Virchows Arch. A Path. Anat. and Histol. 372, 259–285 (1977)

Virchows Archiv A

Intra- and Extrarenal Vascular Changes in the Acute Renal Failure of the Rat Caused by Mercury Chloride*

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Summary. Histologic evidence of intrarenal vasomotor changes were observed in the rat in the course of acute renal failure caused by the injection of $HgCl_2$. Male Wistar rats injected s.c. with 2.5 or 4.7 mg $HgCl_2$ per kg b.wt. developed fibrinoid damage in the media segments of preglomerular renal vessels, mostly in the arcuate and interlobular arteries. The lesions were patchy and irregularly scattered throughout the kidneys. 24 h post-injection the lesions were very rare and of only mild degree, whereas they were fully developed and regularly seen 48 h post-injection. A high percentage of similar changes was found in certain extrarenal vascular areas especially in the mesentery and pancreas. The damaged vascular segments were usually dilated. The results of various trichrome stains and histochemical reactions suggested edema of vascular smooth muscle cells and imbibition of the media by blood plasma substances, sometimes reaching the degree of fibrinoid necrosis. These findings were confirmed by electron microscopy. The imbibition of the smooth muscle cells by blood plasma material was clearly evidenced by the demonstration of intracellular fibrin precipitations. In connection with the degeneration of smooth muscle cells, accumulations of crystal-like fibrin formations could often be shown. Subendothelial fibrin formations were not observed. 96 h after the 2.5 mg injection the changes were already regressing, but edema of the vascular wall and signs of disturbed vasotonia persisted for several days. The maximum of the vascular changes usually coincided with the maximum of azotemia and the formation of debris cylinders in the renal tubules. However, no clear relationship was recognizable in individual cases between vascular damage, extent of tubular necrosis and renal function. The pathogenesis of the vascular changes is obscure, but neurogenic factors, increased release of catecholamines and/or

* Part of this work has been communicated at the 59. meeting of the German Society of Pathology, Kiel, May 20–24, 1975 (Verh. Dtsch. Ges. Path. **59**, 454 (1975)) and at the Hacettepe University of Ankara (Turkey), June 22, 1976

vasoactive agents of renal origin in connection with other factors might play a decisive role. Arterial hypertension was absent. It is assumed that the structural damage of the vascular media is mainly brought about by prolonged or recurring vasospasms, or by alternating spasm and vasodilatation with local ischemia and increased tension of the vascular wall in the dilated segments. The altered function and structure of the vascular wall might, to a certain extent, contribute to renal insufficiency.

Key words: Mercury poisoning – Kidney failure, acute – Vasomotor system – Vascular disease – Permeability.

