THE INTERACTION OF DONOR AND HOST LYMPHOID CELLS IN THE PATHOGENESIS OF RENAL CORTICAL DESTRUCTION INDUCED BY A LOCAL GRAFT VERSUS HOST REACTION

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Plates 15 to 20

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Simonsen has recently emphasized that graft *versus* host reactions (GVHR) are characterized by "the direction of the immunologic process which starts the development" (1). Thus in situations where the GVHR is induced by the injection of immunologically competent lymphoid cells from inbred parental strain (P) donors into an appropriate F_1 hybrid, the initiation of the process is unidirectional, as expressed in the term "graft *versus* host." Since the host is genetically tolerant of the attacking donor cells, it cannot muster an immune response against them; *i.e.*, an isoimmune host *versus* graft response is precluded. However, as Simonsen points out, other modes of participation by host lymphoid cells in the pathogenesis of the ensuing disease are by no means excluded. In fact, most studies have indicated that there is a definite, sometimes preponderant, proliferative response by the host's lymphoid cells as the GVHR develops (1–7). In some cases the host response can be attributed to an isoimmune host *versus* graft reaction, but even in the $P \rightarrow F_1$ hybrid situation, where such is precluded, the host response can be demonstrated (3, 4). The significance of this latter type of host response is shrouded in mystery, but it is almost certainly important in the pathogenesis of many GVHR's.

The experiments described below shed light on the pathogenesis of the GVHR which develops in hybrid rat kidney after the local inoculation of parental strain lymphoid cells. The immunogenetics and histopathology of this GVHR have been detailed elsewhere (8). Although the "unidirectional" immunogenetic circumstance requisite for the development of the lesion proved that donor cells were responsible for the *instigation* of the GVHR; the continuing role of such cells as the lesion developed, and the possibility of host mononuclear participation remained to be elucidated. The present report provides evidence for the mutual interdependence of both donor and host mononuclear cells in the development of the invasive-destructive lesion which is characteristic of this GVHR.

Materials and Methods

Animals.—Inbred Lewis (L), BN, and Buffalo (Bf) rats were obtained from Microbiological Associates, Bethesda, and (LBN)F_1 and (LBf)F_1 hybrids were bred therefrom. DA and

(LDA)F_1 rats were obtained from the Wistar Institute colony, Philadelphia, Pennsylvania, maintained by Dr. H. R. Ramseier and Dr. D. B. Wilson. Each of the 4 parental strains is isogenic as judged by skin grafting, and each strain is sufficiently diverse from the other 3 that skin allografts are all rejected within 10 days. Moreover, doses of 25 to 50 $\times$ 10^6 spleen cells from normal adult donors of each of the parental strains have proven competent to induce a local GVHR in F_1's derived from a cross with any one of the other 3 (9).

Induction of GVHR's in Primary Hosts.—Suspensions of spleen cells from 2- to 3-month-old parental strain donors were prepared in Hanks' balanced salt solution at room temperature. Fifty million cells in 0.1 cc were injected under the capsule of the left kidney of an appropriate F_1 as described previously (8). The hosts were sacrificed on the desired day and

TABLE I

*Baseline Values for Kidney Weight Ratio, Corrected Spleen Weight, and Cardiac Blood Leukocyte Count at Autopsy on 7th day in Negative Controls**

Dose‡	No.	Mean Ki/Kc§ (SE)	Mean S/Kc‖ (SE)	Mean leukocyte count (SE)
rad				
0	11	1.01 (0.01)	0.51 (0.03)	16,300 (3000)
1000	8	1.01 (0.01)	0.33 (0.07)	930 (200)
1200	4	0.98 (0.02)	0.22 (0.03)	560 (200)

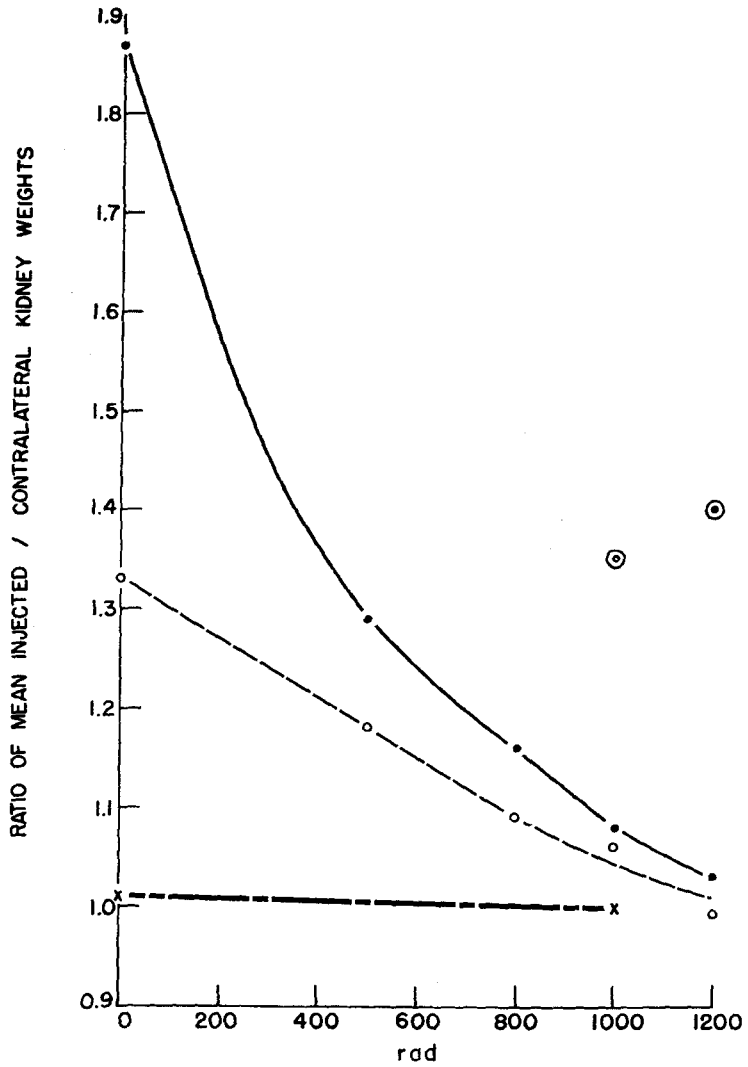
r for Ki/Kc and S/Kc = 0.17 ($p > 0.10$)¶
r for Ki/Kc and leukocyte count = 0.34 ($p > 0.10$)¶

* P $\rightarrow$ P, $F_1 \rightarrow F_1$, $F_1 \rightarrow$ P spleen cell inocula; *i.e., no* GVHR possible.
‡ Total body irradiation.
§ Weight inoculated/contralateral control kidney.
‖ Weight spleen/control kidney.
¶ Correlation coefficient, r, calculated for individual (N.B., not mean) values. The p values relate to probability that r differs from 0.

both kidneys and spleen removed. These were trimmed and weighed individually to the nearest milligram. Specimens of the injected kidney and the spleen were fixed in Tellyesniczky's or Bouin's fluid, embedded in paraffin, sectioned, and stained with hematoxylin-eosin or toluidine blue–eosin (Dominici).

Quantitation of Virulence of GVHR in Primary Hosts.—As shown in Table I and Text-fig. 1, the inoculation of lymphoid cells, which are for immunogenetic reasons incompetent to induce a GVHR in a given host, does not change the weight ratio of the injected/contralateral control kidney (Ki/Kc) from the expected value of 1. When, however, GVHR's are induced with competent inocula and the host autopsied after 7 days, this ratio (Ki/Kc) is elevated due to the tumorous mass of inflammatory tissue which constitutes the GVHR (8). The ratio Ki/Kc can be shown to depend upon the dose and type of parental cells inoculated (9), and provides a quantitative measure of the intensity of the 7th day GVHR.

The corrected spleen weight given by the ratio (S/Kc) of spleen weight to that of the control (*i.e.*, uninjected) kidney also reflects GVHR intensity. In this study the ratio S/Kc is further utilized to indicate the degree of damage to the host lymphoid system attributable to



TEXT-FIG. 1. Inhibition of GVHR by host irradiation prior to inoculation of lymphoid cells. Key: X- - -X incompetent inocula (negative controls); O- - -O L → (LBN)F_1 (whole-body irradiation); ●————● L → (LBf)F_1 (whole-body irradiation); ◎ lumbar irradiated L → (LBN)F_1; ◉ lumbar irradiated L → (LBf)F_1.

irradiation. The cardiac blood leukocyte count was also taken as a measure of the latter phenomenon. The base line values for these two parameters are shown in Table I.

