of detectable damage secondary to GVHRs on the 42nd day, provided the criteria whereby the potency of various inocula were judged. Table

III shows the results obtained with inocula of differing anatomic origin, and Table IV allows comparison of the results with normal, tolerant, and immune donors. The attenuated reactions of thymocytes and the negligible activity of the "tolerant" inocula are in accordance with expectations. The inocula from spleen, lymph node, blood, and thoracic duct of *both normal and sensitized* donors were about equal when evaluated by this method.

V. Extrarenal Effects of the GVHR

The ravages of systemic graft *vs.* host disease, *e.g.* runt disease, extend to many organ systems and may lead to profound illness or even to death (13). It is therefore of interest to remark that signs of systemic graft *vs.* host reactions were absent even in those rats in which severe renal GVHRs had been induced. After convalescing from surgery, all the rats remained lively and gained weight. Diarrhea, dermatitis, and anemia were not clinically evident. Histologic study of the spleen in all cases, and of the thymus, lymph nodes, and liver in many cases revealed no abnormalities.

However there is some evidence in Table II which does suggest that the local GVHRs exerted a systemic effect. The mitotic activity in the spleen and nodes of the host paralleled that within the renal GVHR, although there was no evidence of extensive colonization of the host's lymphoid organs by the injected cells. Thus the presence of a GVHR may induce increased mitotic activity in the lymphoid system of the host, even though the immunologic reactivity of the inoculum is expended within the injected kidney.

VI. Absence of Reactions Following Transfer of Immune and Hyperimmune Sera

Fresh and undiluted anti-Lewis isoimmune serum from BN donors was injected beneath the kidney capsule of six Lewis rats. Two rats were sacrificed on each of the 7th, 14th, and 42nd days. No definitive lesions were noted in any of the injected kidneys.

Hyperimmune anti-BN kidney serum from Lewis donors was similarly injected into six $(L \times BN)F_1$ hybrids. Three rats were sacrificed on the 3rd and 7th days. Again no definitive lesions were noted upon gross or histologic examination.

VII. Failure of Lymphoma Cells to Destroy Renal Parenchyma

During evaluation of the pathogenesis of the renal GVHRs the question arose as to whether or not both the invasive and the destructive components of the reaction were simply the result of a mitogenic effect which the antigenic renal cells would exert on the injected lymphoid cells. The histologic appearance of the early GVHRs suggested that a quasi-neoplastic process might be responsible for the renal destruction. To test this possibility suspensions of malignant Lewis lymphoma cells were injected beneath the renal capsules of five Lewis

and seven F_1 hybrids, and these rats were sacrificed on the 7th and 10th days. Extensive cortical infiltration by malignant lymphoblastic cells was observed in all the Lewis and in five of the F_1. The infiltrates were almost entirely constituted of these cells, which were morphologically similar to the blasts in the GVHRs produced by normal lymphoid cells. However, despite the presence of large numbers of highly invasive cells in the interstitium, the convoluted tubules in the cortex remained intact and showed little evidence of degeneration (Figs. 22 and 23).

Since the Lewis lymphoma cells invaded isologous as well as homologous (hybrid) kidney, it is evident that they were displaying malignant not immunologic behavior. Moreover the failure of the L8 cells to effect destruction of hybrid kidney is strong evidence that they were not immunologically competent. The main point about this experiment, however, is that it demonstrates that the presence of a rapidly dividing, invasive cellular infiltrate does not by itself cause tubular degeneration.

DISCUSSION

Implications of the Immunogenetic Conditions of the Reactions.—The inoculation of immunologically competent lymphoid cells beneath the kidney capsule has been shown to result in invasion and destruction of the underlying renal parenchyma if the transfer is from parental strain donor to F_1 host. Since no reaction is observed with $P \rightarrow P$, $F_1 \rightarrow F_1$, and $F_1 \rightarrow P$ transfer, the observed invasion must be interpreted as a graft *vs.* host reaction. It follows that the subsequent local destruction of renal cortex is a consequence primarily of the activity of the injected lymphoid cells, and cannot have resulted from the development of a state of hypersensitivity by the host.

Implications of the Histopathology of the GVHRs.—Waksman (5) investigated the histologic characteristics of a variety of local reactions of delayed hypersensitivity, including primary homograft rejection, and concluded that perivenous mononuclear cell infiltration in antigen-containing tissue is the hallmark of these reactions and is the primary histologic event therein. Moreover the mononuclears may proceed to invade and destroy the antigenic tissue, where cell necrosis occurs only in intimate relationship to the infiltrating cells. The histopathologic characteristics of the renal GVHRs are in all respects similar to the invasive-destructive reactions described by Waksman.

By the same token it is interesting to note that the histopathology of the local GVHR mimics in many particulars the rejection process of primary renal homografts (7, 8, 21, 22). The two processes have in common (*a*) the distribution and cytologic characteristics of the infiltrating mononuclears, (*b*) the susceptibility of convoluted tubules and resistance of glomeruli, (*c*) invasion and fragmentation as the mode of tubular destruction, and (*d*) the disruption of the intertubular capillary circulation by the invading mononuclears. Since the

transferred lymphoid cells engaged in a local invasive-destructive reaction, which mimicked the rejection of a primary renal homograft, the hypothesis (7) is sustained that such grafts are actively destroyed by infiltrating mononuclears.

If it is true that the renal damage which results from the GVHR is a manifestation of homograft immunity, it is not surprising that the inocula of immune and hyperimmune sera produced no visible effect. These negative results neither support nor contradict the contention of Terasaki *et al.* (23) that injections of hyperimmune isoantiserum into the renal artery produce specific damage to renal parenchyma in the mouse, but one may well question the specificity of the lesions which they reported.

