THE ROLE OF LYMPHOCYTES IN THE SENSITIZATION OF RATS TO RENAL HOMOGRAFTS

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The experiments of Hume and Egdahl (1) suggest that a kidney homograft can sensitize its host by way of the blood because the graft is destroyed even if it is prevented from reestablishing a lymphatic drainage. This observation implies that kidney homografts may not sensitize in the same manner as homografts of skin for Billingham *et al.* (2) and Greene (3) have shown that skin homografts placed in certain "privileged" sites in the hamster and rabbit respectively do not sensitize their hosts even though they become fully vascularized. In addition, histological studies (4) and experiments involving the technique of adoptive immunization (5) have shown that the seat of the reaction to orthotopic homografts of skin is in the regional lymph node; but no particular group of lymph nodes has been shown to play a comparable role in the destruction of kidney homografts.

It is not clear how a kidney homograft could sensitize its host by way of the blood. One possibility is that the lymphoid tissue of the host is activated by "free" antigen which is liberated into the blood. However, experiments employing allogeneic cells (6) or tissue extracts (7) have shown the intravenous route to be an inefficient method of sensitization; indeed, the survival of homografts may actually be prolonged by such injections. Another possibility is that immunologically competent cells in the blood of the host may interact with antigen during their passage through the kidney homograft and initiate a reaction against the graft after they have "homed" into the lymph nodes and spleen.

The present experiments were designed to test the second possibility in rats on the assumption that the cell type in blood which initiates the immunological response to kidney homografts is the small lymphocyte. Justification for this assumption comes from the ability of small lymphocytes to initiate a response to histocompatibility antigens in graft-against-host reactions (8, 9) and probably, in the reaction against orthotopic homografts of skin (10–12). The idea that sensitization to homografts might occur peripherally (as opposed to centrally within regional lymphoid tissue) was first suggested by Medawar (22) following the discovery that cells capable of initiating graft-against-host reactions were present in the blood.

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Two sets of experiments were performed. First, thoracic duct lymphocytes from a rat (X) were perfused *in vitro* through an isolated kidney from a genetically dissimilar rat (Y). The lymphocytes were then injected into a normal rat, syngeneic with X, whose subsequent response to a Y homograft of skin was taken as a measure of sensitization. Second, the vessels of an isolated allogeneic kidney were linked to an artery and vein of a restrained, unanaesthetized rat. The perfusion was discontinued after a number of hours and the degree of sensitization was again tested by applying a graft of skin from the kidney donor.

Methods

The rats used in these experiments were members of an inbred hooded strain (HO) and the F_1 cross between it and an inbred albino strain (AO). "Stock" albino rats from a closed non-inbred colony were also employed.

The thoracic duct was cannulated by the method of Bollman *et al.* (13) and after the operation the rats were maintained, unanaesthetized, in restraining cages. Lymph was collected at room temperature into sterile flasks containing 5 ml of Krebs-Ringer's solution with 100 units of heparin and 0.5 mg of streptomycin.

Radioactive Labelling of Thoracic Duct Cells.—In several experiments thoracic duct cells were labelled *in vitro* with tritiated adenosine in order to determine their fate after intravenous injection into the recipient animals. The cells were incubated at 37°C for 30 minutes in a medium containing tritiated adenosine (Radiochemical Centre, Amersham, England; specific activity 1 c/mmole) at a concentration of 10 μc per ml, employing the method of Gowans (8). The label is incorporated into the RNA of small lymphocytes and into the DNA and RNA of large lymphocytes. The use and limitations of this method for tracing the fate of lymphocytes have been discussed elsewhere (14).

Autoradiography.—Tissues for autoradiography were fixed in formal-alcohol for 24 hours, embedded in paraffin wax and sectioned at 4 μ. Autoradiographs were prepared with Ilford K5 nuclear research emulsion and were exposed for 14 days. After exposure and development the sections were stained with methyl green–pyronin.

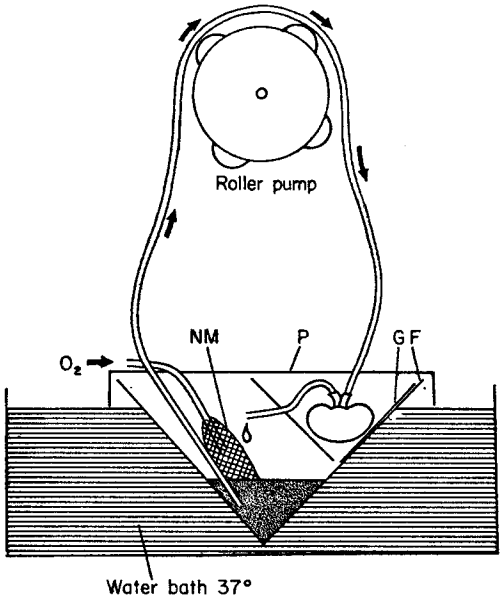
Skin Grafting.—Skin was removed from the ventral thoracic wall of the donor and trimmed of underlying fat and muscle. A graft bed of approximately 2 x 1.5 cm with an intact panniculus was prepared on the lateral thoracic wall of the recipient. The skin was placed on the bed, anchored with silk sutures, and covered with a sterile paraffin gauze dressing which was held in place with an Elastoplast bandage (Smith & Nephew Ltd., Welwyn Garden City, England).

