A STUDY OF ALLOGRAFT KIDNEY REJECTION OCCURRING SIMULTANEOUSLY IN WHOLE ORGAN AND EAR CHAMBER GRAFTS IN THE RABBIT

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Most of the early studies on allograft rejection have been concerned with the morphological changes seen either in biopsy tissue or after removal of whole organs at different time intervals after grafting. Some have studied the organs *in situ* by indirect techniques which monitored physiological capacity or alterations in blood flow characteristics.

Various tissues have been studied microscopically by heterotopic implantation of organ fragments into such sites as brain (1), anterior chamber of the eye (2), renal capsule (3), hamster cheek pouch (4), and the transparent ear chamber of the rabbit (5). However, when these techniques were applied to allografted tissues, few were successful; and all those that did yield a clear microscopic view of the functioning tissues in vivo, such as hamster cheek pouch, the anterior chamber of the eye, or the rabbit ear chamber, exhibited atypical responses which were quite unlike the rejection that occurred when the same tissues were transferred as whole organs.

When pieces of kidney were allografted into the rabbit ear chamber, they reestablished their blood circulation in 2–4 days by anastomosis of their cut vessels to the adjacent vasculature of the ear chamber membrane (6). Autografts appeared to function indefinitely, since autologous renal tissue was observed in the rabbit ear chamber without change for 6 mo after fixation, and functioning myometrial autografts were observed for periods of up to 1 yr (6). Allografted renal tissues in the rabbit ear chamber reject after periods of up to 3 mo (6). A whole kidney allografted in similar rabbits is rejected in 7 days. When a whole kidney is allografted with an established ear chamber allograft from the same donor already *in situ*, both grafts reject synchronously over the following 7 days.

This report describes the changes seen in the unmodified rejection of renal allografts occurring simultaneously in whole organs and minute implants within transparent ear chambers. Microscopic details of the process which results in whole organ renal allograft destruction can thus be studied in vivo by observation of the ear chamber grafts which are being rejected synchronously with the whole organ.

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Materials and Methods

Rabbits.—Recipient rabbits were chosen from a closed colony of half-lop rabbits maintained at the Australian National University. Donor rabbits were unrelated; they were either from the same colony or sometimes from an entirely different strain from outside this colony.

Grafting Procedures.—

Ear chamber grafts: The procedure for transparent ear chamber insertion and the grafting of organ fragments into them was as previously described (6). The ear chamber grafts were taken from the donor's right kidney through a small flank incision. A wedge of cortex approximately 0.5 × 0.8 cm was transferred to the stage of a dissecting microscope, and the fragments for ear chamber implantation were prepared from this by a second operator. A wedge of Gelfoam (Upjohn Co., Kalamazoo, Mich.) placed in the renal incision provided swift hemostasis, and the flank incision was closed with continuous 3/0 chromic gut sutures (Ethicon, Inc., Somerville, N. J.).

As soon as these grafts had their circulation reestablished, within 3 or 4 days of the first procedure, a second operation was performed to transfer the whole left kidney from the same donor to the same recipient. One operator removed the left kidney from the donor through a broad abdominal incision, while a second readied the site in the recipient rabbit by removing its left kidney, and preparing the renal vessels transected close to the excised kidney for anastomosis to the graft vessels.

Whole renal grafts: Whole renal grafts into the abdomen were performed by an adaptation of the method described by Owen (7). Sometimes the whole organ was grafted into the recipient's neck, anastomosing the renal artery end-to-end with the internal carotid and the vein end-to-side to the external jugular vein. The arterial anastomosis was effected by six to eight interrupted sutures of 9.0 nylon (Ethicon) and the venous with a continuous suture of 8.0 braided silk (Ethicon), both inserted with a microvascular needle holder using × 4 magnified vision (Keeler telescopic spectacles, Keeler, London, England). Ischemia time for the transplanted kidney was 45 min–1 h. The kidney was held in place by two to three sutures through the capsule. Transplants in either site had a polythene tube 2 mm in diameter tied into their ureters by a double ligature of 5.0 braided silk (Ethicon) to act as a catheter. The other end of the catheter tube was passed under the skin to a point just behind and between the ears where it was brought out and fixed with a 3.0 silk suture (Ethicon) through the skin. This ensured that the rabbit did not pull the catheter out, and periodic collections of urine could be made by sample bottles attached to the ear. When the graft was placed in the neck, it allowed more direct access for palpation and biopsy. When the allograft was placed in the neck, the recipient rabbit retained both its own kidneys, and when it was grafted into the abdomen, it replaced the left kidney, the right kidney of the recipient remaining *in situ.*

Photomicroscopy.—Photographic observations on the ear chamber grafts and urinary cellular contents were made with a 35 mm Leitz camera and a 16 mm cine camera (Vinten Ltd., London, England) at both normal speed (16 frames/s) and time-lapse (60 frames/min to 4 frames/min).

Whole Organ Graft Observation.—Urine was collected at 2–4-h intervals and observed microscopically without previous centrifugation. Urinary cell numbers and types were recorded, and samples were stored frozen for gel electrophoresis.

Daily needle biopsies were performed on the grafted organ, under mild anesthesia (Nembutal, 0.3 ml/kg in 10 ml saline) with a Franklin-Silverman biopsy needle designed for pediatric use. The core of extracted tissue was immediately fixed as described below for Durcupan embedding. When the whole organ was placed in the neck, it was possible to make daily measurements of the long and short axis of the kidney with calipers through the skin.

Observation of the Recipient Rabbit.—A daily clinical assessment of the rabbit's condition was made and its blood pressure was recorded at least daily with a Grant's capsule on the ear

artery. A peripheral leukocyte count and differential as well as erythrocyte sedimentation rate (ESR)[1] and hematocrit determination were also made daily.

Fixation, Embedding, Sectioning, Staining, and Viewing of Tissues.—The ear chambers and needle biopsies were fixed in 4% glutaraldehyde in cacodylate buffer at pH 7.2, 4°C, for 1 h (8). Whole organs being removed were fixed *in situ* by intra-arterial perfusion of the organ with Millonig's modification of Karnovsky's fixative (9, 10) at room temperature, under a pressure equal to the intraluminal pressure recorded at the time of insertion of the perfusing cannula proximal to the arterial anastomosis. After perfusion for 2 h, the organ was cut lengthwise and blocks trimmed under a dissecting microscope from the cut face.