Origin and Fate of the Cellular Infiltrate.—Since pure suspensions of lymphocytes were as fully active as more heterogeneous lymphoid suspensions, *e.g.* spleen, we may presume that the lymphocytes in the various inocula were the active elements which initiated the ensuing GVHRs. The evidence derived from the sex chromosome marker experiments indicates that the inoculated lymphocytes gave rise to the rapidly dividing, pyroninophilic blasts in the active phase of the invasive-destructive reaction. Other investigators (24–27) have observed similar primitive, pyroninophilic cells to replace the normal lymphocytes in the follicles of lymph nodes and spleen during acute systemic GVHRs. Moreover Gowans (25) and Porter and Cooper (27, 28) have shown that these blasts were derived from small (but see reference 29) lymphocytes in the inoculum. Presumably, then, the blasts noted in the local renal GVHRs and in the lymphoid organs of hosts with systemic GVHRs such as runt disease are of common lineage.

The important role of these blasts in the pathogenesis of the GVHRs is borne out by the failure of tolerant populations of lymphoid cells either to give rise to such forms or to produce any parenchymal infiltration following $P \rightarrow F_1$ transfer. The cytological expression of immunological competence in these reactions thus seems to consist in the ability of the inoculated lymphoid cells to give rise to these primitive cells. This observation substantiates the conclusions of other investigators concerning the failure of such cells to develop in response to specific antigenic stimulation during immunologic unresponsiveness (30, 31).

About the 14th day, as the blasts disappear, the mitotic index of the reaction declines and little further invasion is evident. Where do these cells go, or what do they turn into? It seems likely that they give rise to the lymphocytes and histiocytes present in the 7th and 14th day infiltrates, and/or to the plasma cells which become prominent around the 14th day. The evidence for these transformations is only circumstantial, being derived from the failure of the infiltrates in the $BN \rightleftarrows L$ reactions to become florid or to contain a significant component of pyroninophilic cells. It is, however, consistent with Holub's findings concerning the transformation of lymphocytes, confined in diffusion chambers and cultivated *in vivo* in foreign hosts, into a mixed population of lymphocytes,

histiocytes, plasma cells, and their respective precursors (32). The rapidity with which the reacting mononuclears lose their virulence is interesting. There are a number of possible explanations for this, and these are presently under investigation.

Potency of Cells from Various Sources.—The low incidence of virulent (grade III) GVHRs following transfer of thymocytes is consistent with the reported feeble ability of such cells to induce runt disease in neonatal animals (24). It is interesting to note, however, that the thymocytes characteristically formed lymphoid grafts in the subcapsular space (Fig. 5). Such behavior (grade I GVHR) was also exhibited by lymphoid cells from other sources, but was most typical of thymocytes. These grafts must represent some minimal degree of responsiveness to antigen since thymocytes and other types of lymphoid cells do not exhibit this behavior when transferred to isologous hosts. Although some irregular invasive activity was demonstrated by the thymic inocula, it is possible that this activity was due to a small percentage of "contaminating" blood lymphocytes, and thymoctyes *per se* could be inert.

The results obtained with inocula from *tolerant* donors show that the mere act of injecting parental strain lymphoid cells beneath the kidney capsule of F_1 hybrid hosts does not result in any infiltrative reaction unless the cells are specifically competent to react against the isoantigens of the parenchyma. This finding strengthens the argument from the immunogenetic evidence concerning the nature of the renal reactions that do occur after $P \rightarrow F_1$ transfer.

The evidence in regard to the lack of effect of donor sensitization must be taken with reservation for it is based on retrospective analysis of data from experiments which were not designed to be definitive on this point and minor differences could well have been obscured. Simonsen (33) has shown that the differential effectiveness of sensitized *versus* normal lymphoid cells as inducers of a GVHR in neonatal mice varies inversely with the antigenic disparity of host and donor, and since the disparity between the BN and Lewis rat strains is great (14), one might expect little difference in the potency of cells from sensitized and normal donors to induce GVHRs.

But even if the crudity of technique leaves us unable to distinguish subtle differences in the virulence with which the inoculated cells attacked the underlying kidney, it is puzzling that there was not even a difference in *tempo*. It is not surprising that competent cells from normal donors should have become immunologically active by the 3rd day (34), but one does wonder why the GVHRs produced by cells from immune donors were not relatively more advanced at this early stage.

Possible Mechanisms by Which Lymphocytes Destroy Renal Parenchyma.—We are left with the problem as to just how the infiltrating mononuclears cause damage to homologous renal tissue. It seems certain that the mechanical and metabolic disturbances which the development of an active interstitial infiltra-

tion might set up are not sufficient to explain the destruction of the cortical tubules, for it has been shown that the presence of an immunologically inactive lymphomatous infiltrate is quite harmless in this respect. Moreover the results obtained with *in vitro* systems (10, 11) suggest that immunologically active lymphoid cells possess specific cytocidal properties for homologous cells with which they are in contact.

The histopathology of the renal GVHRs provides no visual suggestion that the invading cells are producing diffusible cytotoxic substances, such as classical humoral antibody, for no lesions were detected in tubules not intimately surrounded by mononuclears (Fig. 8). Whether or not such antibody was produced and then immediately and locally adsorbed by the surrounding parenchyma, or whether "cell-bound antibodies" (35–37) or lytic enzymes (36) were the weapons of the infiltrating mononuclears is beyond the scope of these experiments.

