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The Production of Lesions in the Rabbit Kidney by Xenoperfusion With Fresh Human Blood*

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Summary. Kidneys of 12 heparinized rabbits were perfused with the fresh venous heparinized blood of two healthy human volunteers for 30 s, 1, 2, 5, 15, and 30 min. The renal lesion was investigated by optical, electron, and immunofluorescence microscopy. After 30-s perfusion the first signs of local human IgG fixation appeared, and after 1 min, positive staining for complement (C 4, C 3) was recorded. The intensity of fluorescence increased up to 2 and 5 min, and was strongest on the inner surfaces of arterial vessels. Weaker focal and segmental linear fluorescence was found in glomerular and peritubular capillaries. Anti-human-IgA and -IgM yielded discrete and sporadic fluorescence, while IgD, IgE, and C 1q were always negative. Anti-rabbit-Ig exhibited only vague fluorescence in the arterial adventitia and discrete linear positivity in glomerules, and reacted in perfused as well as in control non-perfused kidneys. Ultrastructurally, moderate focal platelet aggregation appeared after only 30-s perfusion. After 5 min the most striking feature was extensive disorganization with disruption of capillary and arterial and arteriolar endothelium and focal cell sloughing, without apparent topographic relation to platelet aggregation or leukostasis. The disruption of the endothelia and intracapillary blood elements became diffuse and massive after 15 and 30 min; in capillaries focal granular masses and sparse networks reactive with anti-human-fibrin and -fibrinogen appeared. Typical occlusive thrombosis was not found.

The most salient feature of blood xenoperfusion was immediate vascular fixation of human antibodies and of complement, with probable "classical" activation of the latter. Extensive endothelial destruction followed within several minutes. Man thus rejects xenografts as effectively as lower mammals.

Key words: Renal ultrastructure – Immunopathology – Renal xeno/transplantation – Hyperacute graft rejection.

* Dedicated to Prof. MUDr. Vl. Vortel, DrSc., Hradec Králové, Czechoslovakia

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Introduction

Hyperacute rejection (HAR) is a sudden dramatic event leading to rapid graft necrosis. Its essential feature is a specific local fixation of preexisting antibodies to the histocompatibility and possibly other antigens, followed by complement activation (Porter, 1976). The main target structure is the inner surface of arterial vessels and capillaries, and severe vasoconstrictive, cytotoxic, and haemocoagulative disorders cause rapid circulatory breakdown (Colman et al., 1976). In xenogeneic transplantation, the speed and severity of HAR depends largely on the degree of genetic discordance between the donor and the recipient (Starzl et al., 1964; Perper and Najarian, 1966a, b; Chaussy et al., 1975). HAR may even follow an allotransplant both in animals and in man (Dempster, 1974; Kissmeyer-Nielsen et al., 1966), but in clinical transplantation, its differentiation from accidental, "non-immune" lesions may be very difficult; HAR does not seem to be the main cause of human allograft necrosis (Rossmann and Jirka, 1979).

Changes virtually identical with classical HAR may be produced by extracorporal blood perfusion of a xeno- or even allogeneic kidney (Mozes et al., 1971). This experimental model has been used for many functional and haemodynamic observations, studies of changes in renal venous blood and in attempts to attenuate or block the rejection process. In the present study, rabbit kidneys were xenoperfused with fresh human venous blood. This modification was suggested by the availability of a great number of fluorescent anti-human antisera (11 altogether), and by the question whether a human "recipient" is capable of rejecting a highly discordant xenogeneic tissue as efficiently as do lower animals. In the experiments of Mozes et al. (1971), damage of the sheep kidney by human blood had appeared distinctly milder than in combinations of other species. In addition divergent data have been published on immunofluorescence microscopy of HAR and on the importance of haemocoagulation and aggregation in its development; some of these topics and the possible interference of artificial (e.g. perfusion) damage will be dealt with in the discussion.

Material and Methods

Experimental Model. 12 rabbits weighing 2,600–4,600 g (mean, 3,960 g) received i.v. heparin (1,600 iu/kg) and were immediately anaesthetized with i.v. pentobarbital (20–25 mg/kg). Through a flank incision left-side nephrectomy was performed, so that the renal circulation was stopped 5 min after heparin injection. Within 4 min of stoppage the renal artery was connected to the perfusion device, designed by Heller and Horáček in our Centre. Fresh xenogeneic blood was donated by both authors, healthy adult males, who had received heparin (5,000 iu i.v.) at about 10 min before the start of the first perfusion. Blood was withdrawn from the cubital vein with a teflon cannula into a siliconized silastic tubing. The perfusion device maintained a constant pressure of 130 mmHg with the aid of a peristaltic pump, under continuous monitoring with a mercury manometer; no oxygenator was included. The temperature was maintained at 37° C. The kidney was placed in a moist chamber at 37° C. Six pairs of kidneys (one with each of both blood donors) were perfused for 30 s, 1, 2, 5, 15, and 30 min. Two control kidneys, obtained in the same way, were fixed without perfusion. Renal venous blood was collected after 30 s and then at 1-min intervals.

Morphology. Fixation followed within 20–30 s after the end of perfusion. For *electron microscopy* small blocks of renal cortex were fixed by dripping and immersion (2.5% glutardialdehyde in Na^+ cacodylate, pH 7.3, 360 mval/l, 4° C, 60 min), postfixed with 2% OsO_4, and embedded into Vestopal W. Sections within the grey and silver interference area (glass knives, Ultrotome III LKB) were contrasted with uranyl acetate, partly with Pb^{++} citrate (Reynolds, 1963), and examined in a microscope Tesla BS 242 D. For *immunofluorescence microscopy* blocks of renal cortex were frozen with solid CO_2; non-fixed cryostat sections were processed by direct fluorescence with the following FITC-conjugated antisera: rabbit anti-human-IgG, -IgA, -IgM, -IgD, -IgE, -C 1q, -C 4, -C 3, and -fibrinogen (Behringwerke, Marburg/L., FRG); rabbit anti-human-fibrin and -albumin, and goat anti-rabbit-7s Ig (Travenol-Hyland, Costa Mesa, Cal.); swine anti-rabbit- and anti-mouse-7s Ig (Sevac, Prague). Controls were carried out (in addition to the use of the anti-mouse conjugate) by inactivation of antisera with the corresponding antigens: human IgG, IgA, IgM, fibrinogen (Sevac, Prague), and normal rabbit serum (5 mg or 0.25 ml/l ml of antiserum, 30 min at 20° C); further, by heat inactivation of the section (30 min at 56° C), and by elution at pH 7.2 and 3.1 (12 h, 37° C). Sections mounted in buffered glycerine were viewed in the spectrum of a mercury vapour lamp Zeiss HBO 200 (filters BG 12/UG 1 and GG 9) and recorded on film ORWO NP 27. For *optical microscopy* paraffin sections of formol-fixed tissue were stained with H.E., trichrome blue, PAS, and PTAH. Semithin sections from Vestopal W (1 and 0.3 µm) were stained with toluidine blue and impregnated with PASM-method (Movat, 1961).