Assay of Graft Survival.—Skin grafts were excised after 6 days and fixed in Zenker-formal. After fixation each graft was trimmed and divided into three strips of about 3 x 20 mm. One section for histological examination was cut from each strip. The entire epidermis of each section was viewed under a magnification of 320 diameters and the percentage of fields showing epithelial necrosis was noted. A field was judged necrotic if the Malpighian layer of the epidermis did not contain at least 2 layers of closely packed epithelial cells with plump nuclei (Figs. 1 through 4). The 3 sections sampled from each graft provided approximately 250 fields. The score for each graft was taken as the mean per cent epithelial necrosis of 3 sections. Measurements of the degree of epithelial necrosis have been shown to provide an exacting index of the degree of survival of skin homografts (5, 15).

Nephrectomy.—The kidney was approached by a midline abdominal incision under ether anaesthesia and the renal artery and vein and the ureter were gently dissected with a cotton swab. The ureter was tied and cut proximally. An intravenous injection of 100 units of heparin

was administered to the rat and the renal artery and vein were tied and cut across. The kidney was then removed from the animal and placed in a dish of warm saline where the renal artery was separated from the vein with fine forceps. A short length of polyethylene tubing (bore, 0.28 mm) was inserted into the artery and anchored with a silk tie after which 2 ml of warm saline containing 200 units of heparin was injected through it in order to wash the kidney free of blood. The vein was cannulated with polyvinyl tubing (bore, 0.5 mm). The abdominal incision was closed with silk sutures and the animal returned to its cage.

Perfusion of Kidneys with Thoracic Duct Cells.—The thoracic duct of a 180 to 220 gm HO strain rat was cannulated and lymph was collected for 12 hours. The thoracic duct cells were

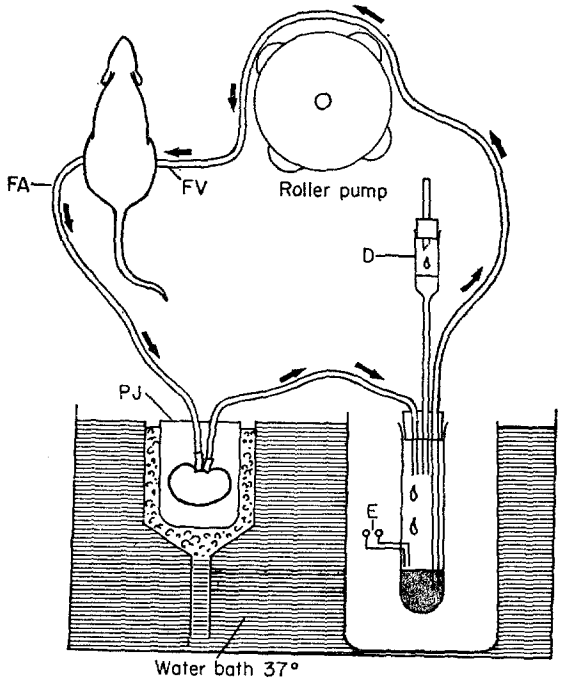


TEXT-FIG. 1. Apparatus for the *in vitro* perfusion of isolated rat kidneys. The nylon mesh and gas line were employed only in perfusions with blood and served to oxygenate the perfusate. *GF*: two glass funnels fused together to support kidney and collect perfusate; *NM*: nylon mesh; *P*: plastic cover.

harvested by centrifugation at about 150 *g* for 10 minutes and were washed once in tissue culture medium 199 (Glaxo Laboratories Ltd., Greenford, England). The cells were resuspended in 3 ml of a medium containing 50 per cent HO rat plasma and 50 per cent medium 199 by volume. Plasma was obtained by centrifuging whole blood which had been collected by aortic cannulation from a 250 to 300 gm rat after an intravenous injection of 200 units of heparin. The total number of cells in the 3 ml of medium was adjusted to 120 to 150 million.

The cell suspension in 3 ml of medium was recycled through a kidney from an (HO × AO) F_1 donor for 2 hours at a flow rate of approximately 25 ml per hour by means of the apparatus illustrated in Text-fig. 1. The organ chamber was constructed by fusing 1 glass funnel to the side of another. The smaller funnel was used to support the kidney so that fluid issuing from the renal vein and oozing from the kidney surface could drain to the bottom of the larger funnel. The interior of the chamber was siliconized.

At the end of the perfusion period, the total volume of perfusate was injected intravenously into a 200 to 250 gm HO rat. In the 10 experiments recorded in Table II the mean number of thoracic duct cells transferred to each recipient was 66 million. A histological study of the kidney showed that the loss of cells from the perfusate was due to embolism of the blood vessels of the renal medulla. The reason for the variability in the recovery of cells was not determined but if the number of cells recovered at the end of a perfusion was less than 50 per cent of the initial number, it was arbitrarily decided to discard the experiment.



TEXT.-FIG. 2. Apparatus for the *in vivo* perfusion of an isolated kidney. *D*: dripper delivering heparin into blood reservoir; *E*: electrodes to relay which controls the roller pump; *FV* and *FA*: cannulae in femoral vein and artery respectively; *PJ*: plastic jacket enclosing kidney and supported in water bath by a wool-packed glass funnel.