However, the finding that positive reactions resulted from injections of thoracic duct lymphocytes from *unsensitized* donors not only confirms Gowans' contention (4) that lymphocytes are competent to initiate as well as to mediate immune reactions, but it also means that the invading cells play a more active role in homologous tissue destruction than is implied by suggestions (36) that immunologically active lymphocytes serve only to *transport* antibody, produced elsewhere, into the target tissue.

SUMMARY

When lymphoid cell suspensions from the spleen, lymph nodes, blood, and thoracic duct of parental strain adult rats were injected beneath the renal capsule of F_1 hybrid hosts, the transferred cells and/or their progeny invaded the underlying renal cortex and destroyed most of the tubules which they surrounded. The immunogenetic conditions under which this reaction was observed defined it as a graft *vs.* host reaction (GVHR). On the 7th day the GVHRs were histologically similar to primary renal homografts undergoing rejection. Lymphoid cells from donors tolerant to the other parental strain were inactive after transfer to the hybrid, whereas cells from either normal or sensitized donors consistently produced reactions of about equal severity. Lewis lymphoma cells displayed malignant, invasive activity but did not destroy either isologous or homologous tissue, showing that the presence of an infiltrate was not *per se* sufficient to damage the parenchyma. These observations indicate that the GVHRs were manifestations of the ability of the transferred lymphocytes to enter into a homograft reaction with consequent destruction of renal parenchyma, and support the hypothesis that at least some of the lymphocytes which are seen infiltrating primary homografts are the agents which effect their destruction.

Dr. R. E. Billingham of The Wistar Institute provided the essential inspiration and opportunity to perform these experiments. To him and also to his colleague, Dr. W. K. Silvers, the author is greatly indebted. Also to be acknowledged is Dr. P. C. Nowell of the University of Pennsylvania School of Medicine for generously providing the guidance and facilities that made possible the chromosome studies.

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EXPLANATION OF PLATES

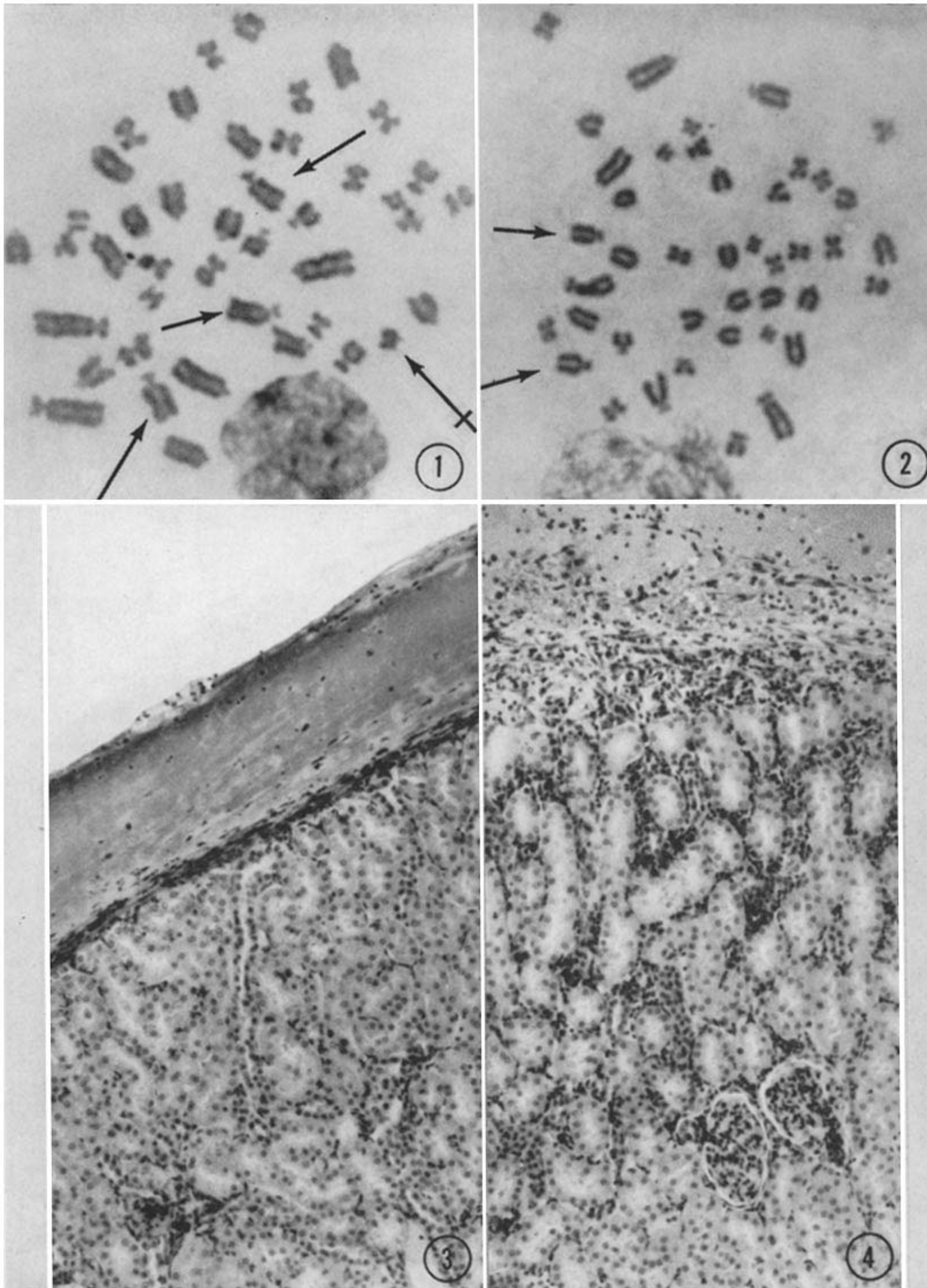
PLATE 30

FIG. 1. Male metaphase chromosome preparation from GVHR on 7th day after injection of 50 million spleen cells from *male Lewis donor* into F_1 female kidney. The smallest acrocentric, the Y chromosome, is indicated by the crossed arrow. The indistinguishable two No. 3 autosomes and one X chromosome are also marked. Aceto-orcein. $\times$ 820.