Results

In all of the pairs of kidneys, perfused for identical times, the renal lesion was almost identical, and the two will be described together.

Gross Findings. After 5-min perfusion hyperaemia of the corticomedullary junction appeared, which progressed (15 and 30 min) to affect the whole cortex and medulla with marked diffuse congestion and cyanosis. Hilar arteries and veins remained patent. – Blood flow alterations will be described in detail elsewhere: in summary, immediately after the start of perfusion the flow rate was comparable with that of a normal kidney (about 2 ml/g/min). After an early decrease (second minute, to 1 ml/g/min) the flow recovered partly for a short period, but from 7 min it decreased irreversibly. After 15 min it had almost stopped (0.3 ml/g/min on the average). In the 30-s and 1-min samples of renal venous blood, Hašková found haemolysis and marked reduction of the heteroagglutinin titer (1:2–1:4, preperfusion levels 1:8–1:16). Later on, haemolysis ceased and the antibody titer returned to 1:4–1:8.

Microscopic Findings. The non-perfused kidneys had a normal structure. There was no significant leukostasis, thrombosis, or granular aggregates. After 30 s solitary leukocytes and moderate segmental hyperaemia without red cell conglutination were found. After 1 min additional sporadic "hyaline" microthrombi appeared in the capillaries, and after 2 min, moderate focal-segmental leukostasis was seen in the glomeruli (granulocytes as well as lymphocytes), but microthrombi were few. Even after 5 min the glomeruli contained only rare hyaline aggregates, but sporadic hypocellular loops displayed loss of nuclear staining and obliteration by granular masses. In both glomerular and peritubular capillaries focal hyperaemia appeared. The picture changed markedly after 15 min, when most glomeruli exhibited numerous, partially coalescent hypo- or acellular

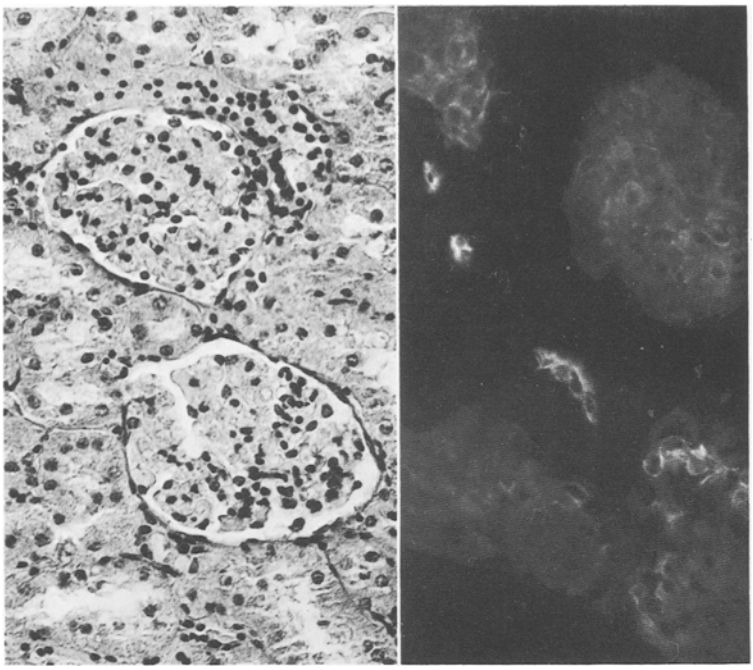

Fig. 1. *Left:* "Acellular" appearance of some glomerular capillaries after 15-min xenoperfusion. Luminal obliteration by granular masses and erythrocytes. Focal leukostasis. Elastic-van Gieson, ×270. *Right:* Fluorescence of arteriolar inner surface, faint and inconstant linear positivity of glomeruli after 1-min xenoperfusion. FITC-anti-human-IgG, ×300

capillary segments with obliterated lumina, erythrocytic aggregates, and granular and hyaline acidophilic masses (Fig. 1, left). The remaining loops showed conspicuous irregular dilatation with hyperaemia and leukostasis. In semithin sections distinct thrombocytic aggregates appeared, with parietal platelet adhaesion. The glomerular lesion varied considerably, as did the peritubular congestion and leukostasis. The arterial vessels exhibited endothelial pyknosis, and the media of interlobular arteries was thickened, with contracted "twisted" nuclei. Some arterial lumina exhibited aggregates of red cells and plasmatic "hyaline" material, but no occlusive thrombosis nor parietal fibrin layers, as seen in necrotic human allografts (Rossmann et al., 1973), were apparent. A similar picture appeared after 30 min, but obliteration and karyolysis were already global in some glomeruli. Part of the afferent arteriole was dilated by erythrocytic masses, and in some Bowman spaces acidophilic precipitates appeared. The basal membrane of dilated loops remained delicate, the mesangium was swollen and expanded, but lacked peripheral interposition. Peritubular capillary hyperaemia was more pronounced. Contracted arteries exhibited pyknosis and focal endothelial sloughing, but no thrombosis.

The results of *immunofluorescence microscopy* are shown in Table 1. In the non-perfused kidneys a faint fluorescence of anti-rabbit-Ig was seen in the adven-

titia of vessels in the form of ill-defined rings. This finding also occurred in perfused kidneys, with weak or minimal linear staining of glomerular capillaries.