Perfusion of Kidneys with Thoracic Duct Cells in Blood.—In order to improve the physiological condition of the perfused kidneys a number of experiments were performed in which the plasma and medium 199 perfusate was replaced with "reconstituted blood." The blood was oxygenated in the organ chamber by allowing the venous effluent from the kidney to flow over a nylon mesh beneath which 95 per cent O_2/5 per cent CO_2 was passing (Text-fig. 1). The blood perfusate was prepared by centrifuging 10 ml of heparinized blood which had been collected in the manner described previously. One ml of packed red blood cells was drawn from the bottom of the centrifuge tube and resuspended in 2.5 ml of the plasma supernatant. This reconstituted blood contained less than 500 white blood cells per mm^3. Between 120 and 150 million thoracic duct cells were suspended in 3.5 ml of reconstituted blood and were recycled through an (HO × AO) F_1 kidney for 1 hour. At the end of the perfusion the

perfusate was injected intravenously into an HO rat. In the 8 experiments recorded in Table III the mean number of cells transferred to each recipient was 101 million.

Perfusion with Labelled Thoracic Duct Cells.—In order to determine the fate of the thoracic duct cells which were injected into HO rats at the end of a kidney perfusion the following experiments were performed. Between 120 and 150 million HO thoracic duct cells were incubated in a plasma and 199 medium together with tritiated adenosine. The cells, suspended in this medium, were then recycled through isolated (HO × AO) F_1 kidneys for 2 hours and subsequently injected intravenously into HO rats. In the first experiment of this series, a 200 gm rat received 2 injections of cells, totalling 140 million, taken from 2 separate perfusions. In each of 3 subsequent experiments a 70 gm rat received 1 injection of 70 to 120 million cells. The recipients were killed 26 hours after the injection of perfusate and the mesenteric nodes, the cervical nodes, and the spleen were removed and processed for autoradiography.

In Vivo Perfusion of Kidneys.—The time required for a renal homograft, devoid of lymphatic drainage, to sensitize its host was studied by linking the renal vessels of an isolated allogeneic kidney to the femoral artery and vein of a normal rat. The femoral artery and vein of a 250 to 300 gm "stock" rat, which had been injected previously with 100 units of heparin, were cannulated with polyethylene tubing (bore, 0.28 mm) and polyvinyl tubing (bore, 0.5 mm) respectively. The arterial cannula was led out through the inguinal incision, and the venous cannula through the posterior surface of the hind limb. In one series of experiments, rats were maintained under ether anaesthesia for one hour during which the blood issuing from the arterial cannula was allowed to flow through an isolated (HO × AO) F_1 kidney by means of the apparatus illustrated in Text-fig. 2. A roller pump was inserted between the renal and femoral veins because it had been found previously that the renal blood flow was negligible when the two veins were connected directly by means of a short plastic cannula. The roller pump was intermittently activated by a relay, so that the blood level in the lucite reservoir remained approximately constant. Protamine sulfate was sprinkled over the hilar fat prior to the perfusion in order to prevent oozing of blood once the perfusion had begun. The ureter was led out of the plastic jacket which contained the kidney and the urine drained away into the water bath.

In another series of experiments, kidneys were perfused for periods of 5 and 12 hours. During these longer perfusions the stock albino rats were maintained, unanaesthetized, in restraining cages because attempts to maintain the rats for prolonged periods under ether anaesthesia had resulted in a high rate of mortality. In order to prevent the formation of clots in the circuit 15 units of heparin per hour were delivered into the lucite reservoir from a dripper. In all the *in vivo* perfusions, the blood flow through the kidney was irregular during the first hour and varied from 15 to 60 ml per hour. However, during the second hour of the longer perfusions, the flow began to increase, and then remained constant at 80 to 120 ml per hour until the termination of the experiment. The high flow rate made it unnecessary to mix the blood in the lucite reservoir. At the end of the perfusions, the rats were released from the restraining cages and the cannulae were removed.

RESULTS

Epithelial Necrosis in First and Second Set Skin Homografts.—The general plan in the present study was to perfuse lymphocytes through an allogeneic kidney and then to return them to an animal syngeneic with the lymphocyte donor. The degree to which the recipients became sensitized was tested by subsequently grafting them with skin from the kidney donor and assaying the degree of epithelial necrosis at 6 days. In order to determine the degree of epithelial necrosis which was to be expected in strongly sensitized and in non-sensitized recipients,

assays were first performed on first and second set skin grafts. This study provided values which could then be compared with those obtained from rats presumptively sensitized with the kidney perfusates.

Grafts of (HO × AO) F_1 skin were removed from HO rats 6 days after implantation. Each HO rat received a further graft on day 20 and this was in turn removed on day 26. Table I shows that the mean percentage epithelial necrosis in the first set grafts was 7 per cent while that in second set grafts was 63 per cent.

TABLE I

Survival of (HO × AO) F_1 Skin Grafts on Normal HO Hosts

Epithelial necrosis	
1st set	2nd set
per cent	*per cent*
19	80
12	77
11	76
8	72
3	69
1	63
0	43
0	22
Mean..... 7	63

Sensitization with Kidney Perfusates Containing Plasma and Medium 199.— Ten (HO × AO) F_1 kidneys were perfused *in vitro* for 2 hours with thoracic duct lymphocytes suspended in plasma and medium 199. The perfusates, containing a mean of 66 million cells, were injected into HO recipients which were grafted 9 days later with F_1 skin. The grafts were removed 6 days after implantation and scored for epithelial necrosis (Table II). Using the student t test, the mean percentage necrosis of the grafts in this group (39 per cent) was found to be significantly different ($P < 0.01$) from the mean of first set skin homografts (7 per cent; see Table I).