Irradiation.—Two- to 3-month-old rats were irradiated in pairs in a lucite box from a Co^{60} source. For whole-body irradiation the factors were; field area, 15 x 15 cm, target distance, 55 cm, dose rate, 54.5 rad/minute, and back scatter factor, 1.034. For lumbar irradia-

tion the rats were sedated with chloral hydrate and stretched prone on a board. The field area was cut to 15 x 7 cm, forming a strip, the long axis of which traversed the lumbar area of 2 rats side by side. The upper border of this field was 1 cm cephalad to the last thoracic vertebra, so as to include the kidneys in the field. Each rat that received local irradiation in this fashion measured 21 to 24 cm from snout to base of tail, thus about ⅓ of the body length was irradiated. The prospective hybrid hosts ($LBNF_1$ and $LBfF_1$) were irradiated 24 hours prior to the injection of 50 million Lewis spleen cells, and the virulence of the ensuing GVHRs evaluated as described above.

TABLE II

Transfer of GVHRs of Various Duration into F_1 Hybrid and Donor Strain Secondary Hosts

Methods of transfer and type of secondary host*	No. positive/no. transfers (by histologic evaluation ‡ of reaction in secondary host)			
	Duration of GVHR before transfer			
	5 to 6 days	7 to 8 days	9 to 10 days	14 days
Suspension of cells (25 to 100 × 10^6) from primary GVHR into:				
Isogeneic F_1	2/2	8/13	1/7	—
Donor strain P	0/1	0/5	0/3	—
Allogeneic F_1	0/2	0/4		
Multiple fragments from primary GVHR into:				
Isogeneic F_1	6/7	18/20	3/8	2/4
Parental strain P	0/2	0/8	0/3	0/2
Allogeneic F_1	1/1	2/12	—	—
Combined Results in:				
Isogeneic F_1	8/9	26/33	4/15	2/4
Parental strain P	0/3	0/13	0/6	0/2
Allogeneic F_1	1/3	2/16	—	—

* The GVHR: (parental donor, P → primary host, F_1) transferred to isogeneic F_1, or allogeneic F_1 or donor strain P secondary hosts.

‡ Criteria for histologic evaluation: *positive;* infiltration and destruction of outer (aglomerular) cortex beneath graft, *equivocal;* infiltration only, *negative;* normal cortex beneath graft.

Transfer of GVHR's.—Virulent, *i.e.* extensive, GVHR's were selected for transfer from primary hosts which had been asphyxiated in ether on days 5 through 14. The tissue comprising the reaction was minced in Hanks' solution and transplanted beneath the renal capsule of isogeneic F_1, allogeneic F_1, or parental strain secondary hosts either as a cell suspension of 25 to 100 million cells in 0.1 cc or simply as multiple small fragment grafts.

Evaluation of Transferred GVHR's.—The secondary hosts were sacrificed 7 days after transfer, and the transverse section of kidney which possessed the graft was excised, fixed, and processed for histologic evaluation. The criterion whereby the transfer was judged positive was infiltration by mononuclears through the outer aglomerular cortical mantle with associated tubule destruction. Cases in which the outer cortex was infiltrated, but in which there was no definite parenchymal destruction, were classed as equivocal. When the cortex

underlying the graft remained free of infiltrate, the transfer was classified negative. Final histologic classification was performed as a blind procedure.

RESULTS

Transfer of the GVHR to Secondary Hosts.—Although previous work had indicated that practically all the *mitotic* cells in the 7th day GVHR were of donor type (8), more direct evidence was sought that these dividing donor cells continued to play an important role in the developing lesion. As Simonsen has pointed out, the successful transfer of a GVHR into a second F_1 hybrid, which is isogeneic with the primary host, constitutes solid evidence for the continuing reactivity of the original donor component against host antigens (1).

The local GVHR induced in hybrid rat kidney is well suited for studies involving transfer to secondary hosts. As the lesion develops from about the 5th day through the 2nd week after inoculation, it presents as a whitish, circumscribed tumorous mass. It is a simple matter to excise the lesion in bulk from the host kidney, mince it, and transfer it beneath the renal capsule of the dedired secondary host. The ability of the transferred "GVHR tissue" to give rise to a similar lesion in the second kidney can then be analyzed histologically.

The success with which the GVHR's were transferred to various secondary hosts is set forth in Table II. When the secondary hosts were isogeneic with the primary hosts, successful transfers were obtained as long as sufficient "reaction tissue" could be harvested for grafting, but the success rate and intensity of the reactions appeared to decline after the 9th day. The method of grafting multiple fragments of the reaction tissue yielded more distinct lesions more often than that involving inoculation of a cell suspension prepared from the GVHR, so most of the experiments were performed by the former technique.

In every case where tissue fragments were grafted, the graft was readily visualized as a whitish lesion on the renal surface. Histologically these *grafts* contained pleiomorphic mononuclear inflammatory cells, necrotic glomeruli, and tubular remnants diffusely scattered in a fibrous matrix. When the secondary host was of the parental strain (donor) type, the cortex remained uninvolved by infiltrate despite the presence of these overlying necrotic grafts (Table II, Figs. 1 *a* and 2 *a*). This indicated that those invasive destructive lesions seen in the kidneys of secondary hybrid hosts (Figs. 1 *b* and 2 *b*) were in fact due to propagation of the GVHR, and did not simply represent a nonspecific inflammatory response to the grafted necrotic tissue.

Furthermore, the histopathology of the positive lesions in hybrid secondary hosts was almost identical with that in the primary hosts (8). The outer cortex contained an interstitial infiltrate of pleomorphic mononuclear inflammatory cells, including "blast" forms (Fig. 3). Indeed, as with the GVHR's in primary host kidneys, these blast forms were most prominent in the more extensive lesions. Cells morphologically similar to plasmocytes were also seen but were

usually less prominent than in GVHR's of similar age (*i.e.*, 14 days) in primary hosts. Destruction of cortical tubules was seen only where infiltrating mononuclears had intimately surrounded a tubule (Fig. 4). Capillaries and venules within the new lesion were often plugged with mononuclears, which were distributed along vessels and around the glomeruli at the deep margin of the lesion (Fig. 1 *b*). These histologic similarities between the reaction in primary

TABLE III

Analysis of Discriminant Transfer by Pairs of Secondary Hosts Receiving Common GVHR of 5 to 8 Days' Duration*

GVHR in primary host; method of transfer		Secondary hosts and histologic results in each	
		Isogeneic F_1	Allogeneic F_1
L *vs.* (LBN)F_1	50×10^6 cells‡	LBN, pos.	(LDA), neg.
" " "	50×10^6 "	" neg.	(LBf), neg.
" " "	50×10^6 "	" pos.	" neg.
" " "	50×10^6 "	" pos.	" neg.
L *vs.* (LBf)F_1	50×10^6 "	(LBf), neg.	(LBN), neg.
" " (LBf)F_1	50×10^6 "	" pos.	" neg.
L *vs.* (LBN)F_1	m.f.§	(LBN), pos.	(LDA), pos.
" " "	"	" pos.	" equiv.
" " "	"	" pos.	" equiv.
" " "	"	" pos.	" pos.
" " "	"	equiv.	(LBf) neg.
" " "	"	" pos.	" neg.
" " "	"	" pos.	" equiv.
L *vs.* (LBF)F_1	"	(LBf), pos.	(LBN) neg.
" " "	"	" pos.	" neg.
" " "	"	" pos.	" neg.
" " "	"	" pos.	" equiv.
L *vs.* (LDA)F_1	"	(LDA), pos.	" equiv
" " "	"	" pos.	" pos.

Summary:

No. pairs in which GVHR in allogeneic secondary host exceeds that in isogenic 0
No. pairs in which GVHR in isogeneic secondary hosts exceeds that in allogeneic.... 14
No. ties..... 5

* Each GVHR transferred into two secondary hosts, one isogeneic and one allogeneic with respect to primary host; but both genetically tolerant of original parental strain donor.

‡ dissociated mononuclear inflammatory cells obtained from minced GVHR.

§ m.f.: multiple minced fragments of GVHR.