Blocks from both methods of fixation were then allowed to soak thoroughly in buffer before being transferred to buffered osmium fixative (11) for 1 h at 4°C, then to uranyl acetate (1% in water) for 1 h at bench temperature. After dehydration with acetone, the tissues were embedded in Durcupan (Fluka AG., Basel, Switzerland).

Sections approximately 1 μm thick were cut with glass knives and stained with Richardson's stain (12) for light microscopy. Sections for ultrastructural examination were cut with a diamond knife (E. I. Dupont de Nemours & Co., Wilmington, Del.), stained with alkaline lead citrate solution (13), and viewed with a Siemens Elmiskop electron microscope (Siemens Corp., Iseling, N. J.).

RESULTS

A total of six rabbits had simultaneous ear chamber and whole organ allografts (Table I). On the first 2 days after whole organ allografting, clear urine flows from the catheter in the transplanted ureter, and microscopically this is usually clear or contains only a few erythrocytes or tubular epithelial cells. There is no change in the ear chamber grafts. (Fig. 1 A).

At 48 h.—The whole organ is still normal to palpation and the urinalysis is normal. The ear chamber grafts begin to show large fluctuations in their blood flow (Figs. 1 and 2). Most of the time there is increased velocity and volume of blood flow which alternates irregularly and sometimes abruptly, at intervals of from 10 min to ½ h, with periods which generally last from 1 to 5 min of greatly decreased general blood flow. The ear chamber host vessels appear normal (Fig. 1). At the same time, the veins and venules of the grafts develop dilations which alternate along their lengths with short constricted regions (Figs. 1 and 2). The diameters of the dilated regions may be up to 20 times that of the constricted regions of the same vessels. The small graft arterioles, such as afferent and efferent glomerular arterioles, show similar, though less pronounced, patterns or irregular dilatation and constriction along their lengths (Fig. 2 A). The larger graft arteries, such as interlobulars, remain uniform along their lengths. However, these larger arteries constrict and dilate as the blood flow varies within the grafts, as described above. Even when the blood is flowing constantly at a high rate, time-lapse cinemicroscopy of such arteries reveals a regular rhythmic constriction and dilatation, which is moderate in degree and uniform along its length, with a periodicity of once every 5 min. This cannot be appreciated by direct observation.

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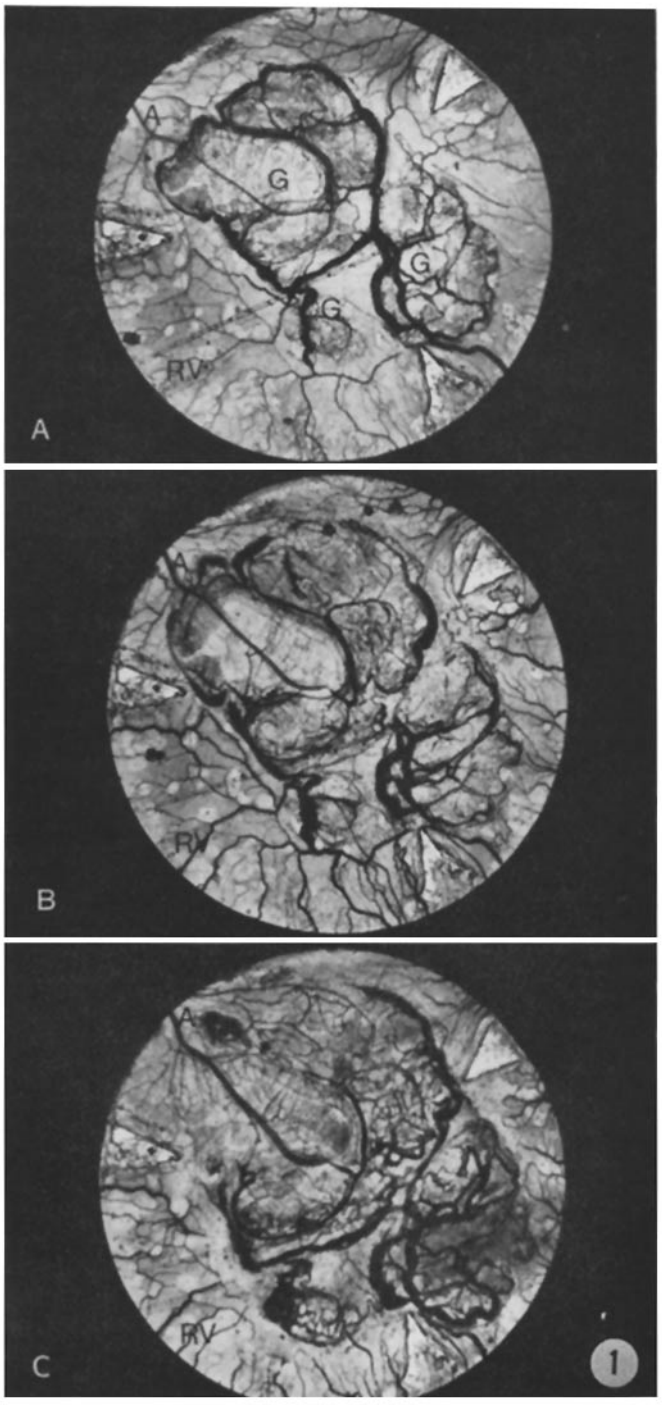
[1] *Abbreviations used in this paper:* ESR, erythrocyte sedimentation rate.