It was necessary to show that the sensitization induced by injecting kidney perfusates was not the result of "free" antigen which had been shed by the organ into the perfusing fluid. To test this point 9 kidneys were perfused with plasma and medium 199 to which no lymphocytes had been added. The recipients of the cell-free perfusate showed a mean epithelial necrosis of 15 per cent (Table II). Although this score was 8 per cent greater than the mean of first set skin grafts, the difference was not significant ($0.05 < P < 0.1$).

In order to be certain that the sensitization of the lymphocyte recipients de-

pended upon an antigenic difference between the lymphocytes and the kidneys which they had perfused, 5 HO kidneys were perfused with HO lymphocytes suspended in plasma and medium 199. The mean percentage epithelial necrosis in skin grafts applied to the HO recipients of this perfusate (4 per cent) was significantly different ($P < 0.01$) from the mean obtained from the perfusion of (HO × AO) F_1 kidneys. However, the mean of 4 per cent was not significantly different ($P > 0.1$) from the mean of 7 per cent noted for first set skin grafts (Tables I and II).

TABLE II

Survival of (HO × AO) F_1 Skin Grafts on HO Hosts Which Had Received a Single Intravenous Injection of Perfusate from a 2 Hour in vitro Kidney Perfusion. Perfusate Consisted of Plasma and Medium 199, with and without HO Lymphocytes

Epithelial necrosis		
HO lymphocytes perfused through		Lymphocyte-free perfusion of (*HO* × *AO*) F_1 kidney
(*HO* × *AO*) F_1 kidney	*HO* kidney	
per cent	*per cent*	*per cent*
65	8	32
65	5	30
42	4	18
42	1	16
40	0	14
36		10
33		6
32		5
30		4
0		
Mean.....39	4	15

Sensitization with Kidney Perfusates Containing Reconstituted Blood.—In the previous experiments the kidneys were anoxic for the 2 hour period of perfusion and it could be argued that their poor physiological condition was responsible for the sensitization which could be induced by injecting the perfusate. Eight (HO × AO) F_1 kidneys were accordingly perfused with reconstituted blood which was oxygenated during a 1 hour perfusion period. The 8 recipients of such perfusates, which contained a mean of 101 million HO thoracic duct cells, were grafted at 9 days with F_1 skin. The percentage epithelial necrosis of these grafts 6 days after implantation is recorded in Table III. The mean of 38 per cent differed significantly from the mean noted in first set skin grafts ($P < 0.01$). The 6 rats in this group which received the greatest number of lymphocytes (> 85 million) also showed the greatest degree of necrosis. The mean per-

centage necrosis in these 6 rats was 48 per cent and was not significantly different from the mean of second set skin grafts ($0.05 < P < 0.1$).

A series of control experiments was performed with a perfusate of reconstituted blood to which no lymphocytes had been added. The results of this group are recorded in Table III. The mean percentage of 12 per cent did not differ significantly from that of the first set grafts ($P > 0.1$).

In comparing these results with those obtained with perfusates composed of

TABLE III

Survival of (HO × AO) F_1 Skin Grafts on HO Hosts Which Have Received a Single Intravenous Injection of Perfusate from a 1 Hour in vitro Kidney Perfusion. Perfusate Consisted of "Reconstituted Blood" (see text), With and without HO Lymphocytes

Epithelial necrosis	
Perfusion of (*HO* × *AO*) F_1 kidney with	
Lymphocyte-free blood	Blood with *HO* lymphocytes
per cent	*per cent*
18	75
15	71
14	45
14	35
13	32
11	20
2	16
	12
Mean.....12	38

plasma and medium 199 it is clear that improving the physiological state of the kidney by shortening the perfusion time from 2 hours to 1 hour and by employing oxygenated blood did not reduce the degree to which the recipients became sensitized.

Contamination of the Perfusate with Leakage from the Kidney Surface.—The reservoir of the organ chamber drained fluid which emerged from the renal vein and from the surface of the kidney. In the perfusions with reconstituted blood, the small volume of fluid which leaked from the surface of the kidneys appeared to consist of blood which had oozed from the kidney capsule and from the hilar fat. However, in order to determine if this fluid contained both blood and lymph, the following experiments were performed.

Three (HO × AO) F_1 kidneys were perfused for one hour with HO thoracic duct cells suspended in reconstituted blood. In each experiment the fluid issuing from the kidney surface and the fluid present in the perfusate reservoir were

sampled at 15, 30, and 60 minutes. The lymphocyte and erythrocyte concentration of each pair of fluid samples differed only within hemocytometer counting errors. It was inferred that very little fluid with a cell content different from that which perfused the vascular bed of the kidney could have mixed with the blood which emerged from the kidney surface; in other words, the contribution of lymph to the efflux from the surface of the kidney must have been negligible. Thus, it is likely that the lymphocytes in the perfusate gained their capacity to

TABLE IV

Survival of (HO × AO) Skin Grafts on Stock Albino Hosts Which Have Received Short-Term (HO × AO) F_1 Renal Homografts (in vivo Perfusions)

Epithelial necrosis	
Renal homografts perfused by host for	
1 hr	5 and 12 hrs.
per cent	*per cent*
27	69
20	62
11	40*
7	29*
7	21
5	13*
5	3
4	0
0	
0	
Mean..... 9	30

* 12 hour perfusions.

sensitize the HO recipients during their repeated passage through the vascular bed of the F_1 kidney, and not by emigrating from its blood vessels and passing through the substance of the kidney itself.