TABLE IV

Effects of Host Irradiation on GVHR Induced by 50 × 10^6 L Spleen Cells in (LBF)F_1 Hosts*

Dose	No.	Mean Ki/Kc (SE)	Mean S/Kc (SE)	Mean leukocyte count (SE)
rad				
0	5	1.78 (0.14)	0.95 (0.04)	7900 (1600)
500	4	1.29 (0.03)	0.51 (0.05)	3200 (500)
800	4	1.16 (0.01)	0.41 (0.05)	900 (250)
1000	3	1.07 (0.01)	0.39 (0.01)	1100 (130)
1200	3	1.03 (0.01)	0.38 (0.07)	320 (80)
1200 (lumbar)	4	1.40 (0.05)	0.67 (0.02)	5000 (1600)

r for Ki/Kc and S/Kc = 0.86 ($p < 0.001$)‡
r for Ki/Kc and leukocyte count = 0.92 ($p < 0.001$)‡

* Whole-body irradiation except where specified otherwise.

‡ Correlation coefficient, r, calculated for individual (N.B., not mean) values. The p values relate to probability that r differs from 0.

TABLE V

Effect of Host Irradiation in GVHR Induced by 50 × 10^6 L Spleen Cells in (LBN)F_1 Hosts*

Dose	No.	Mean (Ki/Kc (SE)	Mean S/Kc (SE)	Mean leukocyte count (SE)
rad				
0	11	1.33 (0.04)	1.01 (0.05)	7700 (1300)
500	4	1.18 (0.01)	0.55 (0.10)	2100 (600)
800	5	1.09 (0.03)	0.47 (0.04)	1600 (600)
1000	8	1.06 (0.02)	0.39 (0.02)	1600 (250)
1200	6	0.98 (0.03)	0.28 (0.03)	1300 (500)
1000 (lumbar)	3	1.36 (0.03)	0.73 (0.08)	6900 (2700)

r for Ki/Kc and S/Kc = 0.83 ($p < 0.001$)‡
r for Ki/Kc and leukocyte count = 0.59 ($p < 0.001$)‡

* Whole-body irradiation except where specified otherwise.

‡ Correlation coefficient, r, calculated for individual (N.B., not mean) values. The p values relate to probability that r differs from 0.

and secondary hybrid hosts substantiate the conclusion that the lesions in the latter were in fact successfully transferred GVHR's. Thus one can conclude that the donor cell population not only persists (8), but that it continues to possess the ability to propagate the GVHR in *isoantigenic* kidney for periods in excess of a week.

Discriminant Transfers.—Some further pertinent information was afforded by the results of experiments which were originally designed to investigate quite another point. It was first thought that the waning of invasive activity noted in GVHR's during the second week (8) might be due to the fact the parental type cells were becoming tolerant in the primary host. To test this possibility GVHR's of 6 to 8 days' duration were subjected to discriminant transfer. The experimental design is illustrated schematically below:

[Donor (P) → primary F_1 host] → secondary hosts

[AA ——→ (AB)F_1] ——→ (AB)F_1 isogeneic hybrid

└——→ (AC)F_1 allogeneic hybrid

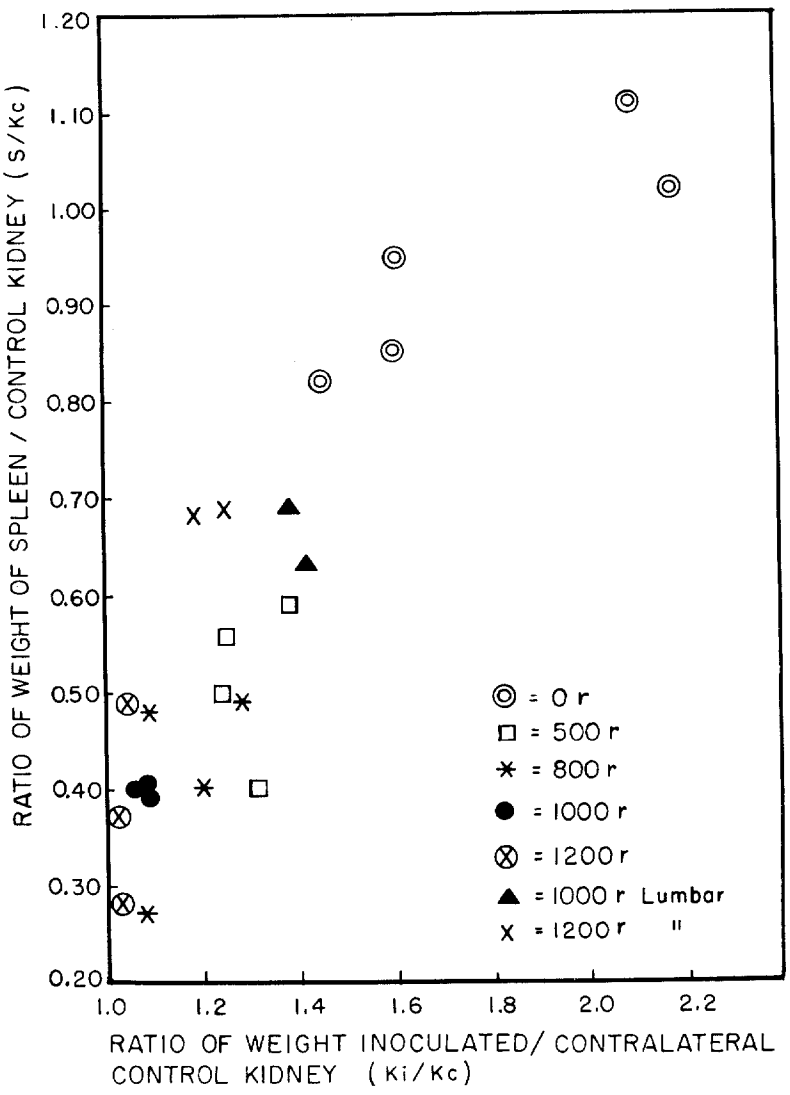
If the AA cells were becoming *specifically* tolerant of the B isoantigens in the primary host, transfers to the (AC)F_1 should manifest greater vigor than to the (AB)F_1 hybrid, for the AA cells would be stimulated anew by exposure to the C antigens. The results of 2 typical experiments are shown in Figs. 5 and 6, and the collective results of 19 such experiments are summarized in Tables II and III. It is immediately apparent that, far from the results predicted above, the GVHR's were not often transferable to the allogeneic (AC)F_1 hybrids. In all but 5 instances, all ties, the GVHR in the isogeneic secondary host was histologically more definitive than that in the allogeneic hybrid; thus one could *not* conclude that the AA cells were acquiring specific immunologic tolerance of B isoantigens. The failure of transferred GVHR's to induce lesions in allogeneic hybrid secondary hosts under such circumstances indicates that successful transfer of the GVHR depends upon continuing stimulation of the donor type cells with tissue of the same isoantigeneic constitution as that to which they reacted initially. If the donor cells are confronted with different antigen(s) their activity ceases and the GVHR comes to a halt. Thus this activity seems to be characterized by a definite immunologic specificity.

Inhibition of the GVHR by Host Irradiation.—Whole-body irradiation of the host *prior* to the injection of parental spleen cells was utilized to reduce selectively the suspected contribution of host mononuclears to the developing GVHR. Lumbar irradiation was employed to evaluate the possible local effects of irradiation on the development of the lesion. The pooled results of several experiments are shown in Tables IV, V, and Text-fig. 1. As the dose of irradiation increased and the host's lymphoid system was increasingly damaged (Tables IV, V, and Text-fig. 2), the virulence of the GVHR's, measured by Ki/Kc, declined as a curvilinear function in both types of hybrid. The inhibiting effect of irradiation was also reflected in the obvious reduction in the extent and density of mononuclear cell infiltration and degree of cortical destruction as viewed histologically (Fig. 7). On the other hand, lumbar irradiation had only a minor inhibitory effect on the development of the invasive-destructive reac-

tion and this was commensurate with the degree of radiation damage to the lymphoid system of the host (Tables IV, V, and Text-figs. 1 and 2). The GVHR is also inhibited in hosts which have been depleted of leukocytes by pretreatment with cyclophosphamide or amethopterin, in preirradiated hosts injected with spleen cells derived from *sensitized* donors, and in irradiated allogeneic hosts which are not genetically tolerant (*i.e.*, not F_1 hybrids) of the donor cells (9). These results indicate quite strongly that radiosensitive host mononuclear cells, presumably lymphocytes, play some essential role in the pathogenesis of the renal lesion. The observation that irradiation suppresses the GVHR *pari passu* as it depletes the host of lymphoid cells supports this conclusion. It should be noted that the requirements of the GVHR for host cells does not involve a threshold effect (Text-fig. 2).