TABLE I

Rabbit no.	No. of ear chambers installed	Days after ear chamber graft inserted; whole organ graft performed	Whole organ graft site	Ear chamber observations								
				Ear	Graft vascular changes	Platelet adherence to endothelium	Leukocyte adherence to endothelium	Platelet adherence to leukocytes	Microhemorrhages	Graft parenchymal destruction	Motile leukocytes observed in urine from whole organ graft first noted	Whole organ graft fate	Recipient rabbit fate
					h	*h*	*h*	*h*	*h*				
EC 167	2	14	Neck	R	40	50	50	50	50	7 days	Not looked for	Fixed in formalin on termination of rabbit day 7	Terminated at removal of whole organ day 7 (very ill)
				L	40 Less pronounced	50	50	50	50	7 days			
EC 168	2	13	Neck	R	53	53	56	58	Not commented upon	12 days	Not looked for	Removed and perfused with Karnovsky fixative day 7	Allowed to recover
				L	53	53	60						
EC 172	2	9	Abdomen	R	50	50	50		70	9 days	Not looked for	Removed day 7 and perfused with Karnovsky fixative	Allowed to recover when terminated 6/12 later had glomerulonephritis in own kidney; IgG deposition on basement membrane
				L	51		51		75	8 days			
EC 174	2	15	Neck	R	46	47	70	70	90	7 days	Not looked for	Day 7 perfused with Karnovsky fixative and removed	Terminated
				L	48	48	48	54	86	6 days			
EC 175	1	10	Neck	R	48	48	49	54	82	7 days	Not looked for	Removed day 6 and fixed with Karnovsky fixative	Recovered; was terminated 10 mo later and found to have glomerulonephritis in own kidneys
EC 171	2	14	Neck	R	48	48	50	51	56	90 h	70 h	Perfused with Karnovsky fixative and removed day 7	Died day 11 with perforated gastric ulcer of "curling" type with hematemesis
				L	48	48	50	52	55	95 h			
AA_1	Nil		Abdomen	No ear chambers installed							57 h	Fixed *in situ* just as rabbit expired	Rabbit moribund and died at 72 h; at autopsy dilated R ventricle
AA^2	Nil		Abdomen								Not looked for	Organ removed and perfused with Karnovsky fixative day 6	Terminated
K_8	Nil		Abdomen								60 h (approx.)	Removed day 6	Allowed to recover
K_{18}	Nil		Neck								70 h	Removed, cut, and fixed in formalin	Allowed to recover
Autografts													
K22B	Nil		Neck									Perfused at 17 days	Terminated
K25	Nil		Neck								Nil at day 7 urine clear	Removed and sectioned at 16 days	Terminated at 16 days urinary fistula developed
CA-1	Nil		Abdomen									Terminated 14 days	Terminated 14 days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At the same time numerous platelets are seen adhering to the graft blood vessel endothelium both in regions which are otherwise normal and also in regions where irregular dilatations and constrictions are present. This platelet adhesion may be seen occurring in the presence or absence of leukocyte adhesion to the vessel endothelium. In this highly characteristic form of platelet adhesion the platelet is fixed by one pole to the endothelium and its free end oscillates in the flowing bloodstream (Figs. 3–5). When flow pauses momentarily, the platelet assumes a position perpendicular to the vessel wall. As many as 20 platelets may be seen adhering individually to the endothelium in this way in one high-power field. Most of the platelets thus observed appear optically denser and larger, and have blunter ends than unattached platelets. Such adherent platelets usually remain firmly attached and have been observed thus for several hours. If such a platelet becomes detached, it is usually replaced within seconds by another platelet or at times by a leukocyte, which adheres apparently to precisely the same point on the endothelium.

Shortly afterwards leukocytes begin to adhere to the swollen venular endothelium, about three to four per high-power field (Figs. 4 and 6). These leukocytes are generally nongranular and vary widely in size from that of small lymphocytes (8 μm) up to four to five times that diameter. The majority lie in the middle of the range ($\sim$25 μm). Time-lapse cinematography indicates that these cells move very slowly when compared with the occasional polymorphs or eosinophils seen adhering in the same fields.

Platelets also adhere progressively to the leukocytes which are sticking to the vessel walls (Fig. 6). Up to 10 platelets may be seen adhering to one leukocyte, producing a pincushion or porcupine effect (see Fig. 5 F). If one such leukocyte is not firmly attached, and commences rolling along the wall, platelets can be seen adhering by one pole to the cell, and by the other to the endothelium on the side away from progression (Fig. 5 E). Subsequently, platelets can also be seen adhering individually by their flat surfaces to both endothelium and adherent leukocytes. Aggregation of platelets into platelet clumps is not common, and when it does occur, usually one adheres to the endothelium by one pole as described above, then three or four others will attach themselves to it, lying

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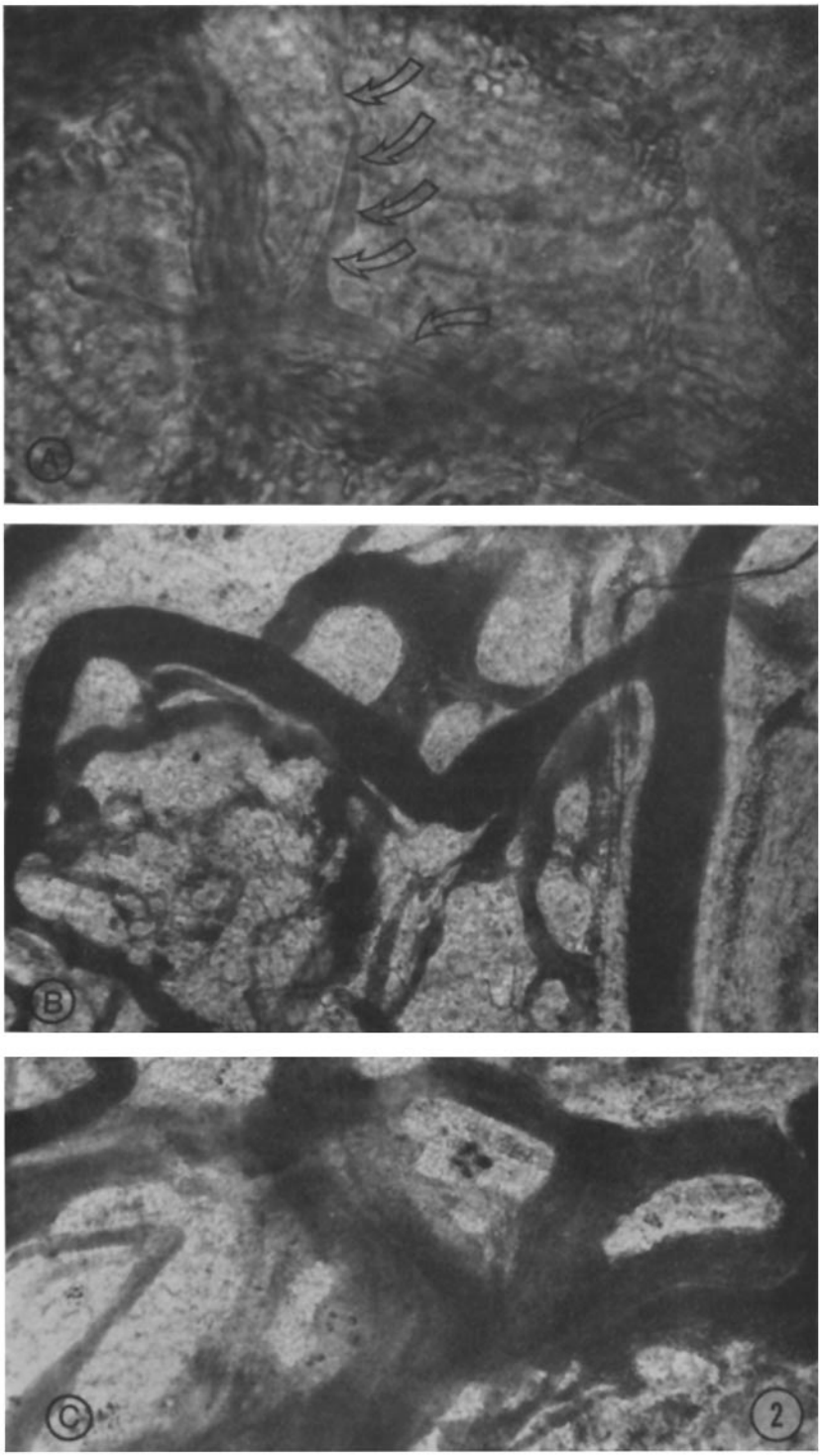
FIG. 1. Three views of a transparent ear chamber membrane showing the changes observed in vivo in early rejection. (A) Three allografts (*G*) well established. The vessels on the periphery of the membrane (*RV*) are of recipient origin. Both they and the graft vessels have good blood flow and the vessel walls are parallel. The arteriole to most of the ear chamber (*A*) has been completely converted to supply the renal grafts. × 10. (B) The same membrane and grafts showing the earliest detectable stage of rejection. The graft vasculature is irregularly dilated and constricted. The recipient vessels (*A* and *RV*) are slightly dilated, reflecting the increased blood flow through the graft, but have uniform lumina. × 10. (C) The same membrane and grafts several hours after Fig. 1 B. The graft vasculature is much more dilated, with irregular areas of constriction. Note uniformity of the recipient vessel walls (*A* and *RV*). × 10.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parallel in the fashion of a shingle roof (Fig. 5 D) but usually only the original one is attached to the wall. True thrombus formation is very rare, but has been observed. Towards the latter part of the period from 48 to 72 h, the irregularity of the graft vessels becomes more accentuated so that the diameter of dilated portions of the veins may be up to 30 times that of the constricted portions of