Perfusion with Labelled Thoracic Duct Cells.—Labelled HO thoracic duct cells were perfused through (HO × AO) F_1 kidneys for 2 hours and subsequently injected into HO recipients which were killed 26 hours later. Labelled cells were found in large numbers in the nodes and in the white pulp of the spleen; in the latter situation they were localized mainly around the central arterioles (Fig. 5). Large cells, about 12 μ in diameter, with strongly pyroninophilic cytoplasm, pale nuclei, and prominent pyroninophilic nucleoli were occasionally found among the labelled cells in both the nodes and the spleen. The nuclei of the large cells were lightly labelled, but the cytoplasm was heavily labelled (Figs. 6, 7, and 8).

In Vivo Perfusions.—The sensitization of normal rats with perfusates from 1 hour *in vitro* perfusions with reconstituted blood suggested that even in the absence of a lymphatic drainage a renal homograft might be able to sensitize its host within 1 hour. To test this point, 10 (HO × AO) F_1 kidneys were perfused *in vivo* for 1 hour with blood from stock albino rats. These rats received skin grafts from the (HO × AO) F_1 kidney donors 14 days later. The mean per cent epithelial necrosis of the 6 day grafts was 9 per cent (Table IV). This score suggests that the stock albino rats were not sensitized by the 1 hour *in vivo* perfusions.

Eight (HO × AO) F_1 kidneys were perfused *in vivo* for 5 and 12 hours in order to determine if an increase in the perfusion time would result in sensitization. The stock albino rats received skin grafts from the (HO × AO) F_1 donors 14 days after the completion of the perfusions. The mean per cent epithelial necrosis of the 6-day grafts was 30 per cent (Table IV) and was significantly different ($0.02 < P < 0.05$) from the mean of the 1 hour perfusion group.

DISCUSSION

The present experiments were designed to test the hypothesis that the sensitization of rats to renal homografts can be mediated by blood-borne lymphocytes. A method for the *in vitro* perfusion of isolated rat kidneys was accordingly developed. Experiments in which the perfusate contained plasma and medium 199 indicated that parental strain thoracic duct lymphocytes, which had made several passages through an F_1 kidney and which were subsequently injected into a normal parental rat, sensitized the recipient to a test graft of F_1 skin. The controls showed that sensitization depended upon an antigenic difference between the lymphocyte and the kidney donor; and that it was probably not due to the liberation of "free" (HO × AO) F_1 antigens from the kidney into the perfusate. Experiments in which the perfusate contained oxygenated blood in place of plasma and medium 199 indicated that the improvement in the physiological condition of the isolated kidney did not diminish the degree of sensitization of perfusate recipients. The mean degree of sensitization conferred by the injection of thoracic duct cells in both series was not as great as that which could be induced by a skin homograft. However, in those rats which received the largest number of lymphocytes the mean percentage epithelial necrosis in the test grafts was not significantly different from that observed in second set skin homografts.

The autoradiographic experiments showed that the injected thoracic duct cells migrated into the spleen and lymph nodes of the recipient. Among the labelled cells found in these organs were large cells, with deep pyroninophilic cytoplasm, open nuclei, and prominent pyroninophilic nucleoli and it is highly probable that they arose from the injected small lymphocytes (8, 16). Large pyroninophilic cells of the kind seen in these experiments are morphologically identical to those which have been observed by Scothorne and McGregor (4)

and Burwell (17) in the nodes draining homografts of skin and cancellous bone respectively. The appearance of these cells in the lymphoid tissues in the present experiments strengthens the view that the injected parental strain small lymphocytes had interacted with the foreign histocompatibility antigens in the F_1 kidney and were responsible for the adoptive sensitization of the recipients.

The results of the *in vitro* perfusions suggested that a renal homograft might be able to sensitize its host within 1 hour. This expectation was not confirmed by experiments in which allogeneic kidneys were linked for 1 hour with the femoral artery and vein of normal rats (*in vivo* perfusions) although increasing the perfusion time to 5 and 12 hours did result in sensitization. Similarly, Nathan (20) found that kidney homografts in dogs had to remain linked to the circulation for more than 6 hours in order to sensitize the majority of hosts. A possible explanation of the discrepancy between the time required for sensitization *in vitro* and *in vivo* is that lymphocytes must remain in contact with kidney tissue for a certain minimal period in order to initiate a homograft reaction. The "contact time" between the lymphocyte and the kidney in any perfusion of a given duration can be shown to be directly proportional to the ratio of the vascular volume of the organ to the volume of the perfusate. Although the 1 hour *in vitro* and *in vivo* perfusates contained equal numbers of cells (about 150 million) the "contact time" in the former (perfusate volume about 3 ml) was considerably greater than in the latter (total blood volume about 15 ml). The opportunity for individual small lymphocytes to make repeated contacts with the kidney during the *in vivo* perfusions would have been reduced further by the fact that lymphocytes are continually entering and leaving the blood as part of the process of recirculation (14). Thus the minimal "contact time" may have been achieved *in vitro* but not *in vivo*. The hypothesis of "contact time" may also explain the paradoxical observation that renal homografts can sensitize by way of the blood while vascularized grafts of skin implanted in the brain or hamster cheek pouch cannot. In the former case a large flow of blood is reestablished immediately and the vascular volume is large, while in the latter, the flow is reestablished more slowly and the vascular volume is comparatively small. The resultant difference between the "contact times" may account for the difference in the ability of each graft to sensitize. The opportunity for either type of graft to sensitize by way of the blood might cease if its vascular endothelium became replaced by that of the host, but there is no decisive evidence on this point.