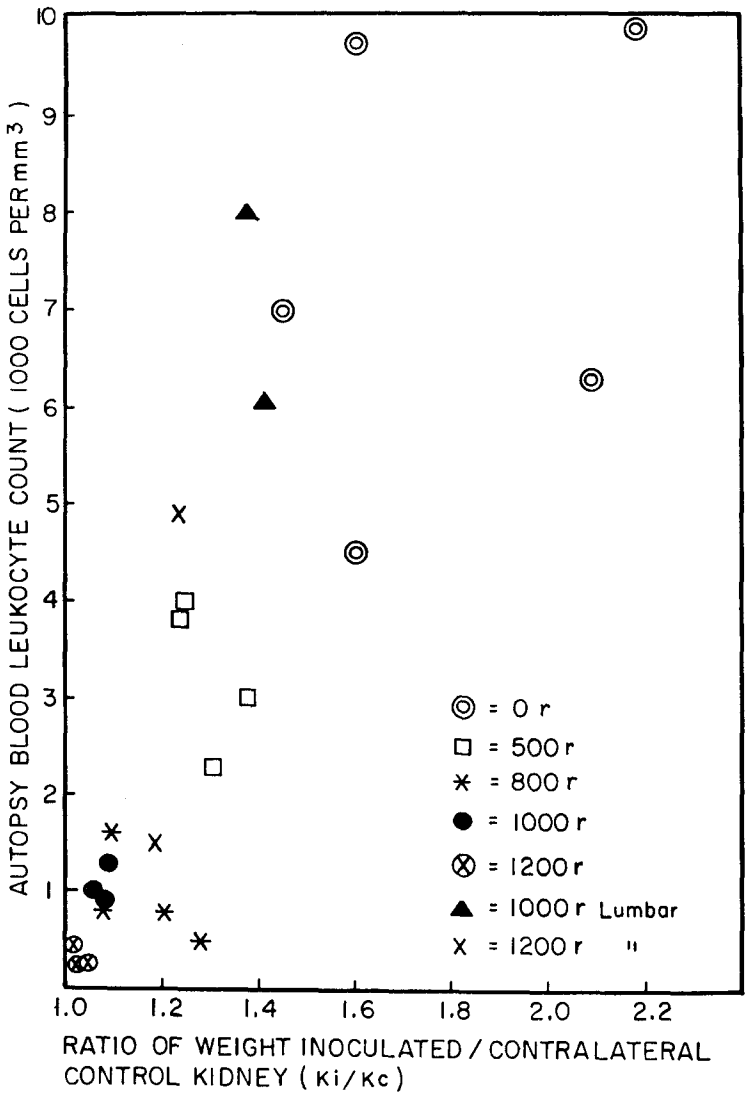
Transfer of GVHR's into Irradiated Secondary Hosts.—Taking the results of the transfer experiments together with those involving primary host irradiation, we can surmise that the 7th day GVHR consists of an immunologically active mononuclear cell infiltrate in which donor-type cells are somehow interacting with host cells with consequent damage to the local renal cortex. Accordingly the reaction tissue grafted into a secondary host consists of; (*a*) donor lymphoid cells, already stimulated by antigen and still responsive thereto, (*b*) that component of host mononuclears necessary to the full development of the GVHR in the primary host, and (*c*) remnant host kidney tissue. Would such an immunologically active, chimaeric complex be sufficient into itself to induce a GVHR in an isogeneic hybrid secondary host depleted of lymphoid cells by prior irradiation? The result of 7 parallel transfers of GVHR's into irradiated *versus* unirradiated, isogeneic hybrids were unequivocal. In no case did the GVHR propagate itself in the kidney of the irradiated secondary hosts, whereas the usual lesions developed in all the unirradiated controls (Fig. 8). This finding shows that the pathogenesis of this GVHR depends on *continuing* interaction of immunologically activated donor cells with a radiosensitive population of host cells within the lesion. In other words, even 7 days of interaction of donor cells with host kidney and/or host mononuclear cells in the primary host does not render the donor cells capable of propagating the lesion in the lethally irradiated secondary host. The continuing requirements for host mononuclears in the presence of appropriately antigenic kidney suggests that their role is not simply explained on the basis of their antigenicity.

The results with irradiated primary and secondary hosts require sophistication of the view that the infiltration is a simple centripetal process in which donor-type cells invade the parenchyma from the subcapsular space. This process no doubt occurs, but at least as important is the centrifugal migration of host mononuclears from the small vessels of the cortex into the interstitial tissue of the cortex.



TEXT–FIG. 2 *a*

TEXT-FIGS. 2 *a* and 2 *b*. Correlation of inhibitory effect of host irradiation on GVHR with suppression of host lymphoid system in L → (LBf)F_1 combination. (*a*) Kidney weight ratio (Ki/Kc) and corrected spleen weight ratio (S/Kc). (*b*) Kidney weight ratio (Ki/Kc) and cardiac blood leukocyte count at autopsy.


AUTOPSY BLOOD LEUKOCYTE COUNT (1000 CELLS PER mm 3)
10
9
8
7
6
5
4
3
2
1
1.0
1.2
1.4
1.6
1.8
2.0
2.2
RATIO OF WEIGHT INOCULATED / CONTRALATERAL CONTROL KIDNEY (Ki/Kc)
◎ = 0 r
□ = 500 r
✳ = 800 r
● = 1000 r
⊗ = 1200 r
▲ = 1000 r Lumbar
X = 1200 r "

TEXT-FIG. 2 *b*

DISCUSSION

The present experiments employed two techniques, host irradiation and transfer of the ongoing GVHR, in an attempt to define the respective roles of host and donor mononuclear cells in the invasive-destructive lesion of a certain GVHR (8). The results suggest that both populations play distinctly different, but mutually interdependent, roles in the pathogenesis of this reaction.

Prior studies (8) had shown that small lymphocytes in the parental strain donors were responsible for initiating a GVHR after inoculation into F_1 hybrid host kidney. Moreover, the presence of dividing donor cells was demonstrated by means of chromosomal markers at the peak of the developmental phase of the reaction, while no evidence was obtained which indicated the participation of host inflammatory cells. But demonstration of presence or absence of *dividing* cell types in the lesion can provide only partial information as to the actual constitution of the infiltrate, and provides no information as to the functions of the different elements therein.

The present studies delineate more clearly the role of the donor cells over and above initiation of the process. When the GVHR is transferred to the kidney of an F_1 hybrid host isogeneic with the primary host, a lesion histologically similar to the original reaction develops. On the other hand, when the GVHR is transferred to secondary hosts of the parental strain, the lesion does not propagate. This shows that the donor elements in the primary GVHR continue to possess reactivity against foreign host antigens and that the reaction ceases if the donor cells are transferred to a non-antigenic kidney. Moreover, the experiments involving discriminant transfer indicate that the transferred donor cells apparently possess the capacity to react only against those foreign isoantigens to which they had originally responded in the primary host. The specificity of propagation of the GVHR is perhaps related to a cell-bound antibody which enables the cell to recognize the histocompatibility factors of other cells.

The role of the donor cells then is recognition of antigen, initiation of the GVHR, and maintenance thereof by continuous, immunologically specific reactivity. Their energies in this respect are obviously limited, for the GVHR begins to wane during its 2nd week, the stage during which the "blast" cells disappear from the infiltrate.

The essential involvement of host cells was uncovered by the irradiation experiments. Clearly the developing GVHR has a requirement for radiosensitive host mononuclears, for any reduction in their availability results in a proportional diminution of the renal lesion. This conclusion is sustained by preliminary studies which indicate that it is possible to achieve renal GVHR's in heavily irradiated hosts by inclusion of hybrid host-type lymphoid cells along with the parental component in the inoculum. The development of other GVHR's, both local and systemic, has in some cases been inhibited by prior

host irradiation (4, 6, 10), whereas the virulence of others has been enhanced by such treatment (11–13). Thus the striking effect observed here is neither unique nor universal, but certainly it raises interesting possibilities about the ways in which inflammatory mononuclear cells can operate.