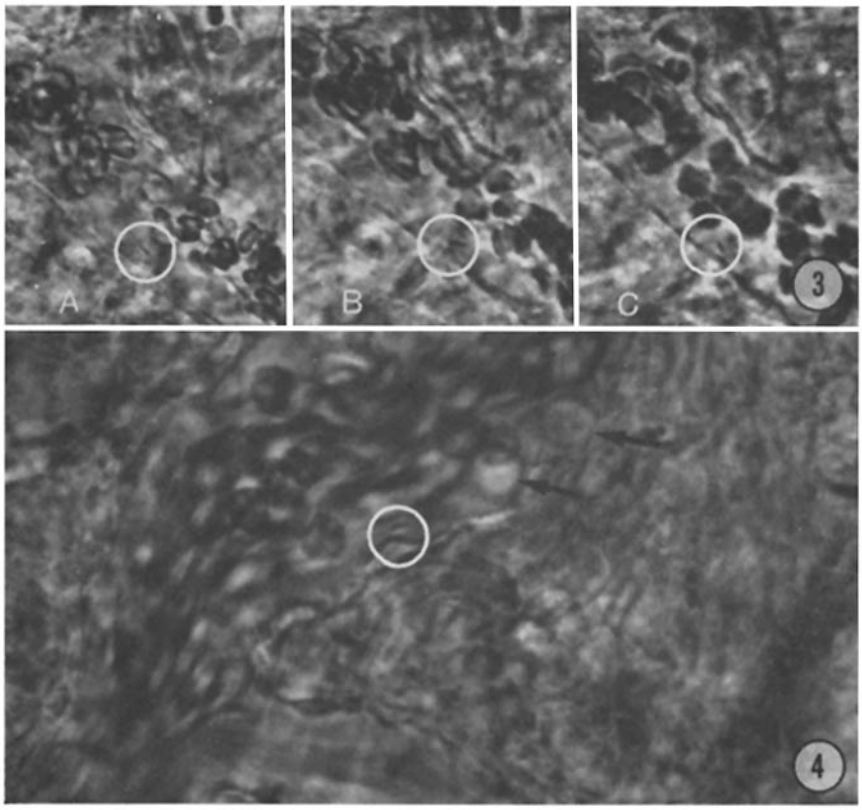


FIG. 3. A platelet (circled) adhering to the graft endothelium as an individual unit by one pole, in the absence of leukocyte adhesion. These pictures were taken within minutes of each other, and illustrate the movement of the unattached pole in the bloodstream. × 1,400.

FIG. 4. A platelet (circled) adhering to the graft endothelium after early nongranular leukocytic adhesion has commenced (arrows). × 1,400.

FIG. 2. (A) In vivo photographs showing the detail of the irregular constrictions (arrows) and dilatations seen in an artery from the graft shown in Fig. 1 B. × 1,200. (B) Detail of the irregular vessels seen elsewhere in the same graft as Fig. 1 B at the same time. × 300. (C) The extreme dilatation of the graft vessels at the stage of rejection shown in Fig. 1 C. There is accentuation of laminar patterns in the blood flowing through these grossly dilated areas. × 300.

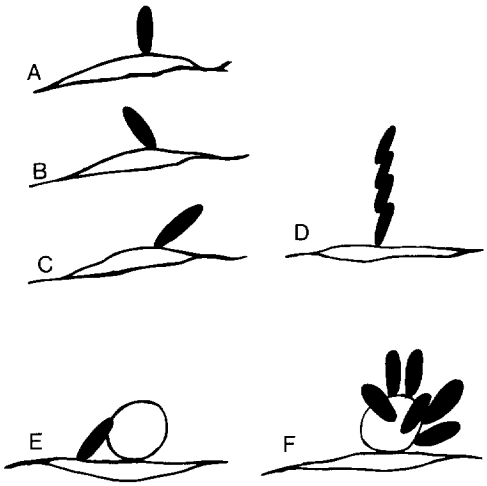


FIG. 5. Diagrammatic representation of the forms of platelet adhesion observed during early rejection. A, B, and C illustrate the oscillating platelet as seen in Figs. 3 A, B, and C. Fig. 5 D shows the form that the rare platelet aggregation took. Fig. 5 E shows the form of platelet adhesion to both endothelium and leukocytes after leukocytic adhesion commenced. Fig. 5 F shows the form of multiple platelet adhesion to an adherent leukocyte. Each is attached as a single unit by one pole and the other pole is free to move independently of the others.