Taken together, the results of the perfusion and the autoradiographic experiments suggest an afferent arc by which renal homografts can sensitize a host. Blood-borne small lymphocytes may pass several times through the vascular bed of the homograft and then migrate from the blood into the spleen or lymph nodes. Once in the lymphoid tissue, those lymphocytes which had interacted with the kidney antigens may transform into large pyroninophilic cells and

thereby complete the arc. A similar scheme has been proposed by Porter *et. al* (21) from the results of a morphological study of kidney homografts in dogs. There is no information about the form and manner in which the kidney antigens become accessible to blood-borne cells but presumably the interaction between lymphocyte and antigen occurs at the luminal border of the vascular endothelium. The efferent limb of the reaction was not the object of the present study but it seems plausible to suggest that the agents which destroy renal homografts (either cells or antibodies or both) may be generated in lymphoid tissue by the large pyroninophilic cells (10, 11).

It is also possible that some blood-borne small lymphocytes may enter renal homografts directly and transform into large pyroninophilic cells. Simonsen *et al.* (18) noted such cells in kidneys 3 days after transplantation and Elkins (19) has shown that the injection of parental strain thoracic duct cells under the capsule of an F_1 hybrid kidney is followed by the appearance locally of numerous large pyroninophilic cells and by extensive damage to the kidney. In the present experiments sensitization did not appear to depend upon the entry of lymphocytes into the substance of the kidney since no cellular infiltration was seen histologically and no traffic of cells through the kidney parenchyma was detected during the perfusions.

If a renal homograft sensitizes its host by the mechanism which has been suggested, then the same mechanism may operate in other homografts, such as lung or heart, which are linked to the host by direct surgical anastomoses of large blood vessels. In these cases, a relatively large blood flow is established immediately and large numbers of blood-borne lymphocytes are able to make contact with the vasculature of the graft.

SUMMARY

In order to study the role of blood-borne small lymphocytes in the sensitization of rats to renal homografts 2 techniques for the perfusion of isolated rat kidneys were employed: (*a*) the *in vitro* perfusion of kidneys with thoracic duct cells suspended in either an artificial medium or in blood; the perfusates were then injected into rats syngeneic with the lymphocyte donors; (*b*) the *in vivo* perfusion of kidneys with blood issuing from the femoral artery and returning to the femoral vein of living rats. The degree of sensitization conferred on the recipients by the perfusates was assessed by applying a skin homograft from the kidney donor and scoring the epithelial necrosis at 6 days. The *in vitro* experiments indicated that parental strain thoracic duct cells, which had passed through an F_1 hybrid kidney could confer upon a parental rat sensitivity to an F_1 skin graft. Several perfusions with radioactively labelled lymphocytes showed that the injected cells migrated to the lymph nodes and spleen of the recipients Labelled large pyroninophilic cells were occasionally seen in the spleen and lymph nodes of recipients, and it was suggested that these had arisen from the

injected cells. Although the *in vitro* perfusions with blood indicated that renal homografts might sensitize their hosts within 1 hour, the *in vivo* perfusions suggested that about 5 to 12 hours were required. The more rapid sensitization *in vitro* was possibly due to the more frequent opportunity for contact between lymphocytes and kidney vascular endothelium which was afforded by the conditions *in vitro*.