FIG. 2. Female metaphase preparation from the spleen of the same recipient. The two X chromosomes are in this instance distinguishable from the No. 3s and are marked with arrows. There is no Y. $\times$ 820.

FIG. 3. Three days after injection of 25 million blood leucocytes $P \rightarrow P$ there is a subcapsular hematoma but no cortical infiltration. Hematoxylin and eosin. $\times$ 200.

FIG. 4. GVHR on 3rd day after inoculation of 25 million blood leucocytes $P \rightarrow F_1$. Note definite but sparse infiltration of outer cortex. Hematoxylin and eosin. $\times$ 200.



(Elkins: Invasion and destruction of homologous kidney)

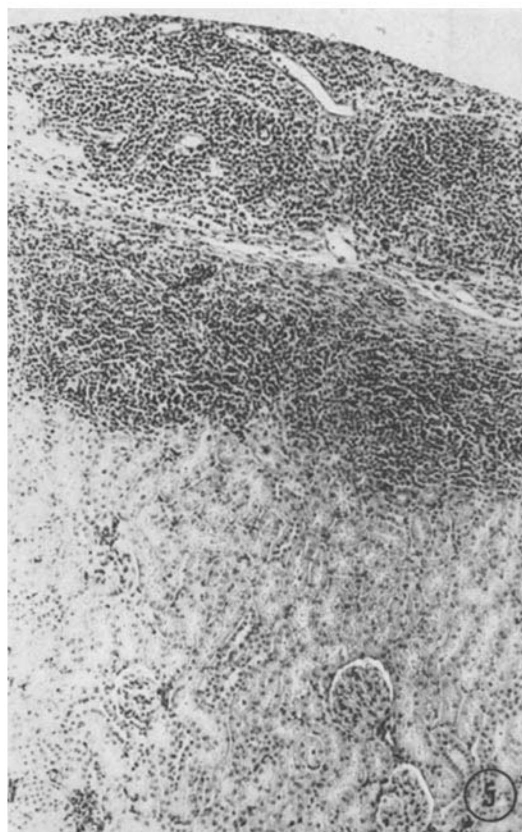
PLATE 31

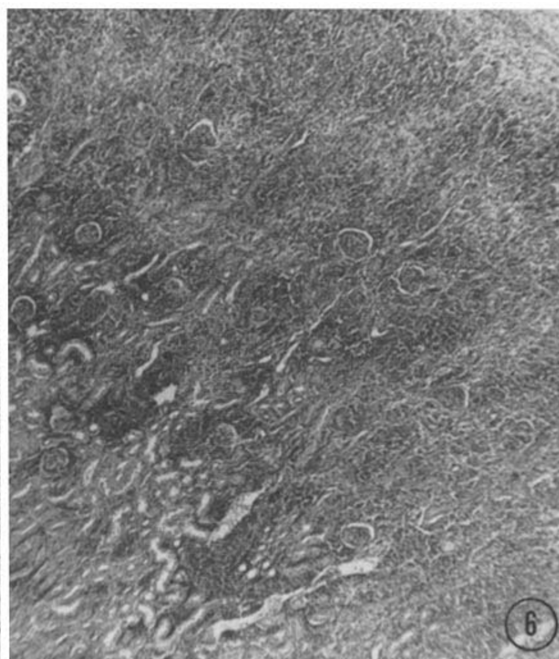
FIG. 5. A grade I GVHR on 7th day after injection of 25 million thymocytes $P \rightarrow F_1$. The subcapsular graft shows no invasive propensity. Hematoxylin and eosin. × 100.

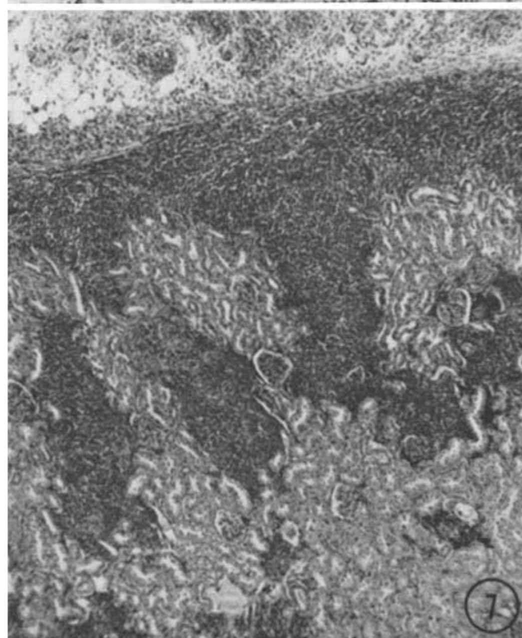
FIG. 6. Grade III GVHR on 7th day after injection of 25 million sensitized leucocytes. The invasive process is diffuse and obliterates normal parenchyma. Hematoxylin and eosin. × 50.