Some human IgG was detected on the inner arterial surfaces after 30-s perfusion. Both the extent and intensity of this deposit increased up to 1 and 2 min and then remained constant; its intensity was only weak or medium (Fig. 1, right). In the glomeruli there was distinct linear fluorescence with negativity of isolated loops or sometimes of the whole glomerulus. Linear fixation of anti-IgG was stronger and more diffuse on the inner surfaces of arteries and arterioles. Discrete fluorescence also appeared after 2 min in groups of peritubular capillaries. Anti-IgA and -IgM presented similar pictures of very faint or barely visible fluorescence, starting from 1- and 2-min perfusion and slightly more distinct in kidneys perfused with blood of K.M. (Fig. 2, left). IgD and IgE were always negative. Staining for complement yielded conspicuous, but divergent results. C 1q was never detected. C 4 deposition appeared simultaneously with that of C 3, with the same morphology, but the fluorescence of anti-C 4 was always weak and inconstant, even in the arterioles, and a considerable number of glomeruli remained negative (Fig. 2, centre). The first traces of C 3 appeared after 1 min (K.M.), and after 2 min the positivity became evident and constant. After 5 min a strong, brilliant fluorescence of anti-C 3 was preponderant. It assumed a mainly linear character, with focal and segmental patterns and considerable local variation. After 15 and 30 min there also appeared minute granules and irregular endocapillary strips. In the arteries and arterioles fluorescence was strongest and almost diffuse, outlining the inner vascular surface (Fig. 2, right). The elastic membrane and the muscular layer

Table 1. Results of immunofluorescence microscopy. Two values mean a difference in a pair of kidneys

Perfusion time	Anti-human									Anti-rabbit Ig	Anti-mouse Ig
	IgG	IgA	IgM	IgD, IgE	C 1q	C 4	C 3	F(BG)	ALB		
0 (Control)	0	0	0	0	0	0	0	0	ND	+	ND
30 s	±	0	0	0	0	0	0	+	0	+	0
1 min	+	0 ±	0 ±	0	0	0 ±	0 +	+	ND	+	0
2 min	++ +	± +	± +	0	0	± +	++	+	0	±	ND
5 min	++ +	±	0 ±	0	0	± +	+++	++	0	+	ND
15 min	++ +	± +	0 ±	0	0	+ +	+++	+++	0	±	ND
30 min	+	± +	0 +	0	0	± +	+++	+++	ND	±	ND

Intensity: 0 = negative, ± = minimal, + = weak, ++ = moderate, +++ = massive, ND = not done

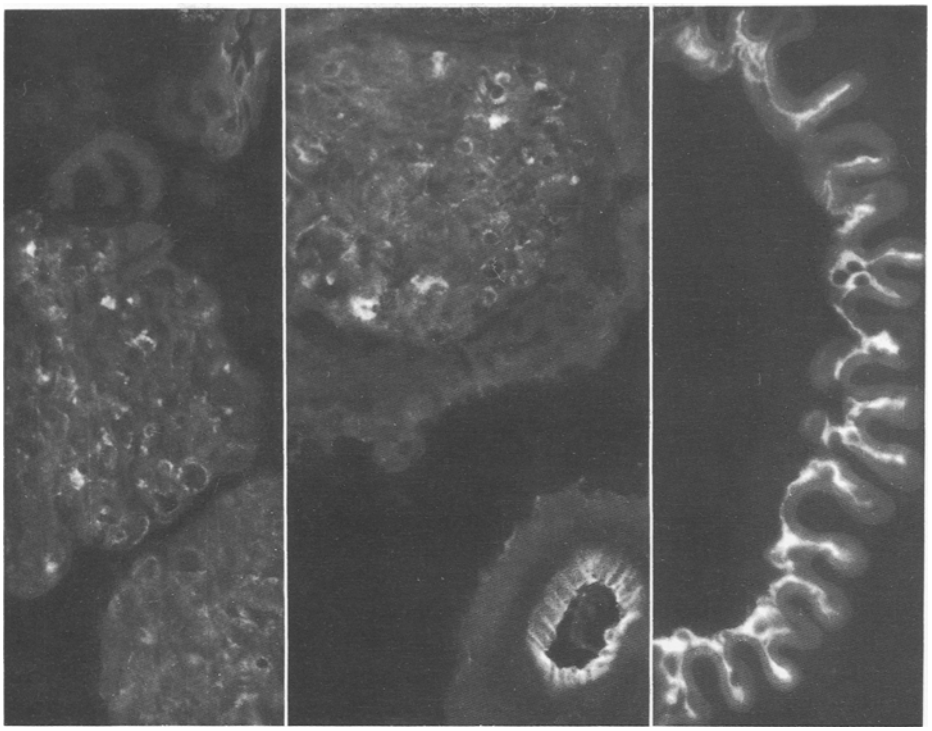

Fig. 2. *Left:* Faint or minimal fluorescence of IgM after 30-min xenoperfusion. Discrete linearities and granules in glomeruli; arterial endothelium is seen at the top. FITC-anti-human-IgM, ×300. *Centre*: Segmental positivity of C 4 in glomerulus, stronger linear fixation in arteriole after 15-min xenoperfusion. FITC-anti-human-C 4, ×300. *Right:* Diffuse and massive fixation of C 3 to endothelial membranes in muscular artery after 15-min xenoperfusion. Blue autofluorescence of inner elastic membrane, negativity of muscular layer. FITC-anti-human-C 3, ×600

always remained negative. After 30 min polymorphism and desquamation of fluorescent endothelial cells was apparent (Fig. 3, cf. left and centre). A strong, but focal positivity was seen in peritubular capillaries. Anti-fibrin and -fibrinogen displayed minute granules in some glomerular loops, arteries, and sinuses after 30 s, and branched mesangial strips and small interstitial pools of staining appeared after 2–5 min. A different picture prevailed after 15 and 30 min: dilated glomerular loops contained plentiful, strongly fluorescent granular masses, filling capillary segments of even whole tufts. Sporadic granular and fibrillar aggregates also appeared within peritubular capillaries, but massive thrombosis was absent. Tests for human albumin and incubation with anti-mouse-Ig were always negative.