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BIBLIOGRAPHY

1. Hume, D. M., and Egdahl, R. H., Progressive destruction of renal homografts isolated from the regional lymphatics of the host, *Surgery*, 1955, **38,** 194.
2. Billingham, R. E., Ferrigan, L. W., and Silvers, W. K., Cheek pouch of the Syrian hamster and tissue transplantation immunity, *Science*, 1960, **132,** 1488.
3. Greene, H. S. N., Compatibility and non-compatibility, *Ann. New York Acad. Sc.*, 1955, **59,** 311.
4. Scothorne, R. J., and McGregor, I. A., Cellular changes in lymph nodes and spleen following skin homografting in the rabbit, *J. Anat., London*, 1955, **89,** 283.
5. Billingham, R. E., Brent, L., and Medawar, P. B., Quantitative studies on tissue transplantation immunity. II. The origin, strength and duration of actively and adoptively acquired immunity, *Proc. Roy. Soc. London, Series B*, 1954, **143,** 58.
6. Medawar, P. B., Immunity to homologous grafted skin. II. Relation between antigens of blood and skin, *Brit. J. Exp. Path.*, 1946, **27,** 15.
7. Medawar, P. B., Use of antigenic tissue extracts to weaken immunological reactions against skin homografts in mice, *Transplantation*, 1963, **1,** 21.
8. Gowans, J. L., The fate of parental strain small lymphocytes in F_1 hybrid rats, *Ann. New York Acad. Sc.*, 1962, **99,** 432.
9. Hildemann, W. H., Linscott, W. D., and Morlino, M. J., Immunological competence of small lymphocytes in the graft-*versus*-host reaction in mice, *Ciba Foundation Symp. Transplantation*, 1962, 236.
10. Gowans, J. L., McGregor, D. D., Cowen, D. M., and Ford, C. E., Initiation of immune responses by small lymphocytes, *Nature*, 1962, **196,** 651.
11. Gowans, J. L., McGregor, D. D., and Cowen, D. M., The role of small lymphocytes in the rejection of homografts of skin, *in* The immunologically competent cell, *Ciba Foundation Study Group*, 1963, 16, 20.
12. Billingham, R. E., and Silvers, W. K., Sensitivity to homografts of normal tissue and cells, *Ann. Rev. Microbiol.*, 1963, **17,** 531.
13. Bollman, J. L., Cain, J. C., and Grindlay, J. H., Techniques for the collection of lymph from the liver, small intestine, or thoracic duct of the rat, *J. Lab. and Clin. Med.*, 1948, **33,** 1349.
14. Gowans, J. L., and Knight, E. J., The route of re-circulation of lymphocytes in the rat, *Proc. Roy. Soc. London, Series B*, 1964, **159,** 257.

15. Steinmuller, D., and Weiner, L. J., Evocation and persistence of transplantation immunity, *Transplantation*, 1963, **1**, 97.
16. Gowans, J. L., and McGregor, D. D., The immunological activities of lymphocytes, *Progr. Allergy*, 1965, **9**, 1.
17. Burwell, R. G., Studies of the primary and the secondary immune responses of lymph nodes draining homografts of fresh cancellous bone, *Ann. New York Acad. Sc.*, 1962, **99**, 821.
18. Simonsen, M., Buemann, J., Gammeltoft, A., Jensen, F., and Jørgensen, K., Biological incompatibility in kidney transplantation in dogs. I. Experimental and morphological investigations, *Acta Path. et Microbiol., Scand.*, 1953, **32**, 1.
19. Elkins, W. L., Invasion and destruction of homologous kidney by locally inoculated lymphoid cells, *J. Exp. Med.*, 1964, **120**, 329.
20. Nathan, P., Antigen release from the transplanted dog kidney, *Ann. N. Y. Acad. Sc.*, 1964, **120**, 458.
21. Porter, K. A., Peart, W. S., Kenyon, J. R., Joseph, N. H., Hoehn, R. J., and Calne, R. Y., Rejection of kidney homotransplants, *Ann. New York Acad. Sc.*, 1964, **120**, 472.
22. Medawar, P. B., The homograft reaction, *Proc. Roy. Soc. London, Series B*, 1958, **149**, 145.