The function of the host cells in this GVHR is not immediately evident. Three alternative, but not mutually exclusive, hypotheses will be considered here.

(*a*) The host mononuclears are the effectively antigenic cells to which donor cells respond, the kidney being simply bland in this respect. This explanation seems unlikely for Gowans has reported the sensitization of lymphocytes as a consequence of their perfusion through an isolated allogeneic kidney (14), and Wilson has employed rat kidney cells as antigenic targets for isoimmune lymphoid cells *in vitro* (15). Assuming the kidney is effective as antigen, it is difficult to imagine why the requirement for host mononuclears should be a *continuing* one, unless they serve some function other than as antigen.

(*b*) The host cells may be required in increasing numbers as the GVHR develops in order to permit increasing numbers of donor cells bring about the full expression of the GVHR. In other words, the host mononuclears may play an important trophic role for the attacking donor force. This possibility seems unlikely but must be experimentally evaluated.

(*c*) The host cells are the constituents of an inflammatory process evoked by the activity of the donor cells, and are in this role somehow the effectors of parenchymal destruction. This hypothesis is entertained more fully below.

Whatever its role, the host component in the renal infiltrate must be considered "non-specific" in the sense that it cannot be there as an immune response to foreign antigen. The hybrid host is theoretically genetically tolerant of the donor component, and in fact no immune response against parental lymphoid cells, analogous to that observed by Cudkowicz and Stimpfling in certain mice (16), can be demonstrated in these rats (9). The situation here appears similar to that demonstrated in the transfer reaction in rabbits and hamsters (5, 10), in experimental allergic uveitis (17), in skin allograft rejection, and in certain lesions of classical delayed hypersensitivity (see reviews; 14, 18, 19). In each of these cases the mononuclear cell infiltrate has been found to consist largely of non-specific cells, and the specificity of these reactions has therefore usually been attributed to the activities of a certain minority of specifically sensitized cells which may recruit or instruct the former component (18, 19).

The situation in the renal GVHR may be pertinent in this regard, for here the separate function of each population is at least partially delineated. The donor component, derived from the small lymphocytes which initiated the reaction, is active all during the development of the lesion and since the driving force of the reaction is specific, this property is conferred upon the whole process.

However, by themselves the donor-type cells cannot do much damage to the kidney or even generate more than a very sparse local infiltrate. The host component is somehow necessary for the full development of interstitial infiltration and parenchymal destruction.

The implication of two populations of mononuclear cells of differing, but interdependent, function in the pathogenesis of this GVHR renders it comparable to that of experimental secondary allergic uveitis. Silverstein (17) has clearly shown that the pathogenesis of the mononuclear inflammatory lesion characteristic of this disease also involves the interaction of two populations of cells. The disease is induced *per primum* by the injection of foreign protein into the anterior chamber of the rabbit eye. After the primary reaction subsides the eye appears normal, but a sparse population of antigen-sensitive mononunuclears has been seeded in the uveal tissue. If antigen is subsequently administered by some other route, when it reaches the eye it activates these specifically sensitive "memory" cells. The secondary reaction develops within the next 24 hours, and by appropriate autoradiographic technique Silverstein showed that most of the inflammatory mononuclear cell infiltrate was derived from circulating cells. Since the secondary lesion develops during the period of antigen excess in the blood, he reasoned that this latter infiltrate must represent a nonspecific component which had been evoked by the activated memory cells. The inflammatory condition in the eye was thought to result from the presence and activity of the recruited cells.

Certainly more experimental work will be required before it is clear as to the nature of the interaction between specific and non-specific (or donor and host) mononuclear cells in these and similar lesions. For obvious reasons the interaction of donor and host lymphoid cells cannot be incriminated for every lesion in every GVHR. Nevertheless, interactions of the sort postulated here may be important in the pathogenesis of some inflammatory processes which are mediated by immunologically active mononuclear cell infiltrates.

SUMMARY

The graft *versus* host reaction (GVHR), which results from the injection of parental strain spleen cells beneath the kidney capsule of F_1 hybrid rats, is transferable during its developmental phase into F_1 hybrid hosts isogeneic with the primary host, but not into secondary hosts of the parental (donor) strain. Furthermore, the GVHR propagates but rarely in secondary hybrid hosts which are allogeneic with respect to the primary hosts, but which are also genetically tolerant of donor-type cells. These findings indicate that the donor cells not only initiate the GVHR but also maintain it by virtue of immunologically specific activity.

Whole-body irradiation of (LBf)F_1 and (LBN)F_1 hosts 24 hours prior to the injection of parental (L) spleen cells results in inhibition of the subsequent

GVHR to a degree commensurate with the radiation damage sustained by the lymphoid system of the host. Furthermore, propagation of transferred GVHRs did not occur if susceptible secondary hybrid hosts had been previously irradiated. These findings indicate that radiosensitive host cells play a continuing and essential role in the pathogenesis of the invasive-destructive lesion. It is concluded that the development of this lesion depends upon the continuous interaction of the specifically reactive donor-type cells with an immunologically non-specific population of host mononuclears.

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BIBLIOGRAPHY

1. Simonsen, M., Graft versus host reactions, their natural history, and applicability as tools of research, *Progr. Allergy*, 1962, **6**, 349.
2. Davies, A. J. S., and Doak, S. M. A., Fate of homologous adult spleen cells injected into newborn mice, *Nature*, 1960, **187**, 610.
3. Fox, M., Cytological estimation of proliferating donor cells during graft vs. host disease in F_1 hybrid mice injected with parental spleen cells, *Immunology*, 1962, **5**, 489.
4. Howard, J. G., Michie, D., and Simonsen, M., Splenomegaly as a host response in graft-versus-host disease, *Brit. J. Exp. Path.*, 1961, **42**, 478.
5. Kosunen, T. V., and Dvorak, H. F., The "transfer reaction" in the rabbit, II. Radioautographic study, *Lab. Inv.*, 1963, **12**, 628.
6. Seto, F., and Albright, J. F., An analysis of host and donor contributions to splenic enlargement in chick embryos inoculated with adult chicken spleen cells, *Develop. Biol.*, 1965, **11**, 1.
7. Mun, A. M., and Burns, E. R., Donor-host cell interaction in homologous splenomegaly in the chick embryo, *Biol. Bull.*, 1964, **127**, 467.
8. Elkins, W. L., Invasion and destruction of homologous kidney by locally inoculated lymphoid cells, *J. Exp. Med.*, 1964, **120**, 329.
9. Elkins, W. L., unpublished observations.
10. Ramseier, H., and Streilein, J. W., Homograft sensitivity reactions in irradiated hamsters, *Lancet*, 1965, **1**, 622.
11. Cole, L. J., and Ellis, M. E., Delayed deaths in sublethally x-rayed F_1 hybrid mice injected with parental strain spleen cells, *Science*, 1958, **128**, 32.
12. Cosgrove, G. E., Upton, A. C., and Congdon, C. C., Influence of radiation dose on induction of foreign spleen reaction in F_1 mice, *Trans. Bull.*, 1962, **30**, 29.
13. Medawar, P. B., and Brent, L., Nature of the normal lymphocyte transfer reaction, *Nature*, 1964, **204**, 90.
14. Gowans, J. L., Role of lymphocytes in destruction of homografts, *Brit. Med. Bull.*, 1965, **21**, 106.
15. Wilson, D. B., The reaction of immunologically activated lymphoid cells against