Pretreatment of antisera with the corresponding antigens (human IgG, IgA, IgM, fibrinogen, rabbit serum) caused disappearance of fluorescence, but exposure of renal sections to 56° C for 30 min had no effect on the positivity of anti-C 3. Elution of sections at pH 3.1 led to substantial decrease or negativity of anti-IgG and -C 3 (Fig. 3, right), whereas anti-fibrin or -fibrinogen remained

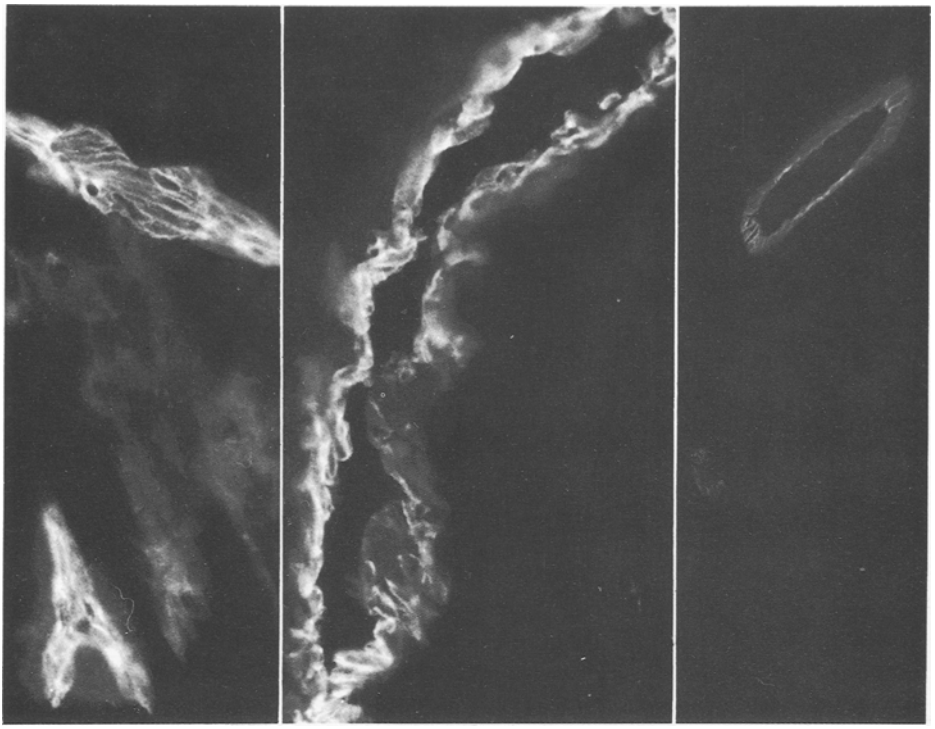

Fig. 3. *Left:* Strong fluorescence of endothelial membranes in longitudinal section after 15-min xenoperfusion. Uniformity of cell contours. FITC-anti-human-C 3, ×300. *Centre:* Massive fluorescence of C 3 after 30-min xenoperfusion. Polymorphism and disarray of endothelium (cf. with left). FITC-anti-human-C 3, ×300. *Right:* Result of elution at pH 3.1. 30-min xenoperfusion. Negativity of glomerulus (*lower right*), faint residual fluorescence of arteri(ol)es. Cf. with central part. FITC-anti-human-C 3, ×150

unchanged. Elution at pH 7.2 had either no effect or caused only a slight diminution of intensity.

Electron microscopy was mainly focused on lesions of glomeruli, arterioles, small muscle arteries, and cortical peritubular capillaries. Non-perfused kidneys had normal fenestrated glomerular endothelium with discrete focal sloughing, and the mesangium exhibited only minute subendothelial cytoplasmic protrusions. The capillary lumina contained isolated leukocytes, few erythrocytes, and minute membrane fragments. The arterial endothelium had a normal appearance.

After 30-s perfusion the picture was unchanged, with isolated or sparsely clumped platelets. After 1 min the glomerular loops contained isolated polymorphonuclear leukocytes with short cytoplasmic protrusions, adherent to endothelium, but the fenestrated membrane was not damaged, and the arterial endothelium remained intact. Following 2-min perfusion by the blood of K.M. the endothelia did not change, although local increase of smooth-membrane-limited vesicles (SMV) was recorded in some places (Fig. 4). In another kidney (P.R.),

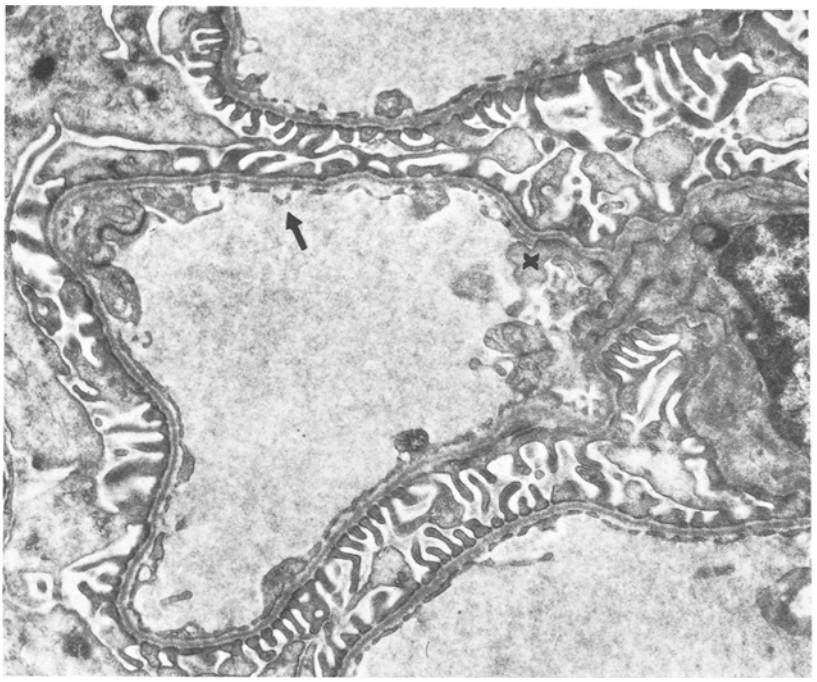

Fig. 4. Normal ultrastructure of glomerule after 2-min xenoperfusion (K.M.). Same picture was found in control non-perfused kidneys. Very slight irregularities of endothelia (*arrow*) and focal mesangial protrusions (×), possibly signs of brief preperfusion ex-vivo ischaemia. ×11,900

there appeared focal swelling, polymorphism, and small defects of the endothelial fenestrated membrane (Fig. 5); some capillary loops displayed loose platelet aggregates, focal hyperaemia, and isolated leukocytes with short microvilli. The cellular membranes of granulocytes showed focal ruptures, and capillary lumina contained free leukocytic granules, membrane fragments, and myelin bodies. A similar picture was also found in peritubular capillaries. Some of the arterial endothelial cells exhibited numerous SMV and a dense shrunken cytoplasmic matrix.