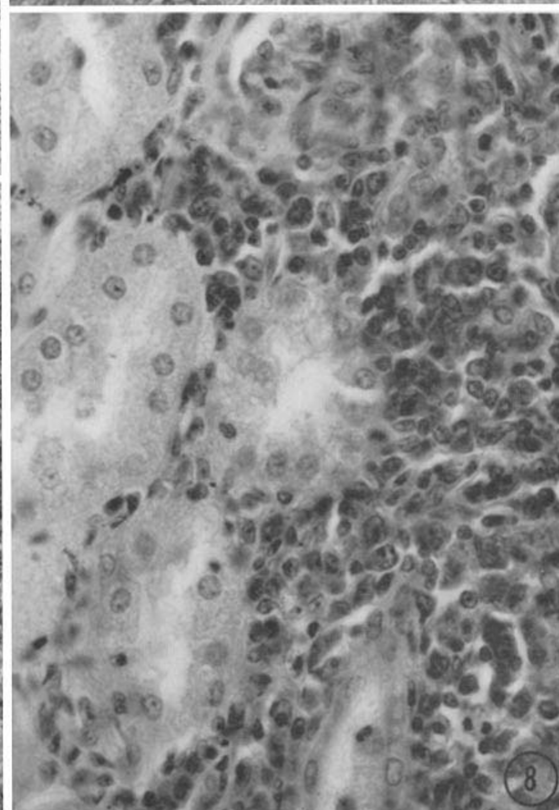
FIG. 7. A grade II GVHR on 7th day after injection of 50 million lymph node cells from sensitized donor. There are discrete invasive tongues. Hematoxylin and eosin. × 50.

FIG. 8. High-power view of margin of invasive tongue and normal parenchyma in the same grade II reaction depicted in Fig. 7. Degenerative changes are present only in tubule surrounded by mononuclears. Hematoxylin and eosin. × 350.









(Elkins: Invasion and destruction of homologous kidney)

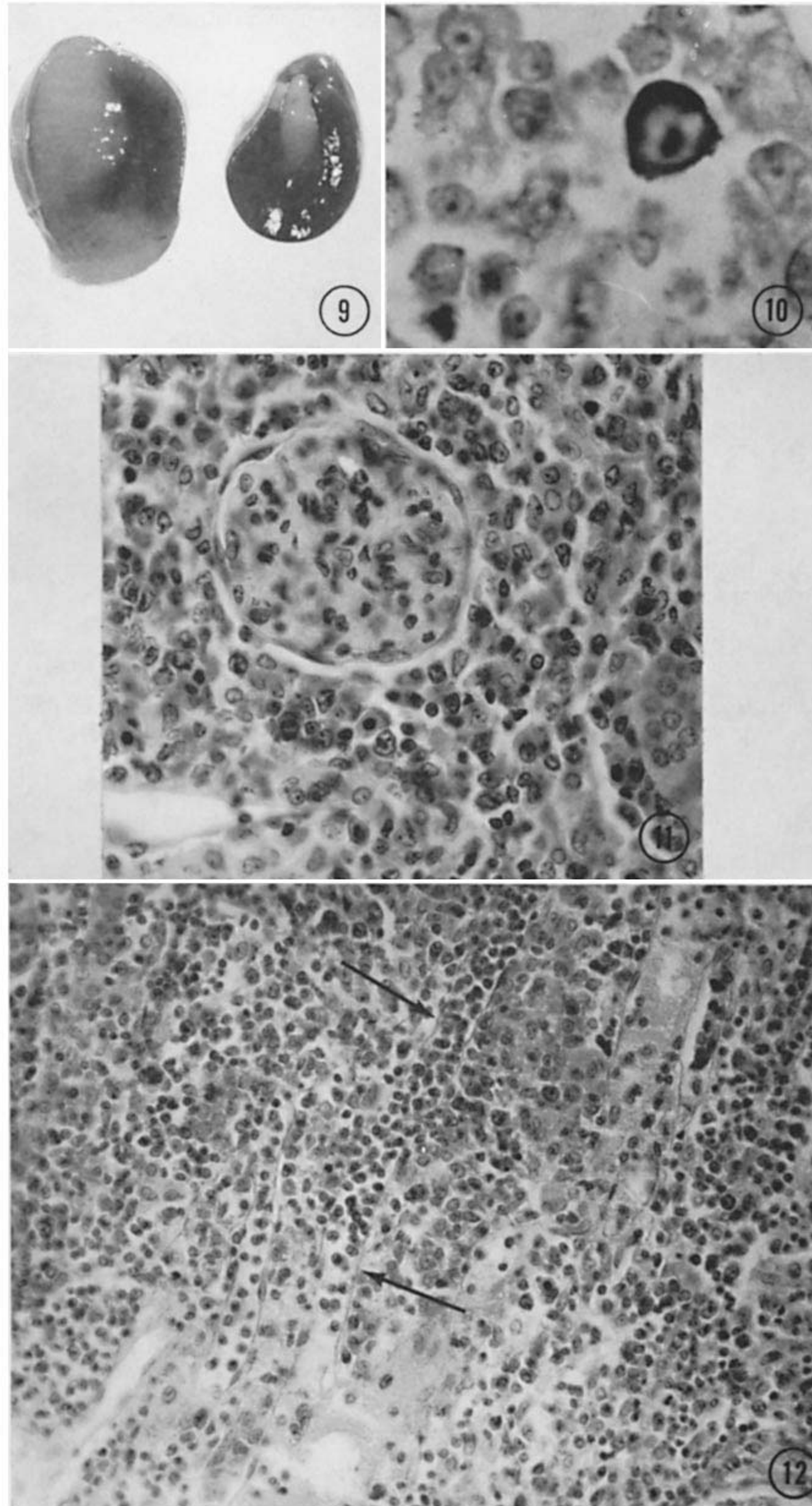
PLATE 32

FIG. 9. Macroscopic appearance of GVHR on 7th day after injection of 50 million spleen cells $P \rightarrow F_1$. The uninjected contralateral kidney is shown on right for comparison. $\times$ 2.