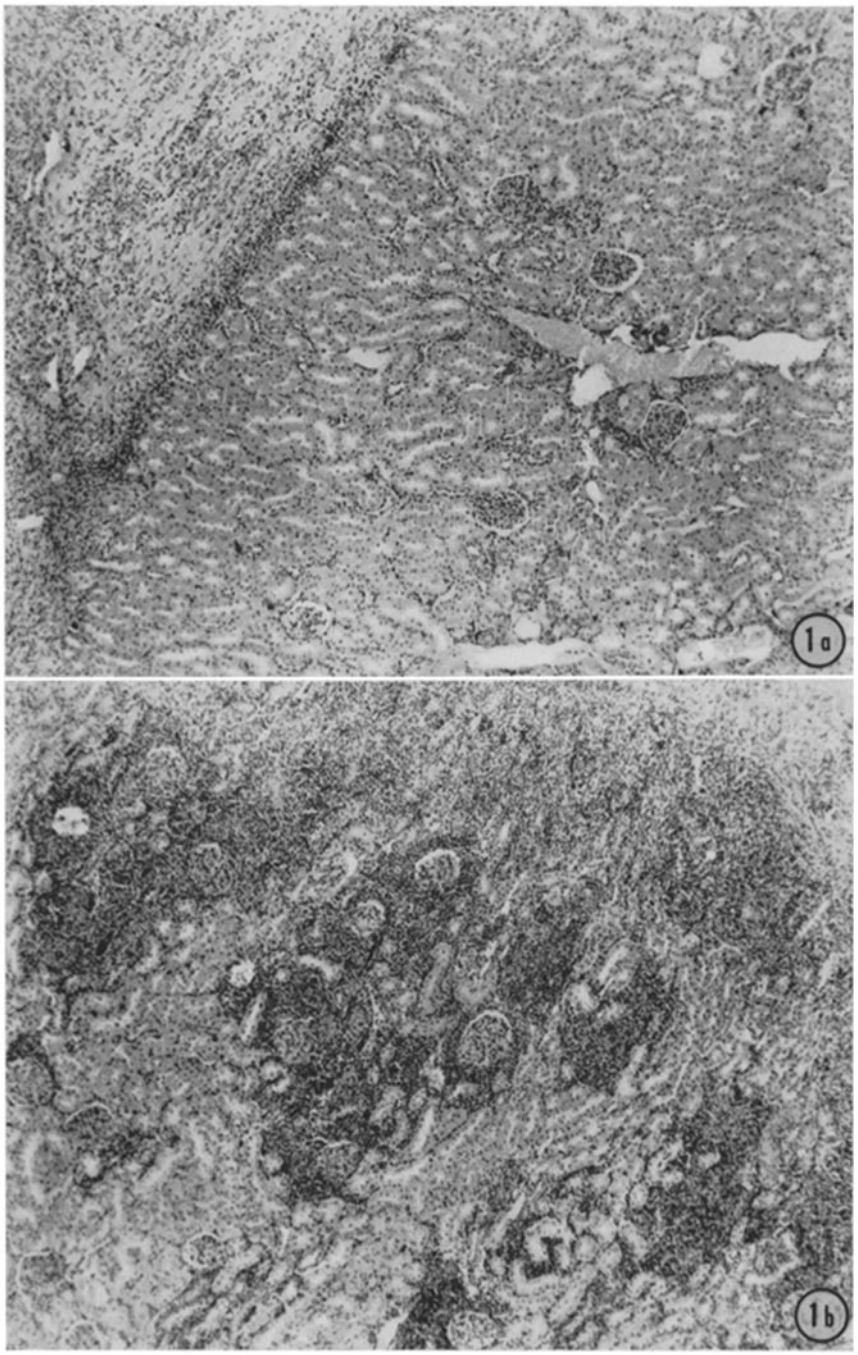
homologous target tissue cells in vitro, *J. Cell. and Comp. Physiol.*, 1963, **62,** 273.

16. Cudkowicz, G., and Stimpfling, J. H., Induction of immunity and of unresponsiveness to parental marrow grafts in adult F_1 hybrid mice, *Nature*, 1964, **204,** 450.
17. Silverstein, A. M., Ectopic antibody formation in the eye: pathologic implications, *in* Immunopathology of Uveitis, (A. E. Maumenee and A. M. Silverstein, editors), Baltimore, Williams & Wilkins, 1965, 83.
18. Benacerraf, B., and McCluskey, R. T., Methods of immunologic injury to tissues, *Ann. Rev. Microbiol.*, 1963, **17,** 263.
19. Waksman, B. H., The local reaction of cellular hypersensitivity, *Ann. New York Acad. Sc.*, 1964, **116,** 1045.

EXPLANATION OF PLATES

PLATE 15

FIG. 1. Results of transfer of BN *versus* (LBN)F_1 GVHR on 7th day into BN (*a*) and into (LBN)F_1 (*b*) secondary hosts. Hematoxylin and eosin, × 61.



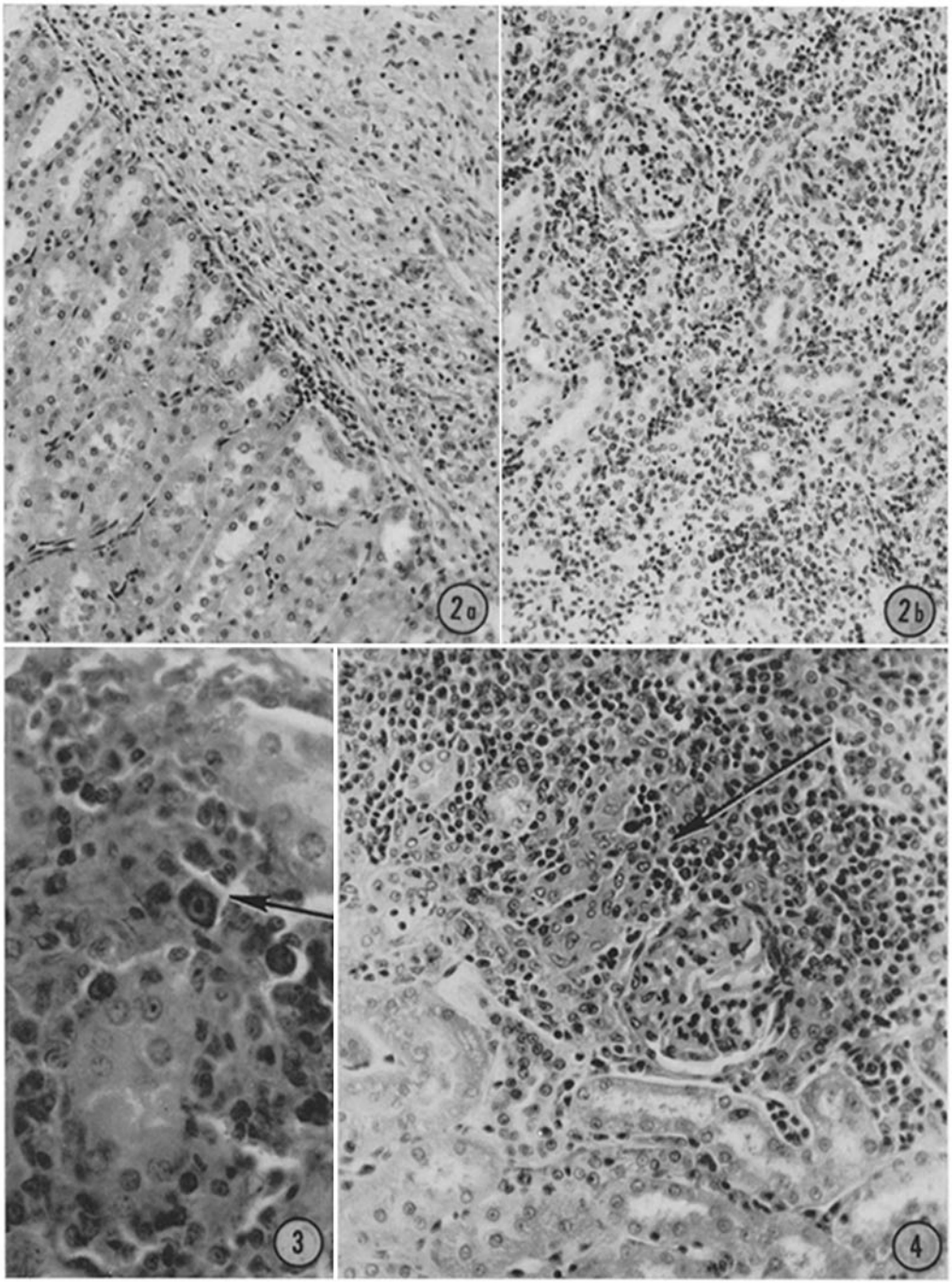
(Elkins: Donor and host lymphoid cells)

PLATE 16

FIG. 2. Results of transfer of L *versus* (LBf)F_1 GVHR on 7th day into L (*a*) and into (LBf)F_1 (*b*) secondary hosts. Hematoxylin and eosin, × 209.

FIG. 3. Pleomorphic mononuclear cell infiltrate and degenerating tubules in kidney of secondary host following transfer of GVHR. The arrow indicates cell referred to in text as a "blast." Dominici, × 343.