5-min perfusion caused extensive, though not diffuse endothelial damage. The glomerular fenestrated membrane had turned into clusters of chaotic sloughing vesicles with thickened, blurred, densely contoured limiting membranes. Numerous loops harboured aggregates of platelets with plentiful pseudopodia, accasionally adhering to the disintegrating endothelial layer or to the basal membrane, as did granulocytes and lymphocytes. Solitary strips of dense intracapillary material resembled fibrin, but did not show typical periodicity. The endothelial lesions had the same appearance both in obstructed and in patent capillaries, enclosing neither leukocytes nor platelets. Similar destructive lesions were also found in peritubular capillaries. The arterial endothelia showed rugged, spiky cell membranes with indistinct blurred contours and dense, shrunken cytoplasmic matrix, especially in the superficial zone (Fig. 6). The cytoplasm contained plenti-

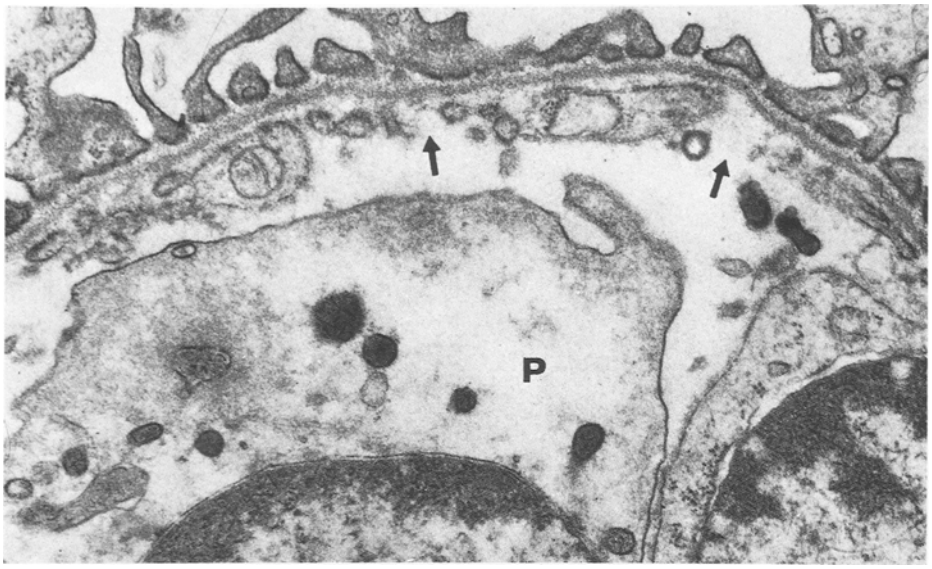

Fig. 5. Early endothelial lesion after 2-min xenoperfusion (P.R.). Irregular endothelial defects (*arrows*). Thickened, dense, blurred contours of endothelial cytoplasmic membrane. Intraluminal polymorphonuclear leukocytes (*P*). ×28,600

ful SMV and GER with dense limiting membranes, partially bulging and sloughing into the lumen. No intraluminal aggregates of platelets, leukocytes, or fibrin nets were detected. After 15 and 30 min the endothelial disruption became total. Extensive areas of glomerular basal membranes were completely denuded, and the cell residues formed scattered clusters of swollen vesicles with blurred, darkly contoured membranes. The cell bodies had transformed to dense areas of fragmented organels, many of which obliterated capillary lumina. Most of capillary loops were occluded by platelets, erythrocytes, leukocytes, amorphous debris, and dense fibrin-like masses. The majority of platelets formed compact aggregates, with numerous pseudopods and vacuoles, and exhibited loss of α-granules; dense bodies were scarce or absent. In some capillaries the basal membrane was covered by a single, continuous, focally interrupted layer of thrombocytes with irregular humpy surface and discoid protrusions (Fig. 7). Polymorphonuclear leukocytes showed multiple vacuoles and cisterns, partially enclosing fragments of membranes, and their cytoplasmic protrusions adhered to the basal membrane as well as to the luminal endothelial residues. The laminae densa and rara externa had a normal appearance despite total destruction of endothelial cells, the foot proceses were also preserved. The mesangial matrix exhibited a honeycombed oedematous structure, and the axial cells had dark, condensed cytoplasm. The residues of arterial endothelial cells had the form of dense, coagulated layers and clumps of vesicles and cytoplasmic fragments, partly sloughed from the basal membrane (Fig. 8). Their nuclei were hyperchromatic, and perinuclear spaces were dilated. Arterial lumina enclosed dense granulovesicular masses, apparently of endothelial origin, but platelet