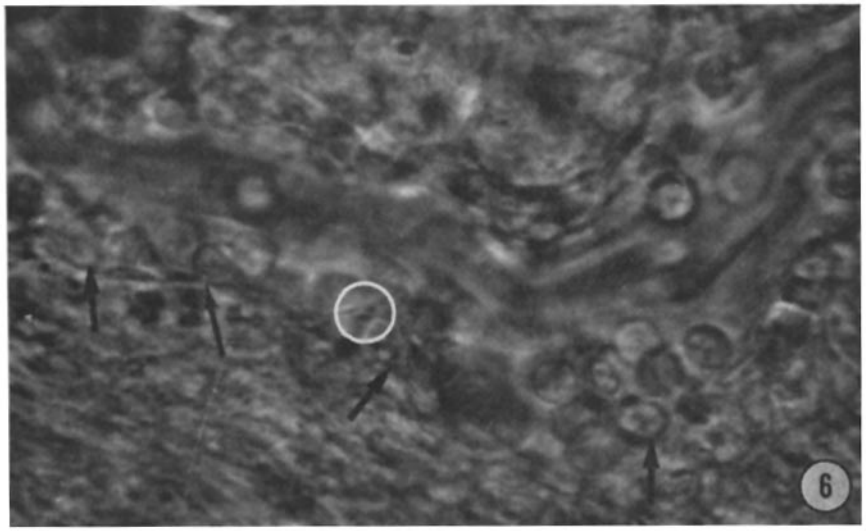


FIG. 6. Platelets (circled) adhering to leukocytes (arrows) which are accumulating along the graft endothelium. × 1,400.

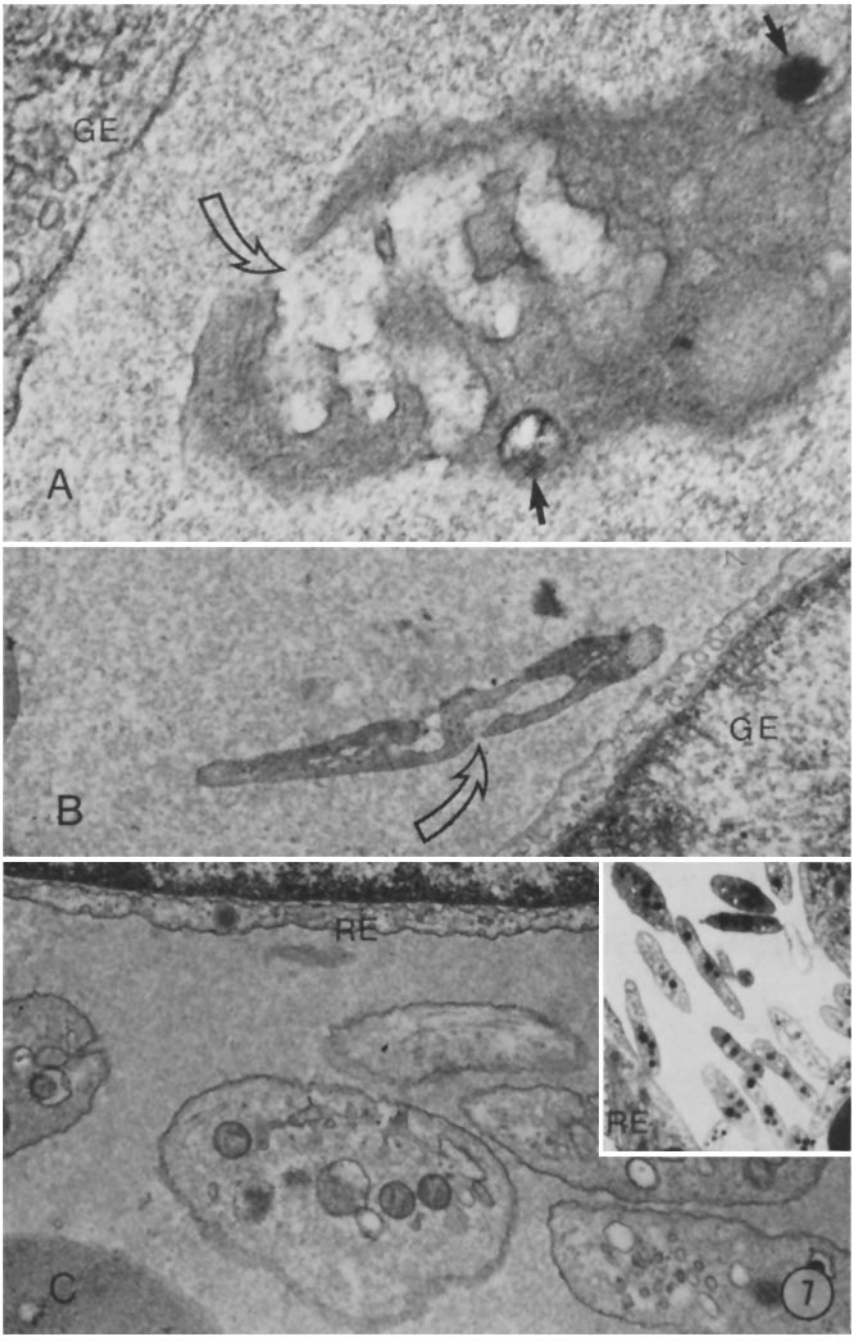
the same vein. The dilated walls are barely visible and where it is possible to see them in vivo they have the appearance of single layers of plump endothelium. Even though the blood is still flowing briskly, it can be readily seen that many of the erythrocytes are becoming aggregated into irregular clumps, and that more than the usual number of single erythrocytes are poikilocytes. These erythrocyte aggregates are also seen in the ear chamber host vessels. At the same time, the peripheral blood ESR rises abruptly. Toward the end of this period, the finer details of the grafts become clouded by developing edema. Large numbers of lymphocytes can be seen within the tubular lumen drifting towards the collecting ducts. One or two microhemorrhages consisting of from 10 to 50 erythrocytes can be seen within the graft parenchyma in the succeeding few hours, apparently occurring from capillaries. Some graft vessels develop intermittent stasis.

A needle biopsy of the whole organ at this stage shows some ultrastructural vacuolation of the tubular cells. The endothelium of intertubular capillaries appears well preserved but some have pointed luminal margins. Occasional lymphoid cells containing numerous cytoplasmic polyribosomes can be seen in contact with endothelial cells. Platelets in very close proximity to endothelium show vacuolation and loss of some or all α-granules and very dense granules (Fig. 7).