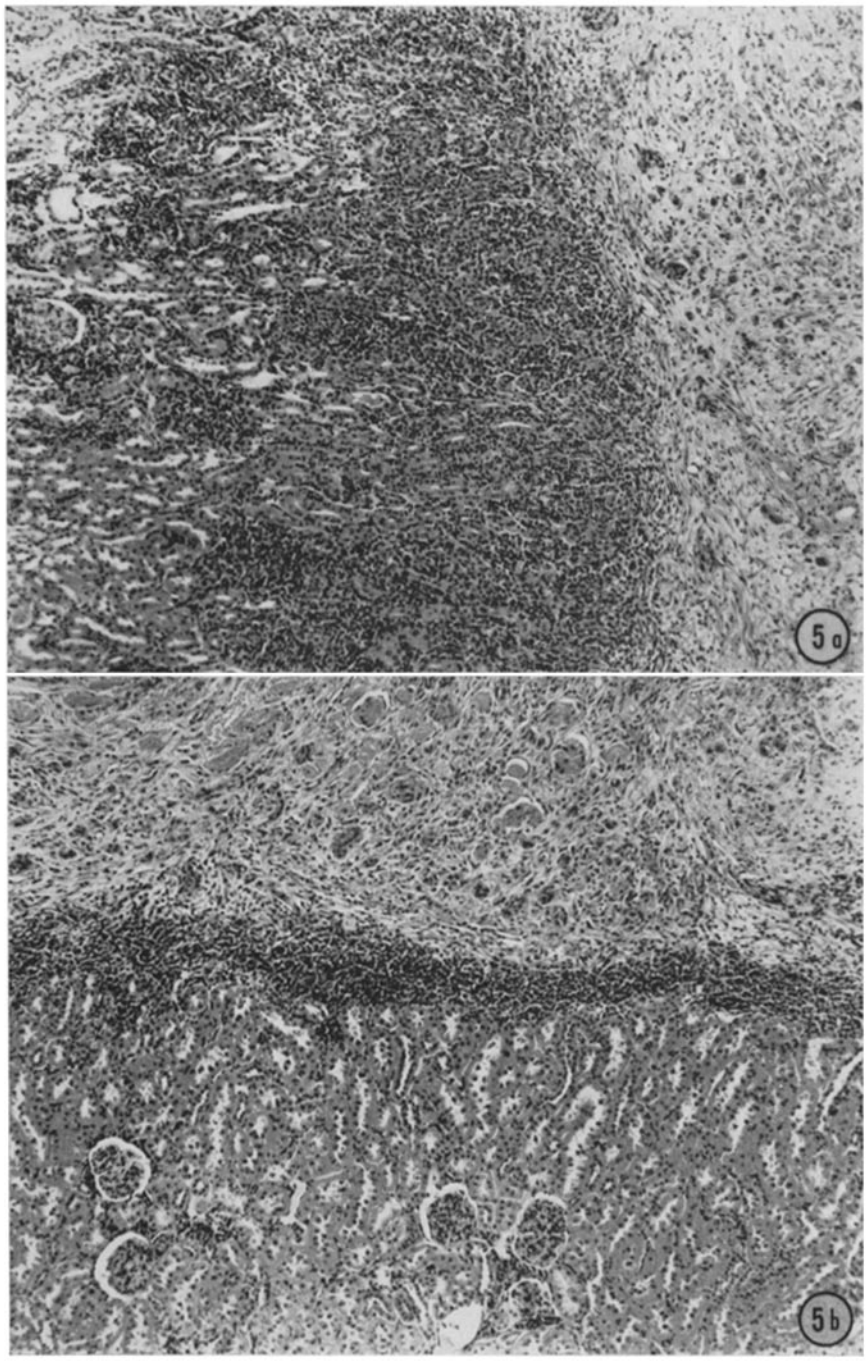
FIG. 4. Only those tubules which have been intimately invested by mononuclears appear to undergo destruction. The lesion developed in (LBN)F_1 secondary host kidney following transfer of L *versus* (LBN)F_1 GVHR. Hematoxylin and eosin, × 209.



(Elkins: Donor and host lymphoid cells)

PLATE 17

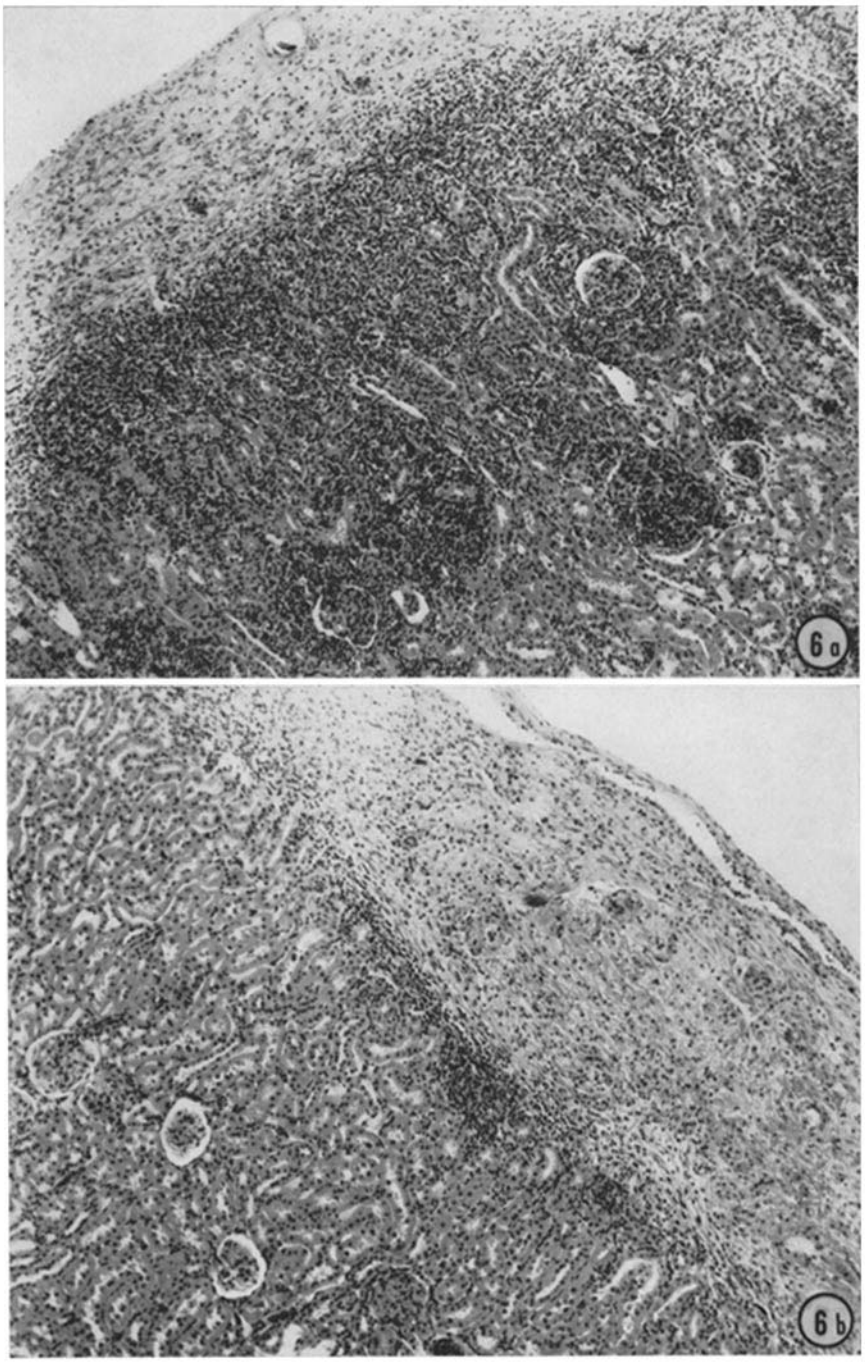
FIG. 5. Results of differential transfer of L *versus* (LDA)F_1 GVHR on 7th day into (LDA)F_1 (*a*) and (LBN)F_1 (*b*) hybrid secondary hosts. Hematoxylin and eosin, × 61.