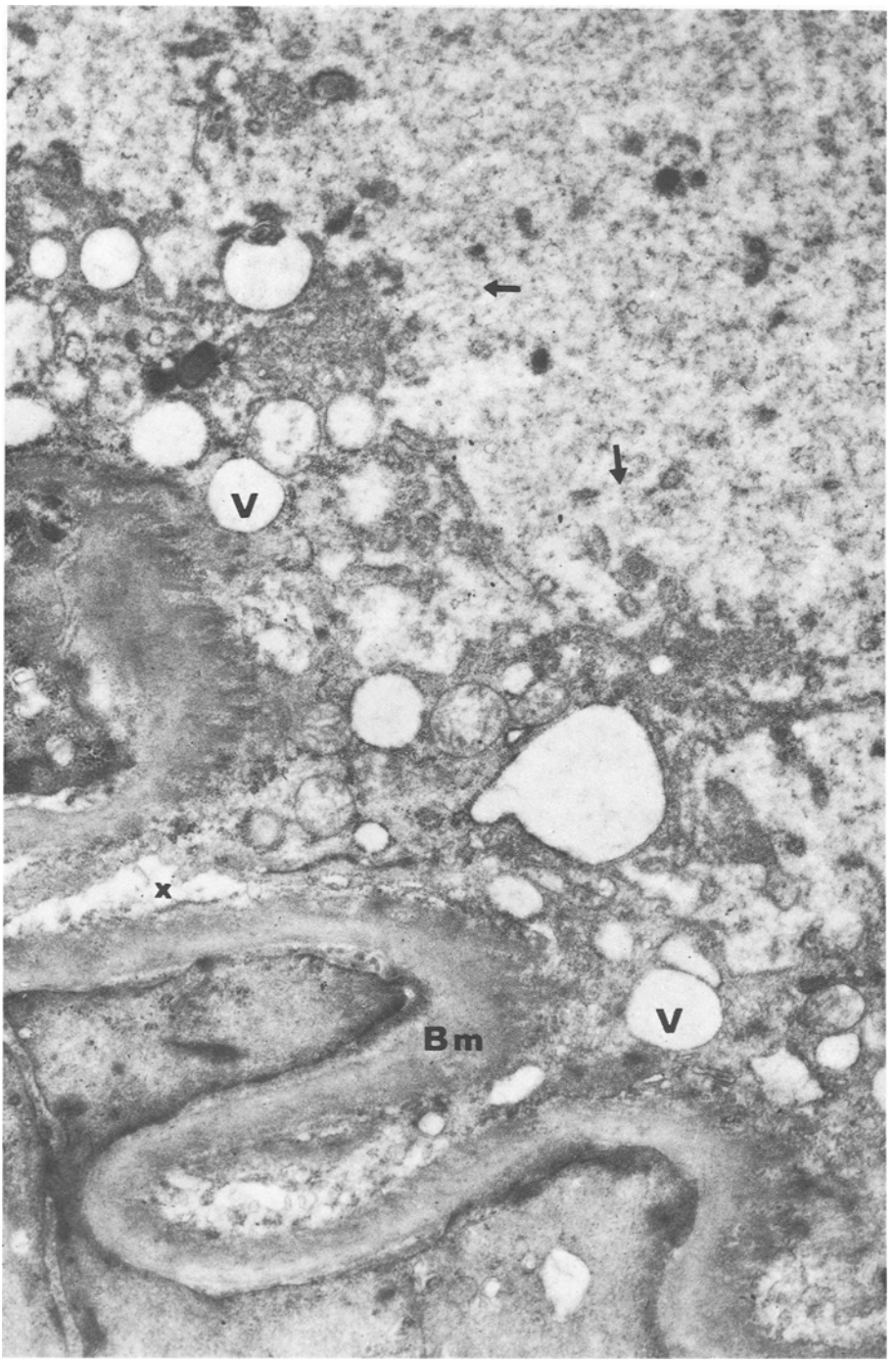

Fig. 6. Endothelium of muscular artery after 5-min xenoperfusion. Granular shrinkage of cytoplasmic matrix. Numerous dilated vesicles and vacuoles (*V*) of smooth and granular endoplasmic reticulum. Disintegration of cell membrane with intraluminal extrusion of dark, densely contoured granulovesicular debris (*arrows*). Focal endothelial sloughing (×). Basement membrane (*Bm*) is unaffected. ×20,000