The Period 72–96 h.—The whole organ is now approximately double its original size on palpation. Urine flow is reduced, in one case to 1 ml/h. Microscopically the uncentrifuged urine has 5 leukocytes and 20 erythrocytes per high power field. When the urine is examined fresh from the catheter, fibrin strands are seen forming on the slide. The ESR continues to rise (see Fig. 8).

The ear chamber grafts show more extensive nongranular and occasional granular leukocytic adhesion in the graft venules and occasional leukocytes adhering in the arterioles. Stasis becomes more pronounced, the microhemorrhages enlarge in the graft tissues, and hemorrhages into Bowman's space can also be observed. Leukocytes are discernible in the graft tissue but their detail is not clear. Platelets are observed adhering as before and nearly all adherent leukocytes have platelets attached. The erythrocytes in both the ear chamber vessels of host origin and graft vessels are now very clumped. The host vessels of the ear chamber show no reaction except those "downstream" from anastomoses with graft veins which show leukocytic rolling but not adhesion.

The needle biopsies of the whole organs examined by electron microscopy show increased cytoplasmic vesiculation of tubular epithelial cells. The endothelial cells are swollen in most vessels and numerous lymphoid cells rich in ribosomes are in contact with their luminal surface. Within the whole graft and ear chamber graft tissue, large numbers of lymphocytes and some polymorphs are seen together with hemorrhage in the graft tissues, particularly in regions adjacent to blood vessels.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The Period 96–120 h.—Urine output from the transplanted whole kidney has almost stopped. The peripheral blood has a rising leukocyte count (Fig. 8) and the ESR is 40–50%. The ear chamber host and graft vessels both have extreme clumping of the contained erythrocytes and the lumina of graft vessels of all sizes are greatly narrowed by adherent leukocytes, so much so that some are completely filled with leukocytes and no blood flows through them. There is a

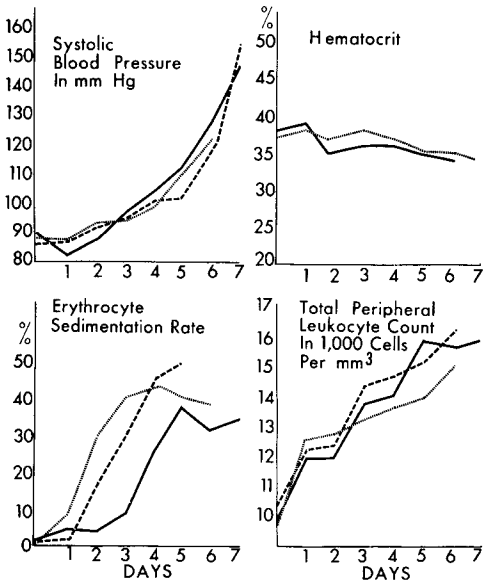


FIG. 8. The relative changes in blood pressure, hematocrit, ESR, and peripheral blood leukocyte count of three recipient rabbits in the days after both ear chamber and whole renal allografting procedures.

FIG. 7. (A) An electron micrograph of a blood vessel in a renal allograft fixed in the earliest stage of rejection when platelet adhesion was first noticed. The single platelet adjacent to the graft endothelium (*GE*) shows extensive dilatation of the surface-connected tubular system (open arrows) and a comparative lack of granules. Two of these (filled arrows) appear to be on the point of discharge from the platelet. Note absence of pseudopodia. × 61,000. (B) An electron micrograph of a similar platelet in the vessel of a rejecting ear chamber allograft. Here there is pronounced dilatation of the surface-connected tubular system (open arrows), with apparent absence of granules. It is particularly noteworthy that the platelet has retained its disk shape, with good preservation of the microtubules and no pseudopodia formation. The adjacent graft endothelium appears well preserved. × 25,000. (C) The form of the platelets present in the ear chamber (recipient vessels) under the same conditions as Figs. 7 A and B. There is good preservation of the platelet granules, even when adjacent to the recipient endothelium (*RE*). × 22,000. Inset, × 6,000.

rapid diminution of recognizable renal parenchyma remaining and the microhemorrhages are extending and coalescing to form confluent areas of hemorrhage limited to the graft tissues. Some collecting ducts remain and begin to form cystic spaces. The epithelial cells lining these spaces at times develop cilia which beat vigorously in vivo and after fixation have a typical 9 + 2 ultrastructural appearance. Even within the hemorrhagic areas, large numbers of leukocytes can be seen within the renal parenchyma. Within the vessels platelets can still be observed adhering to leukocytes.

A needle biopsy of the whole organ shows a progression of the leukocytic infiltration of the tissues seen in the previous period, with extensive deterioration of renal tubular epithelial cells. Glomeruli have some swelling of the epithelium of Bowman's space and occasional leukocytes within tuft capillaries. In some areas endothelial cells appear to have separated from their glomerular basement membrane. For the most part, however, the glomeruli are relatively well preserved.

The Period 120–144 h.—The host rabbit's blood pressure rises abruptly to almost double its previous systolic level (Fig. 8) and urine flow from the whole organ has almost ceased. In the ear chamber the graft parenchyma is very difficult to identify and few tubules can be seen. Blood flows in only a few of the organ capillaries and then only as an erratic trickle through an irregular channel between rows of adherent, mainly nongranular leukocytes three to four deep on the endothelium. Platelets continue to adhere to these leukocytes in large numbers as described previously. Most of the glomeruli in the ear chamber grafts are now bloodless apparently because of lack of afferent blood supply. However a few glomeruli in stasis have their capillaries distended with erythrocytes. Electron microscopy of a needle biopsy of the whole organ at this stage shows very few recognizable tubules. The bulk of the tissue is made up of interstitial lymphoid cells, with some polymorphs and hemorrhage.

The Period 144–168 h.—The recipient rabbits are now quite ill. One died on this day of a perforated gastric ulcer with hematemesis (Table I). The whole bloodstream is coarsely granular and microscopically composed of large masses of aggregated erythrocytes, about 250 μm in greatest diameter. Blood pressure is now up to 165 mm Hg systolic, double the normal resting value (Fig. 8). The ear chamber grafts have stasis of all vessels and no recognizable graft parenchyma remains.