FIG. 10. Blast cell in infiltrate of 7th day GVHR. Transitional forms and lymphocytes are also present. Toluidine blue. $\times$ 880.

FIG. 11. Degenerating tubules and ischemic glomerulus in grade III GVHR on 7th day after injection of 25 million normal thoracic duct lymphocytes. Note the pleiomorphic mononuclear cell infiltrate and disaggregated epithelial cells therein. Hematoxylin and eosin. $\times$ 350.

FIG. 12. Illustrates plugging of dilated cortical venule by mononuclear cells in grade III reaction 7 days after injection of 200 million leucocytes from sensitized donor. Other vessels show earlier stages of occlusion. Note the absence of tubules. Hematoxylin and eosin. $\times$ 220.

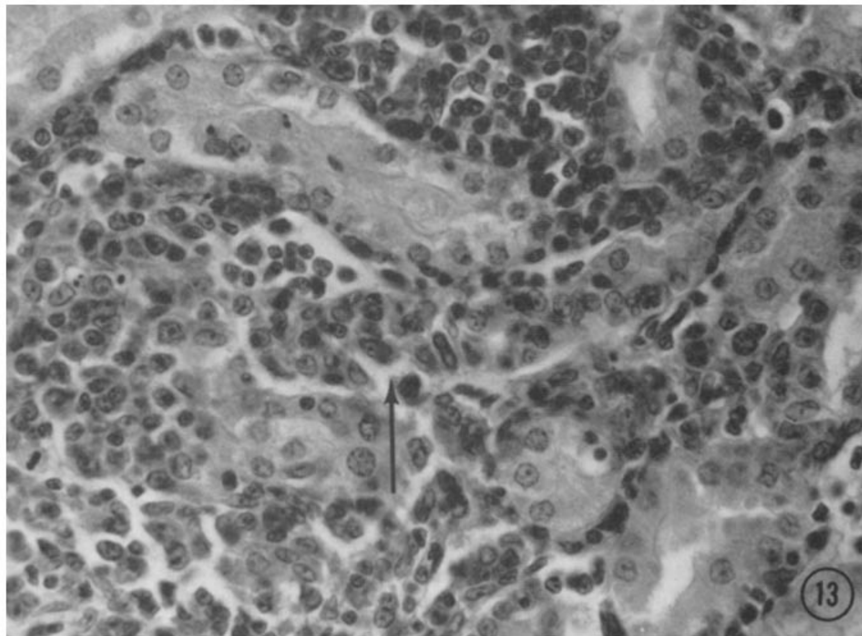


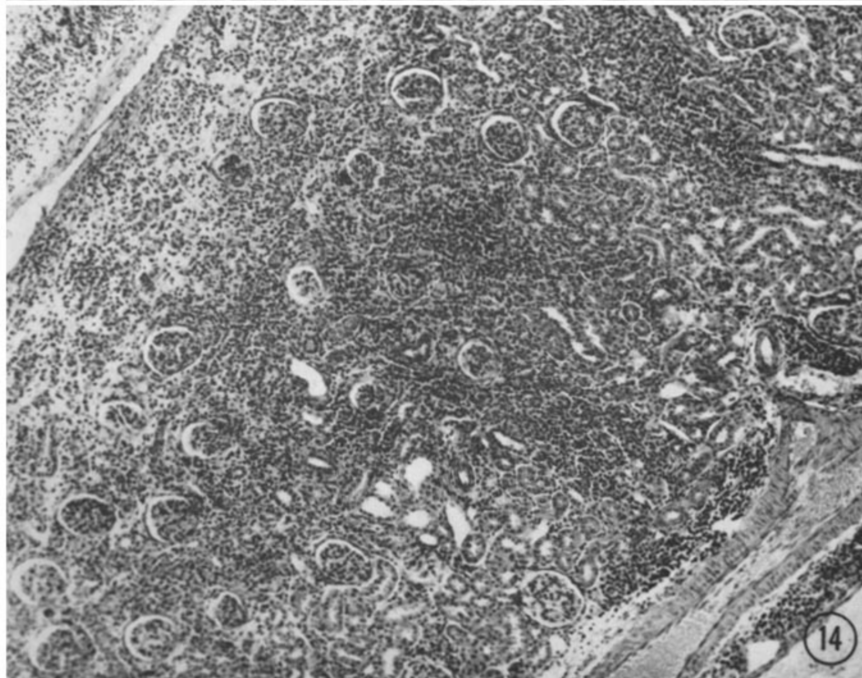
(Elkins: Invasion and destruction of homologous kidney)

PLATE 33

FIG. 13. Invasion of tubule by mononuclears (arrow), and atypical, atrophic tubules in grade III 7th day GVHR induced by 100 million leucocytes from sensitized donor. Hematoxylin and eosin. × 350.

FIG. 14. Grade III GVHR 14 days after injection of 60 million sensitized lymph node cells. The arcuate artery in lower right corner marks the corticomedullary junction. Practically all the cortical tubules have been destroyed in this sector. Hematoxylin and eosin. × 50.