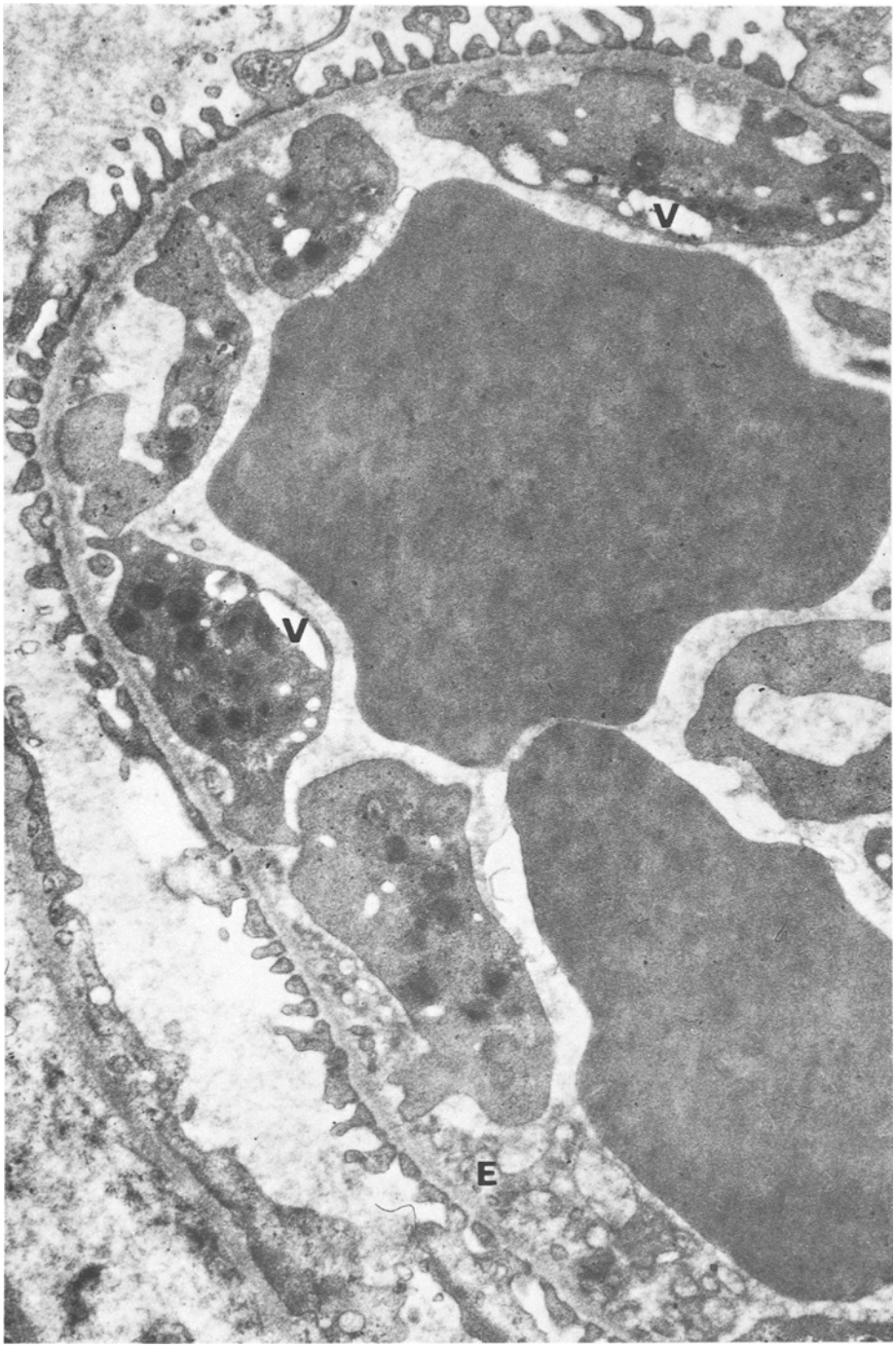

Fig. 7. Detail of glomerular capillary after 30-min xenoperfusion. Denuded basal membrane is "endothelized" by a layer of platelets. At right bottom, residues of genuine endothelia (*E*). Platelet vacuolization and degranulation (*V*). Lamina densa, visceral and parietal epithelia are preserved. ×20,000

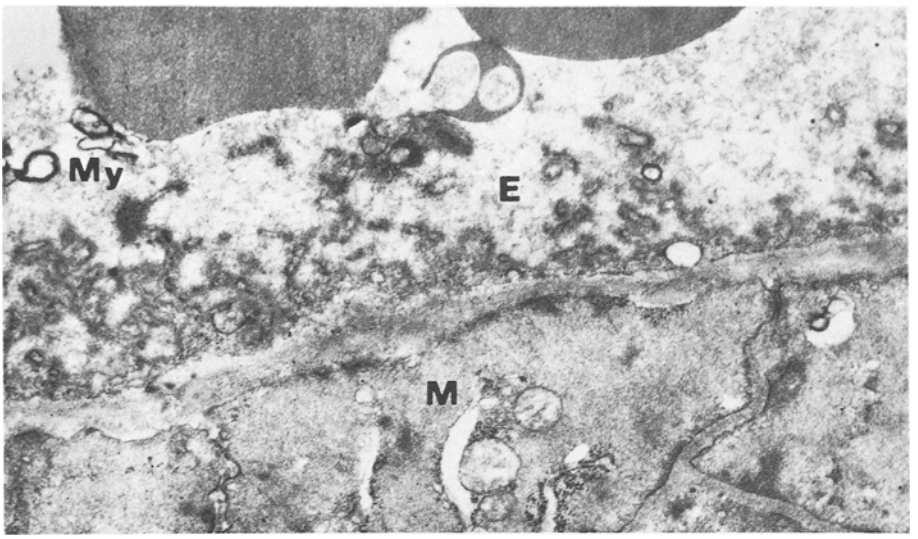

Fig. 8. Disintegration of arterial endothelium after 15-min xenoperfusion. Rests of coagulated cytoplasm are seen as an irregular layer, adherent to the basal membrane. Chaotic dense membranovesicular residues and myelin figures (*My*) in lumen. Muscle cells (*M*) without conspicuous damage. ×20,000

aggregates and leukostasis were absent. The elastic membranes were preserved, and the muscle cells contained small numbers of SMV. The epithelia of proximal convoluted tubules showed dilatation of the basal labyrinth, apparent also in non-perfused kidneys, swelling of mitochondrial matrix, vacuolization and focal formation of myelin bodies, but no structural breakdown comparable to that of the endothelium.

Discussion

The xenoperfusion-induced renal lesion is virtually identical with the picture observed in HAR following intracorporal xenotransplantation (Milgrom, 1977). The endothelium represents the main target structure of the rejection attack (Rosenberg et al., 1971; Friedman et al., 1975). Although the primary pathogenetic factor of HAR is a humoral immune reaction, the final picture is modified by secondary effector mechanisms, such as leukostasis, platelet aggregation, and haemocoagulation processes (Rowlands et al., 1967; Vassalli and Cluskey, 1971). The following comment will be directed towards the main features of the immunopathology, and to some secondary and intercurrent events.

Immunopathology. The earliest sign of the immune response seems to be the local fixation of *immunoglobulins*: in the present experiments it was detectable after a 30-s perfusion. Arteriolar positivity preceded the deposition in the glomeruli, and the strongest fluorescence was displayed by anti-IgG; IgA and IgM

remained discrete, while IgD and IgE were absent. The data on immunofluorescence in HAR are divergent: linear deposition of IgM prevailed in renal allografts of presensitized Macaca speciosa (Busch et al., 1975b), whereas other authors noted only IgG, both in clinical allotransplantation (Williams et al., 1969) and in xenoperfused canine kidneys (Pielsticker et al., 1972). In numerous studies of HAR, however, the deposition of IgG was found to be very weak or non-existant in both man and experimental animals (Lund and Sommer-Hansen, 1972; Berger et al., 1975). Quantitative evaluation of immunofluorescence is influenced of course by the properties of antisera and by other technical details but the quantitative aspects of our results are not the most important. As stated above, the intensity of anti-IgG staining did not increase after a perfusion time exceeding 2 min; even after 30-min perfusion some glomeruli or segments thereof remained unstained. In many clinical cases, the original picture of HAR may be distorted by long intervals between the onset of graft destruction and needle biopsy and/or excision (Rossmann and Jirka, 1973). Furthermore, moderate linear fixation of IgG is a frequent, if not constant, finding in the glomeruli of prospective "zero-hour" allografts (Busch et al., 1975a), preserved either by simple hypothermia or by continual perfusion (Dupont et al., 1974; Filo et al., 1974). The pathogenic basis of immunoglobulin deposition is probably different in this instance (Tourville et al., 1977), but the fixation of IgM and complement from the cryoprecipitated perfused plasma may stimulate the subsequent rejection attack (Light et al., 1975).