(Elkins: Donor and host lymphoid cells)

PLATE 18

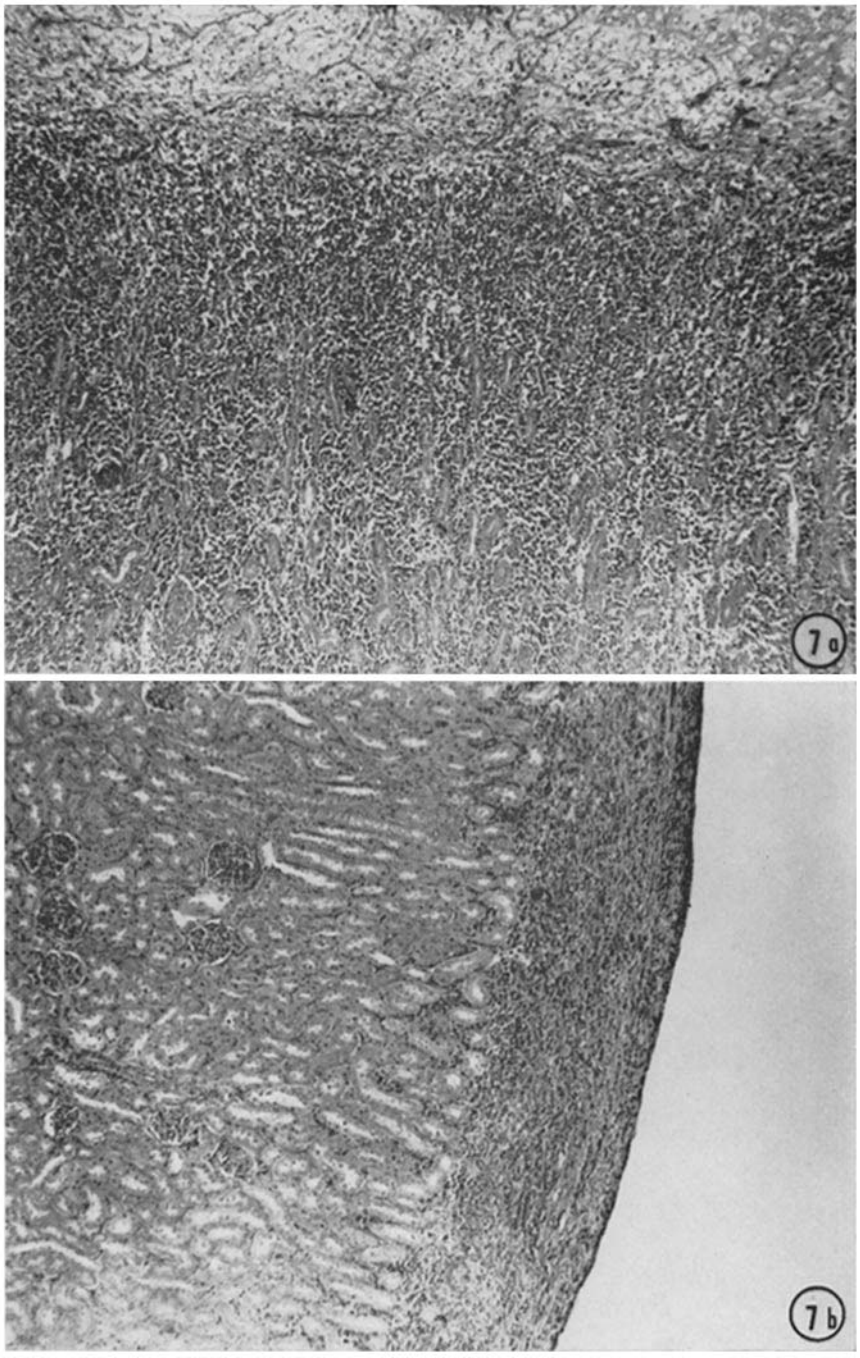
FIG. 6. Results of differential transfer of L *versus* (LBN)F_1 GVHR on 7th day into (LBN)F_1 (*a*) and (LDA)F_1 (*b*) hybrid secondary hosts. Hematoxylin and eosin, × 61.



(Elkins: Donor and host lymphoid cells)

PLATE 19

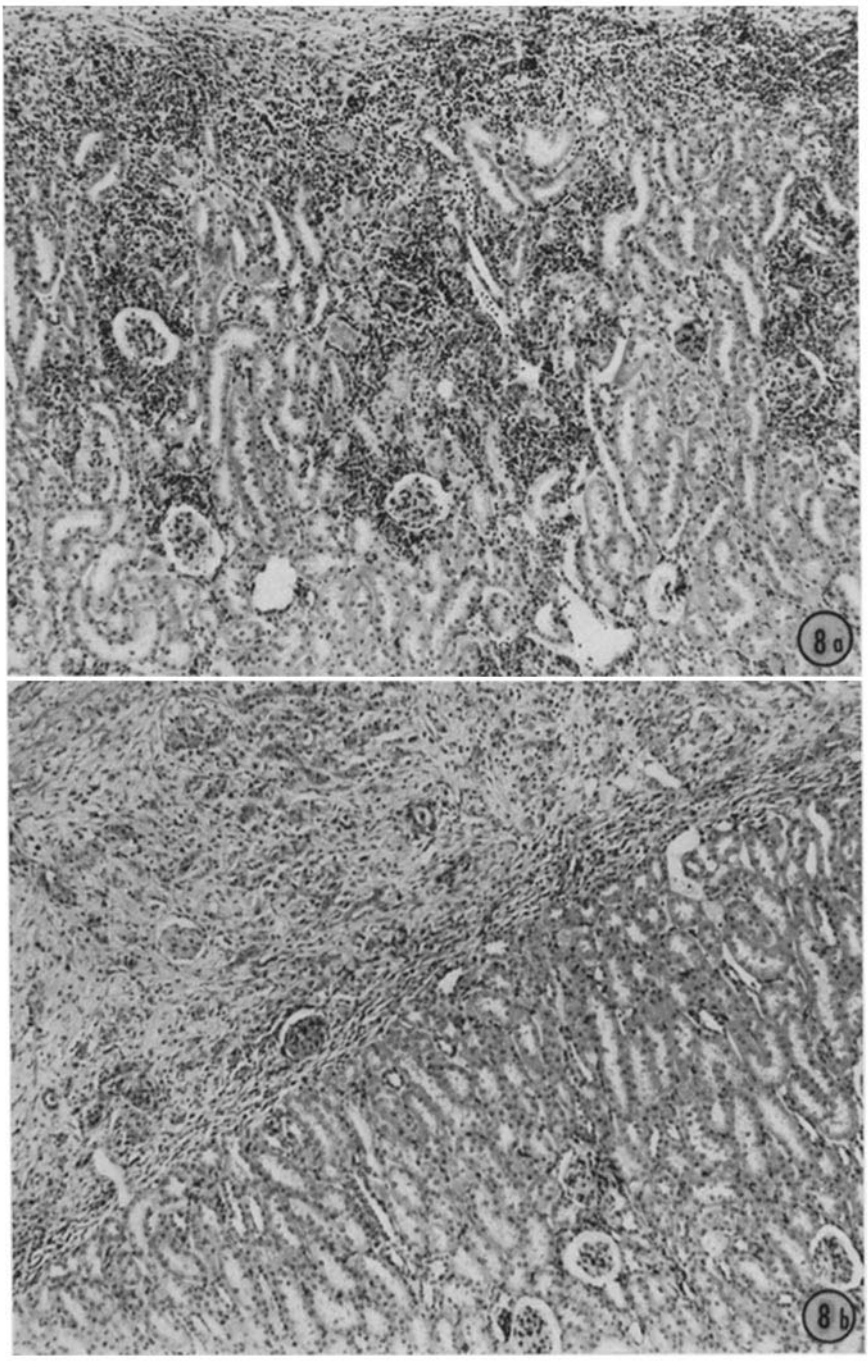
FIG. 7. Effect of whole-body irradiation of prospective host on development of L *versus* (LBN)F_1 GVHR. The host in (*a*) was the unirradiated control, that in (*b*) received 1000 r. Dominici, × 61.



(Elkins: Donor and host lymphoid cells)

PLATE 20

FIG. 8. Inhibition of transferred GVHR by prior irradiation (1000 r) of the prospective secondary host (*b*). Host in (*a*) is unirradiated control. Hematoxylin and eosin, × 61.



(Elkins: Donor and host lymphoid cells)