The whole organ is perfused with Karnovsky fixative as described above, and in one case the animal was allowed to recover (Table I). Sections from paraffin-embedded tissues show relative sparing of the glomeruli which, however, contain occasional polymorphonuclear leukocytes and lymphocytes. There is extensive tubular atrophy and the urinary space from Bowman's space to the collecting tubules contains eosinophilic material and clear vacuoles suggesting lipid. The surviving tubular epithelial cells are very swollen. There is extensive and confluent infiltration of the renal tissue by large lymphoid cells which form

cuffs that are widest around the large arteries and taper in width with the size of vessels, almost disappearing just where the afferent arterioles join the glomeruli (Fig. 9). Several large arteries show leukocytes in their walls (Fig. 9).

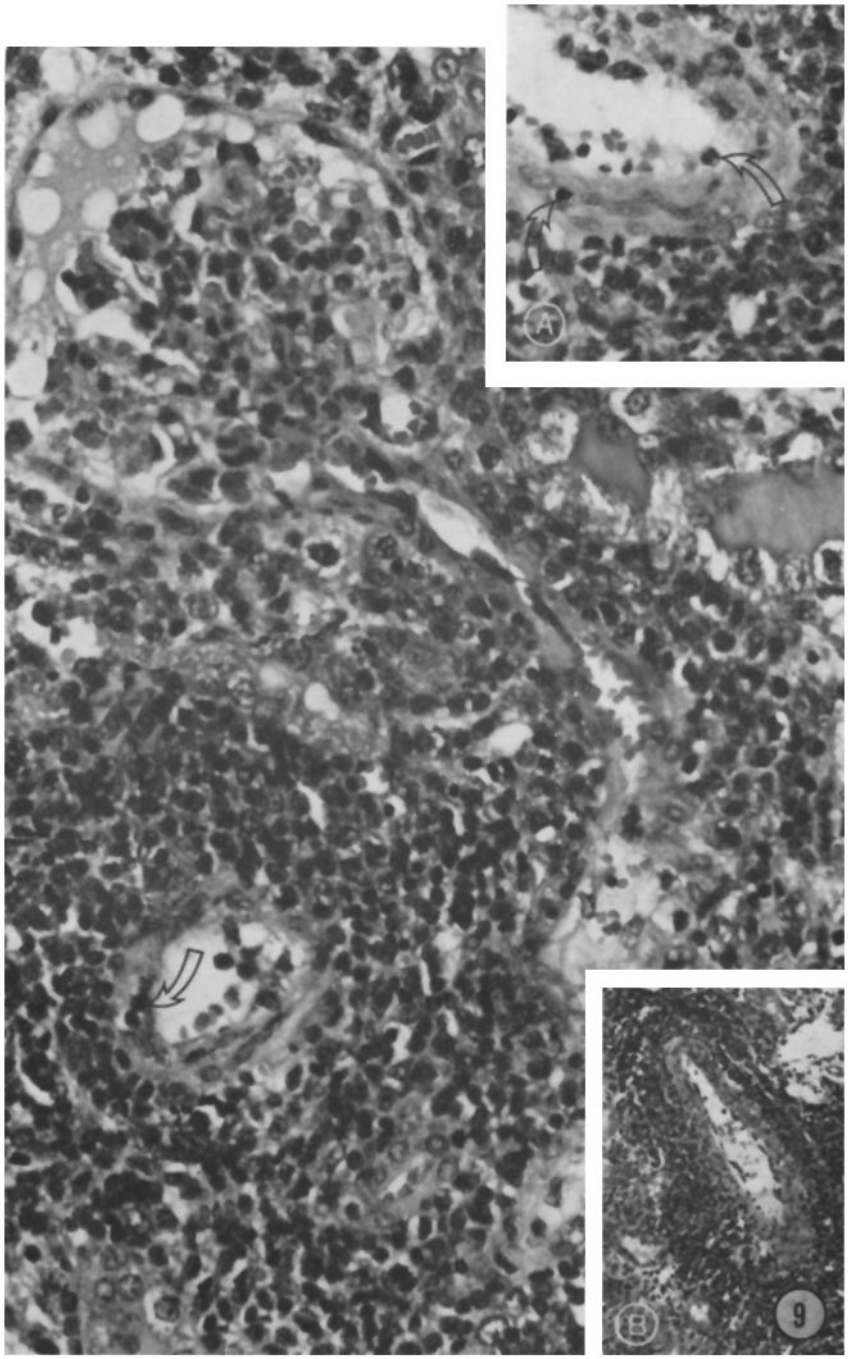
DISCUSSION

In the absence of a simultaneous allograft stimulus using tissue from the same donor, ear chamber allografts from adult donors routinely survive for several months, without modification of the rejection process (6). The presence of whole organ grafts, like that of skin grafts or intraperitoneal injections of spleen cells obtained from the same donor, triggers a more rapid rejection of the ear chamber grafts (6), which is completed within 7 days of the grafting of the whole kidney.

The correspondence of in vivo microscopic observation in the ear chamber grafts to the histological preparations of serial needle biopsies of the whole organ establishes that the process of rejection in the whole organ can be monitored in the ear chamber grafts. Using this method, it is clear that changes can be observed in vivo principally in the graft vessels long before early histological evidence of rejection is present in biopsies of the whole organ. Graft vascular constrictions and dilatations, varying patterns of blood flow, and adhesion of individual platelets without aggregation of platelets, behavior occurring at about 48 h accompanied by mild nongranular leukocytic adhesion, is the earliest evidence of rejection observed in vivo. In the following period (72–96 h) when the usual criteria of whole organ rejection first appear, such as increasing organ size, rising ESR, and reduced volume of urine which contains both protein and leukocytes, biopsy of the whole organ shows platelet and leukocytic adherence to the graft endothelium.

The ear chamber grafts reflect these changes in vivo. The rising ESR is paralleled by the increasing aggregation of erythrocytes observed in both graft and recipient vessels and the hematuria by the presence of erythrocytes in Bowman's space of the ear chamber graft glomeruli. The drop in urinary output is reflected in the in vivo observation of increasing irregularity of graft blood flow and intermittent stasis. The parenchymal destruction seen in whole organ biopsies in the period 96–120 h is reflected in the ear chamber grafts by a diminution in the number of recognizable tubules and an extension of the microhemorrhages. It is interesting that the collecting ducts are relatively resistant to this process and many commence to dilate into cystic spaces. Since embryologically they are derived from the ureteric bud and not the metanephros (14, 15) it could be that, although ureters do reject (16), they may evoke a less severe rejection process.