(Elkins: Invasion and destruction of homologous kidney)

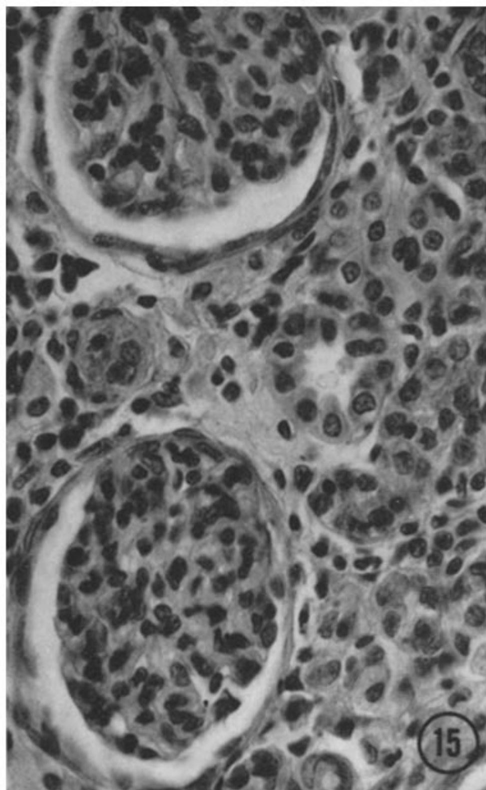
Plate 34

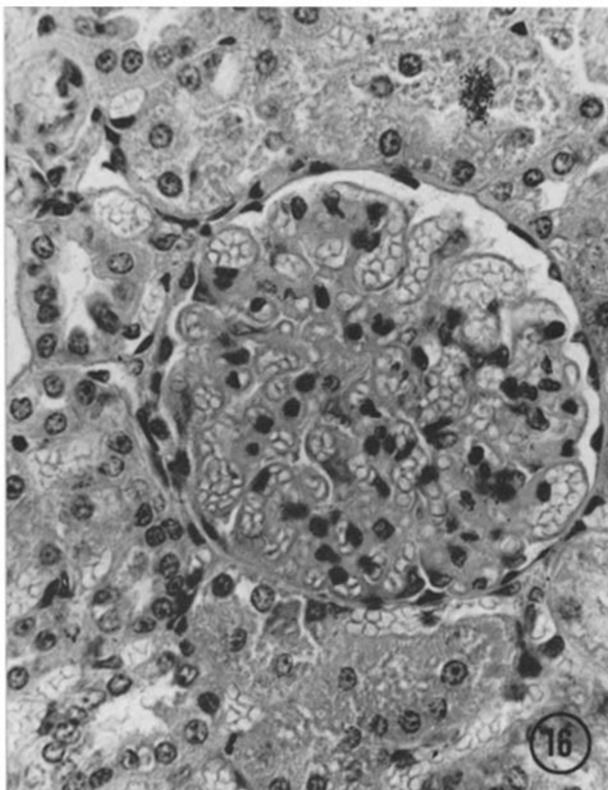
Fig. 15. GVHR on 14th day after injection of 60 million sensitized lymph node cells. The tubules are atrophic, the glomeruli ischemic, and the infiltrate has thinned. Hematoxylin and eosin. × 350.

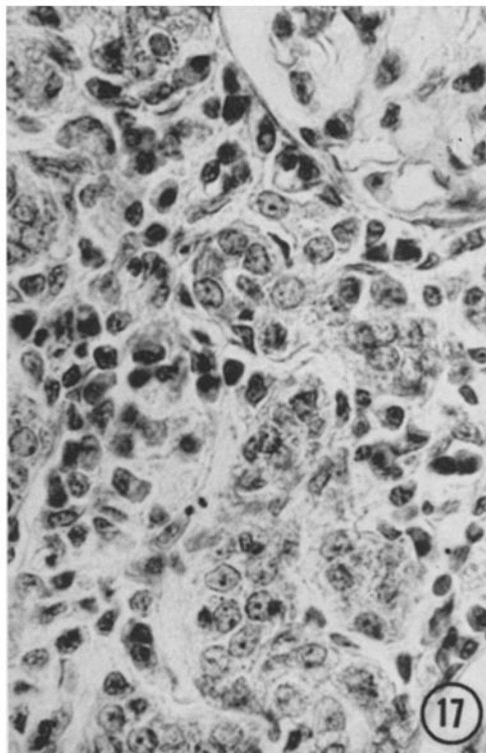
Fig. 16. Normal glomerulus and tubules outside reaction zone. The capillaries show to advantage. (Compare to Figs. 11 and 15.) Hematoxylin and eosin. × 350.

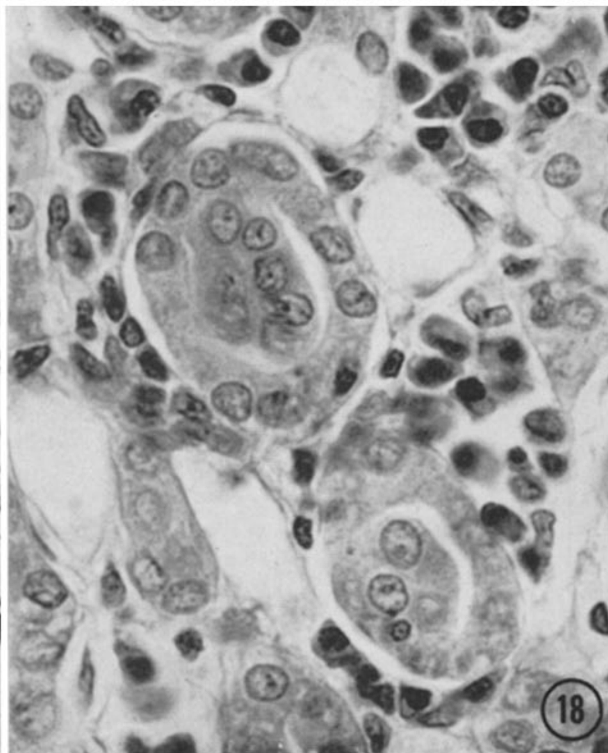
Fig. 17. Atrophic tubules and sparse infiltrate of an involuting GVHR 14 days after injection of 50 million spleen cells. Note mature pyroninophilic cells in the infiltrate. Pyronin and methyl-green. × 500.