EXPLANATION OF PLATES

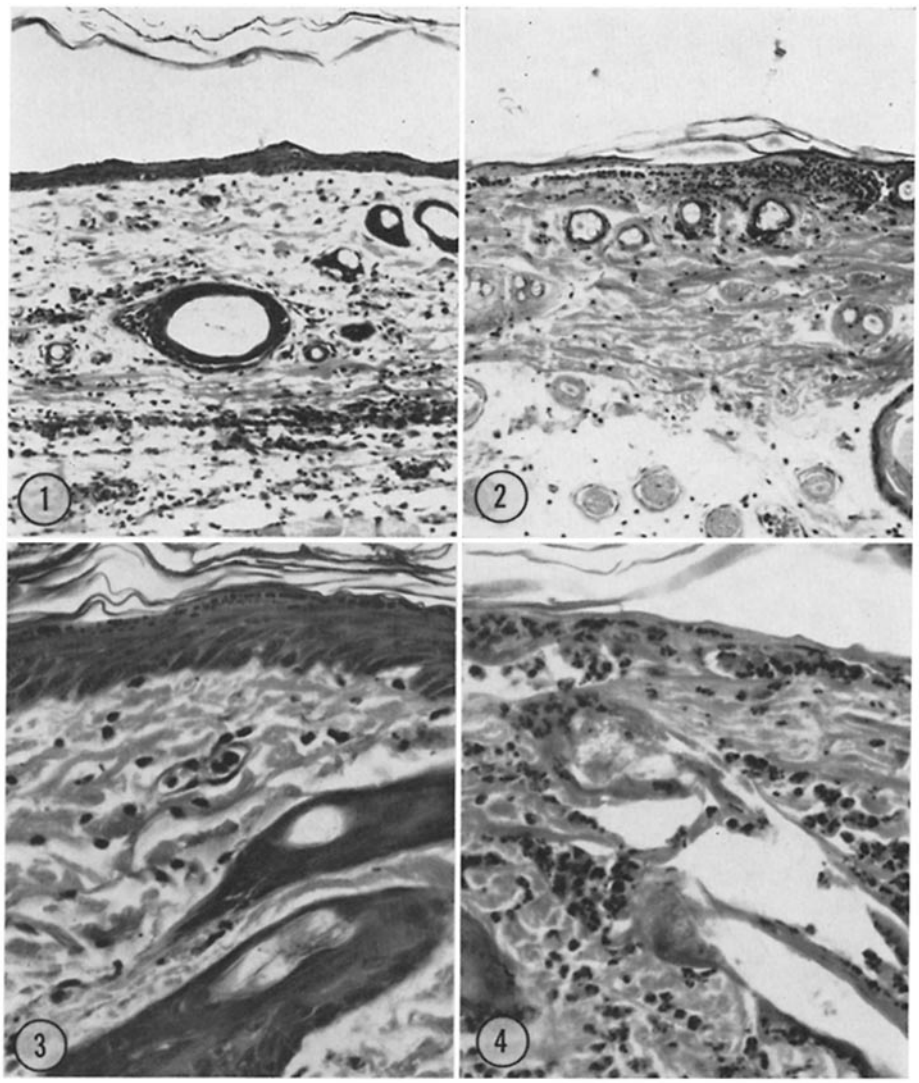
PLATE 23

FIG. 1. First set (HO × AO) F_1 → HO skin homograft at 6 days. The surviving epidermis contains several layers of closely packed cells. Haematoxylin and eosin. × 140.

FIG. 2. Second set (HO × AO) F_1 → HO skin homograft at 6 days. The epithelium is necrotic. Haematoxylin and eosin. × 140.

FIG. 3. Intact epithelium in a 6 day skin homograft. Haematoxylin and eosin. × 320.

FIG. 4. An area of necrotic epithelium in a 6 day skin homograft. Haematoxylin and eosin. × 320.

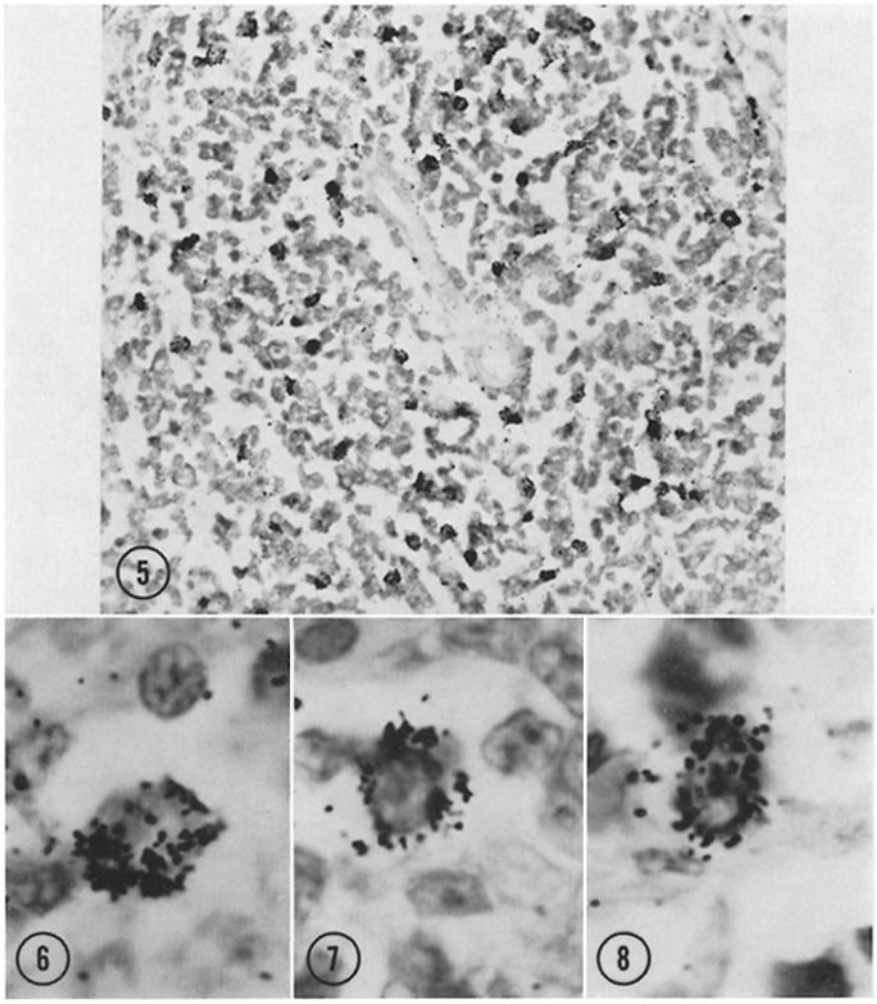


(Strober and Gowans: Sensitization of rats to renal homografts)

PLATE 24

FIG. 5. ^{3}H-adenosine-labelled lymphocytes had been perfused through an allogeneic kidney *in vitro* and then injected into a rat syngeneic with the lymphocyte donor. Many labelled small lymphocytes are localized around the central arteriole of the splenic white pulp 26 hours after the injection. Methyl green–pyronin. × 360.

FIGS. 6–8. Single labelled large pyroninophilic cells in the splenic white pulp 26 hours after the injection of ^{3}H-adenosine–labelled lymphocytes as described in Fig. 5. The labelling is predominantly cytoplasmic and the cells have large nucleoli. Methyl green–pyronin. × 2250.



(Strober and Gowans: Sensitization of rats to renal homografts)