Glomeruli have a relatively intact structure in biopsies of the rejecting whole organ and this corresponds to the in vivo observation that, although glomeruli of the ear chamber graft have little blood flow through them, they are clearly visible, and it is rare to find leukocytes in them either by direct observation or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by time-lapse cinemicroscopy. The continuing increase in ESR reflects the progression of intravascular erythrocyte agglutination observed in the blood vessels of both graft and donor origin in the ear chambers. In the period 120–144 h, the circulatory stasis observed in the ear chamber graft vessels and the progressive obstruction of their lumina by leukocytes is reflected by the great reduction in urine being produced by the whole organ and agrees with the appearance of the needle biopsy.

For some time there has been increasing emphasis on the role of graft vessel damage in allograft destruction (17–31). The dramatic changes in vessel form and function described above, occurring when the platelet adhesion is developing in the earliest stages of rejection, suggest that these adhesive platelets play some role in the rejection process. Porter et al. (18) drew attention to intravascular platelet aggregates in human kidney allografts, and others, using sensitized animals (32–34), have commented on the possible role of platelets in canine renal rejection. Platelets have also been suggested as pathogenic factors in rapid human allograft rejection (35). Recently Busch et al. (31), describing the vascular lesions in early human renal rejection, commented on the possibility of platelets producing vessel damage without necessarily forming thrombi.

Apart from some of the early descriptions of the platelet (36–38) and some more modern studies in vivo (39, 40), by far the bulk of knowledge concerning the properties of the platelets has come from in vitro studies. These have revealed that under various stimuli resulting in aggregation, platelets may release some or all of their pharmacological contents which include histamine (41), serotonin (42, 43), ATP (44), ADP (45), fibrinogen (46), adrenaline (47), noradrenaline (48), lysosomes (49), acid phosphatase (49), elastase (50), and a so-far unidentified heat-stable factor, of mol wt less than 10,000, which causes increased vascular permeability (51, 52).

The morphological changes observed in vivo and reported here to occur in the adherent platelets, such as darkening and loss of refractibility, are similar to those platelet changes observed by Bizzozero (36, 37) and others (38, 40) in vivo in the course of producing hemostasis and viscous metamorphosis during which platelets undergo the release reaction (53). Further, platelets which have undergone the release reaction in vitro (54–57) are very similar in ultrastructural appearance to the platelets in graft vessels described in this report.

FIG. 9. Photograph showing a section from the whole renal allograft removed at day 6. The larger area shows the cuffing of lymphoid cells widest around the larger vessels, and tapering proportionately with the diameter of a branch which supplies a glomerulus. The perivascular cuff almost disappears just where the afferent arteriole enters the glomerular tuft. The open arrow indicates several lymphoid cells present within the vessel wall many of which are pyroninophilic. The tubular epithelium is swollen and vacuolated, but the glomerulus is relatively well preserved. Karnovsky fixative, paraffin embedded, hematoxylin and eosin stain. × 1,200. Inset A shows detail of a larger vessel in the same section with several cells, one resembling a neutrophil in its wall (open arrows). × 1,200. Inset B indicates the evenness of the perivascular infiltrate in another area from the same specimen. × 300.

Evidence has accumulated dramatically that platelets can release their contents under the influence of immune complexes (58–62) and by interaction of the platelet with antigenically activated leukocytes (53, 63–66). This striking similarity with the circumstances under which we observed platelet adherence described in this report becomes even closer to Henson's report that there was no lysis of the platelets during the in vitro release of their vasoactive amines (55). The identity of the leukocytes involved in these studies is disputed. Henson suggests that they are monocytes (63) but also believes neutrophils can behave in this way (55), and Barbaro and Schoenbechler felt that the small lymphocytes were responsible (64); but Aikawa takes the view that several cell types may be responsible (54). Aikawa's (54) ultrastructural studies showed pseudopodia of the adherent platelet in vitro inserted into the interacting leukocyte cytoplasm, with discharge of platelet granules. The resultant ultrastructural reduction of granules in adherent platelets resembled the appearance of platelets within graft vessels described in the electron micrographs in this report (Fig. 7).

There is little doubt that, once liberated, platelet constituents have the capacity to alter vessels (51, 67–72) and may well account for the graft vasoactivity and vessel destruction described in this report. Cochrane and Hawkins (73, 74) have postulated a similar role for platelets in the vasculitis occurring in the Arthus reaction to that occurring in anaphylaxis or serum sickness (75–78). They felt that histamine released from platelets is responsible for increased permeability, which may then allow implantation of circulating immune complexes into vessel walls and thus provoke a vasculitis (73, 74). Horowitz et al. (79) have demonstrated in canine renal allografts that there is deposition of immune complexes within the walls of the larger graft arteries such as the interlobular and afferent arterioles by the 3rd day. This is about the time we observe the vascular reaction in similar vessels in rabbit allografts and could result from the observed adherent platelet release reaction. Hughes and Tonks (80) have shown that platelet aggregation can produce vasculitis in the myocardial circulation, and others have shown that platelet aggregation by antigen-antibody complexes, viruses, bacteria, and endotoxin can cause blood vessel damage (52, 81–84) and focal lesions in the rabbit aorta (76).

From this it is clear that agents with proven vasoactivity and others with at least a potential for tissue destruction may be liberated from platelets under circumstances similar to those described in this report at the earliest evidence of rejection in vivo. This is strong evidence that platelets may play a large part in pathogenesis of diseases manifesting vascular damage without forming aggregates or taking part in thrombosis. Furthermore, in the earliest stages of unmodified rejection, the platelets may play several roles in the destructive process which results in vascular derangement and subseqeuent graft elimination.

SUMMARY

When portions of adult renal tissue are allografted into the rabbit ear chamber, they usually survive for periods of up to several months (6). When a kidney from the same donor is grafted as a whole organ, the ear chamber grafts then reject with the whole organ in 7 days. During that time serial needle biopsies of the whole organ are compared with the in vivo appearance of the ear chamber grafts. This establishes that the changes occurring in the ear chamber grafts are monitoring the rejection process proceeding in the whole organ grafts.

Dramatic vascular changes herald the earliest stages of unmodified rejection. A highly characteristic form of individual discrete platelet adhesion to both endothelium and adherent leukocytes is observed which is associated with the release reaction. At times as many as 20 such discrete platelets are clearly visible in profile in one high-power field. This demonstrates in vivo a mechanism whereby vascular and parenchymal damage may be produced by platelet contents, without previous aggregation or thrombus formation being necessary.

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