Fig. 18. Nests of mature pyroninophilic cells with cytologic characteristics of plasmacytes, 14 days after injection of 100 million thoracic duct lymphocytes. Note also the intraluminal epithelial cell masses in collecting tubules. Pyronin and methyl-green. × 880.









(Elkins: Invasion and destruction of homologous kidney)

Plate 35

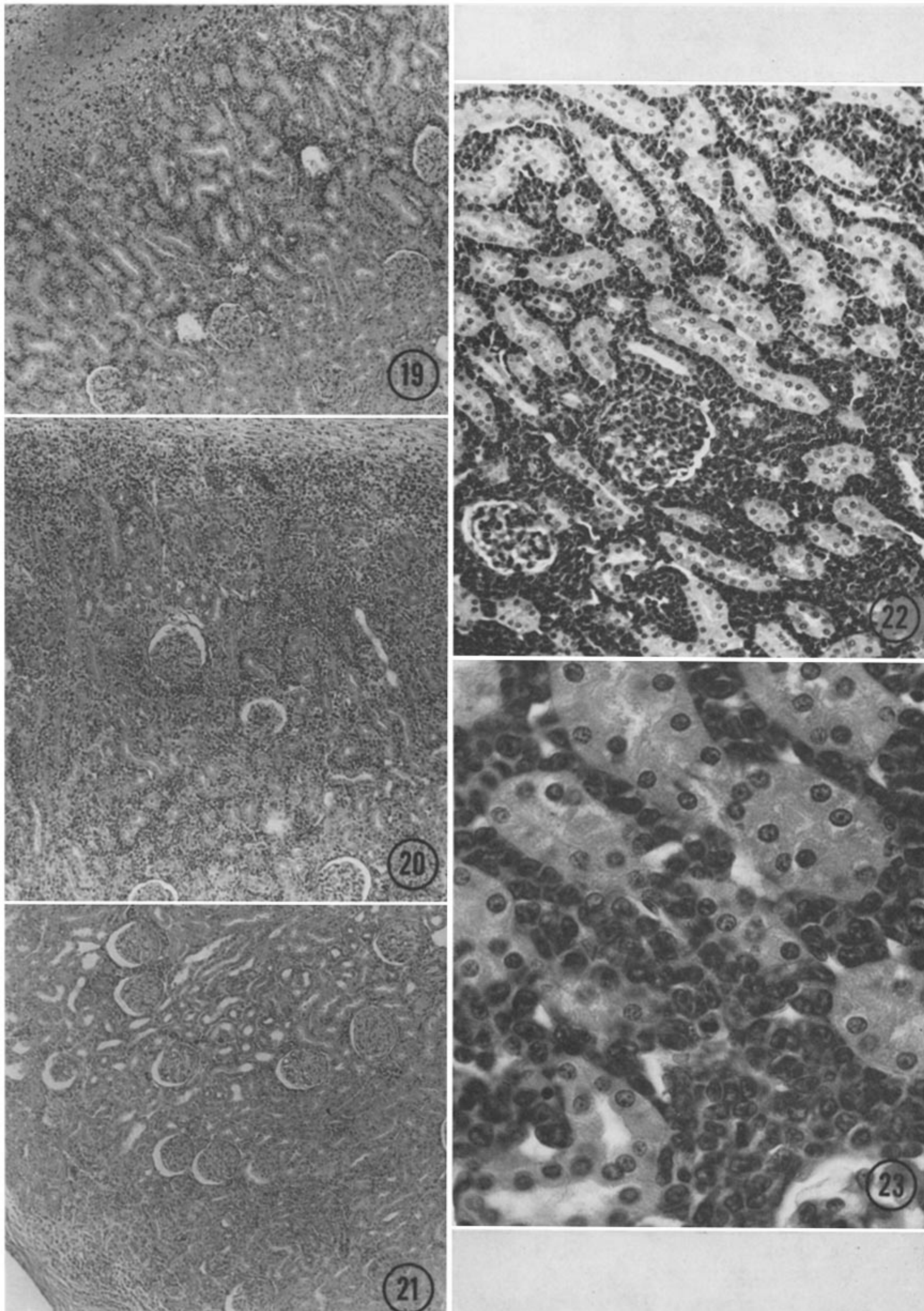
Fig. 19. Infiltration of outer cortex on 3rd day following transfer of 100 million lymph node cells from BN to Lewis ($P \rightarrow P'$). The reaction is similar to that shown in Fig. 4. Hematoxylin and eosin. × 100.

Fig. 20. Infiltration on 7th day following transfer of 50 million lymph node cells $P \rightarrow P'$. The mononuclear cells have invaded widely, but they are not so dense as in $P \rightarrow F_1$ reactions. Many tubules in outer rim have survived. Hematoxylin and eosin. × 100.

Fig. 21. Fourteen days after injection of 50 million leucocytes $P \rightarrow P'$. The infiltrate has practically cleared. Hematoxylin and eosin. × 100.

Fig. 22. Outer cortex of Lewis kidney 7 days after injection of isologous lymphoma cells. Note that many tubules persist despite the dense interstitial infiltrate. Hematoxylin and eosin. × 50.

Fig. 23. Malignant lymphoblasts and normal tubules from the same specimen. Hematoxylin and eosin. × 350.



(Elkins: Invasion and destruction of homologous kidney)