Antibody deposition by itself lacks potent destructive properties unless followed by the local fixation and activation of *complement* (C) (Müller-Eberhard, 1968). Even here, however, assessment of immunofluorescence is difficult. In the present series, C was visualized slightly later than IgG, but after 5 min striking and brilliant fluorescence of anti-C 3 was seen. As with IgG, the initial and strongest deposition was in arterial vessels, whereas in glomeruli focal and segmental involvement persisted even after 30-min xenoperfusion. Strong positivity with anti-C 3 probably corresponds a high concentration of C 3 in the serum (Fearon et al., 1977). With the aid of anti-C 1q and -C 4 we tried to confirm the assumption that activation of C in HAR follows the complete, "classical" pathway (Alper and Rosen, 1975); evidence for this has been yielded by the proof of sequestration of C 1, C 4, and C 2 in the rejected graft (Porter, 1974).

Berger (1975) has demonstrated frequent fluorescence with anti-C 3 in biopsies of various human nephropathies, but it was usually unaccompanied by positivity of early fractions. The presumed "alternative" activation of C 3 seems to result in less noxious effects and to possess limited cytotoxic and inflammatory properties (Verroust et al., 1974). In our biopsies of autologous and allografted human kidneys, positivity for anti-C 3 is a common finding, in the form of scattered granules and strips within the mesangium, most frequently in the media of arteries. Local detection of C 3 does not necessarily reflect precipitation of immune complexes or an immune lesion (Ward, 1971), and C 3 may persist in situ long after eventual disappearance of Ig (Rosenau et al., 1969). In our human allografts, arterial C 3 positivity was never accompanied by staining of anti-C 1q or -C 4 (Rossmann and Jirka, 1979). Local

("innocuous") C 3 fixation was also observed in non-used prospective grafts (Sterling et al., 1972; Busch et al., 1975b).

In the present series, the participation of the C system seems to be of different kind, and two conspicuous morphological features have to be stressed. The first is positive staining for an early fraction -C 4. Anti-C 1q failed to react, but this may only reflect insufficient sensitivity of the direct method in poorly-concentrated C 1, or perhaps a very rapid decay of this initial fraction. Anti-C 1q of the same make and batch had yielded a positive reaction in deposits of L.E. nephropathy, so that false negativity is unlikely. Secondly, in xenoperfusion nephropathy, fluorescence of C 4 and C 3 affected the inner arterial surface exclusively, while the muscular layer and the inner elastic membranes remained negative. Thus, it is probable that complete, "classical" activation of C occurs in blood xenoperfusion. Depletion or inactivation of C can considerably delay the onset of HAR (Moberg et al., 1971; Meija-Laguna et al., 1972; Belitsky et al., 1973), and these processes are much more efficient in this respect than heparinization (Rosenberg et al., 1971) or depletion of leukocytes and platelets (Forbes et al., 1976). Nevertheless, HAR cannot be suppressed even by elimination of C, and the graft tissue is possibly also attacked by different types of humoral response (Schilling et al., 1975). Nor can pharmacological stabilization of endothelial membranes prevent the eventual cell disruption (Busch et al., 1976).

Secondary Destructive Mechanisms and the Experimental Model. Extracorporal renal perfusion will generate several artifacts and secondary disturbances. Initially, the excised kidney is submitted to a short ex-vivo autolysis, but the 4-min "warm" ischaemia in our schedule is acceptable and is hardly achievable in a clinical cadaver transplantation programme. Minute endothelial defects and mesangial protrusions without cytoplasmic shrinkage and cell disruption correspond to analogous (reversible) findings in human prospective allografts, and are apparently of the same origin. Secondly, in continually perfused human kidneys, segmental necrobiosis of glomerular capillaries and disseminated microthrombi compromising subsequent graft function have been repeatedly observed (Spector et al., 1975). As documented by Limas et al. (1977), hypocellular occluded capillary segments, found in 1-hr posttransplantation biopsy of such grafts, resemble glomerular damage in HAR. A more detailed examination disclosed considerable differences. In ultrastructural studies, massive intravascular coagulation was found to be accompanied by swelling and local defects of endothelium, but without massive cytoplasmic condensation or disruption of cell membranes comparable to the xenoperfusion damage. Moreover, normally fenestrated endothelial cells persisted in many capillary loops even 1 hr posttransplant. By immunoflurescence microscopy, insudates of IgM within the arterial media were seen, whereas consistent linear positivity of IgG and C was absent. Several series of comparative experiments of extracorporal auto- and alloperfusion (dogs, rabbits) are being performed by Heller in our Centre. Preliminary results show a remarkable stability of renal blood flow beyond a 30-min interval, as opposed to its rapid failure in xenoperfusion. Consequently, most of the lesions described in this paper seem to depend on xenogeneic

properties of perfused blood rather than on mechanical and/or other non-immune disturbancies. Pathogenic and morphological differencies between disseminated intravascular coagulation and HAR have been the subject of many detailed studies (e.g. Colman et al., 1969; Watanabe and Tanaka, 1977).

Preliminary perfusion by isotonic saline, Collins's or other solution was omitted in order to shorten the initial ischaemia and to avoid additional endothelial artifacts. Admittedly, initial haemolysis may influence the renal lesion, but in our experiments it did not cause an irreversible vascular occlusion. The first-minute blood flow (about 20 ml) seems adequate to withdraw the rests of rabbit blood, so that most intravascular elements described above are of xenogeneic, i.e. human origin.

The premedication of both the "donor" and the "recipient" by heparin is indispensable for maintaining a good perfusion even in the extracorporal auto- and alloperfusion model. In canine xenoperfusion, however, massive (tenfold) doses did not prevent rapid decline of the renal circulation. Anticomplementary effects of heparin cannot be excluded, but previous studies have stressed the inefficacy of heparin in HAR (Rosenberg et al., 1969, 1971), and local fixation of C was intensive in the "heparinized" conditions of our experiments. Here again, considerable differences exist between HAR and disseminated coagulation in anaphylactic reactions, e.g. the Shwartzman phenomenon (Klassen and Milgrom, 1971).

In conclusion, man appears to be endowed with potent destructive tools to reject xenogeneic tissues. The individual mechanisms evidently work with considerable autonomy, and a weakening or elimination of one or more of them cannot modify the final catastrophic outcome. The primary impulse consists of the formation of a high-molecular, insoluble, and very aggressive complex of antigen(s), antibodies, and classically activated complement. The target endothelia are hit with a tremendous *velocity and efficacy* and our observations fully support the opinion of Dempster (1974), that HAR is the most violent defensive response known in general immunology.

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