Zusammenfassung. Histologische Untersuchungen ergaben Hinweise dafür, daß bei Ratten im Verlauf des durch $HgCl_2$-Injektion erzeugten akuten Nierenversagens Störungen der intrarenalen Vasomotorik auftreten. Männliche Wistar-Ratten entwickelten nach einmaliger subkutaner Injektion von 2,5 oder 4,7 mg $HgCl_2$/kg KG segmentale fibrinoide Läsionen in der Media präglomerulärer Nierengefäße, am häufigsten in Aa. arcuatae and Aa. interlobulares. Die Läsionen traten fleckförmig und unregelmäßig über die Nieren verteilt auf. Sie waren nach 24 Stunden sehr selten und gering ausgeprägt, dagegen nach 48 Stunden voll entwickelt und regelmäßig anzutreffen. In bestimmten extrarenalen Gefäßprovinzen (vorzugsweise Mesenterium und Pankreas) fanden sich in einem hohen Prozentsatz gleichartige Veränderungen. Die geschädigten Wandabschnitte waren meist dilatiert. Das Ergebnis verschiedener Trichromfärbungen und histochemischer Reaktionen ließ auf ein Ödem der Gefäßmuskelzellen und eine Imbibierung der Media mit Blutplasmasubstanzen bis hin zur fibrinoiden Nekrose schließen. Dies konnte elektronenmikroskopisch bestätigt werden. Die Imbibierung von Muskelzellen mit Blutplasmasubstanzen wurde besonders durch den Nachweis intrazellulärer Fibrinpräzipitate deutlich. In Verbindung mit Muskelzelluntergängen waren oft Anhäufungen kristallartiger Fibrinformationen nachweisbar. Subendotheliale Fibrinablagerungen fanden sich nicht. 96 Stunden nach der Injektion (2,5 mg-Dosis) waren die Veränderungen bereits rückläufig, jedoch konnten Gefäßwandödem und Zeichen eines gestörten Vasotonus noch nach mehreren Tagen beobachtet werden. Zeitlich korreliert das Maximum der Gefäßveränderungen im allgemeinen mit dem Höhepunkt der Azotämie und der Ausbildung von Detrituszylindern in den Harnkanälchen, jedoch bestanden im Einzelfall keine strengen Beziehungen zwischen Gefäßwandschaden, Ausdehnung der Tubuluszellnekrosen und der Nierenfunktion. Die Pathogenese des Gefäßwandschadens ist unklar. Neurogene Faktoren, vermehrte Ausschüttung von Katecholaminen und/oder von vasoaktiven Substanzen renalen Ursprungs könnten im Verein mit anderen Faktoren eine entscheidende Rolle spielen. Ein arterieller Hypertonus war nicht nachweisbar. Es wird vermutet, daß für die Entwicklung der Gefäßwandschäden anhaltende oder rezidivierende Vasospasmen oder der Wechsel von Spasmus und Vasodilatation mit lokaler Ischämie und vermehrter Wandspannung in dilatierten Gefäßwandabschnitten ausschlaggebend sind. Die gestörte Funktion und

Struktur der Gefäßwand könnte bis zu einem gewissen Grade zur Niereninsuffizienz beitragen.

Introduction

For many years, mercury chloride (MC) poisoning has been used as an experimental model for acute renal failure induced by nephrotoxic agents. However, the pathogenetic mechanism of the resulting oliguria or anuria is still imperfectly understood. While tubular obstruction by cellular debris or interstitial edema are thought to play no role in the initiation of MC-induced renal insufficiency (Oken, 1972; Balint et al., 1973; Flamenbaum, 1973), the passive backflow hypothesis (Bank et al., 1967; Steinhausen et al., 1969) has been maintained more recently (Wada et al., 1974). On the other hand, evidence has been found that MC and other nephrotoxins (Oliver et al., 1951; Oliver, 1953; Conn et al., 1954; Hollenberg et al., 1970) cause considerable disturbances of renal circulation. Balint et al. (1973) have suggested that alterations in pre- and postglomerular vascular tone might be responsible for the functional impairment in MC-induced renal failure. On arteriography, Sherwood et al. (1974) found a dose-dependent vascular shut-down in the dog renal cortex and concluded that "a primary vascular disorder" might be of importance. In studies on isolated perfused rat kidney, Russell (1975) has presented evidence that MC may evoke an increase in vascular resistance. Preuss et al. (1975) found that early functional alterations associated with MC injection (4.7 mg/kg) in the rat were similar to those after total renal ischemia.

In contrast, structural abnormalities of the intrarenal vessels have not been reported in most of the morphologic investigations of MC poisoning. To the best of our knowledge, vascular lesions have been mentioned in only two papers: Staemmler (1956) investigating rat kidney on the second day after injection of 3 mg MC/kg b.wt. i.p. reported lipid infiltrations in the media of single small arteries of the renal cortex, and Kosmider et al. (1968) observed endothelial proliferation in medium-sized and small renal arteries with thickening of the vascular wall and perivascular fibrosis 30 days after i.v. injection of 3 mg MC per kg b.wt. in the rabbit.

In the following, we deal with structural lesions, especially fibrinoid damage in intrarenal and certain extrarenal arterial vessels, previously undescribed in MC poisoning but comparable to those reported in the intrarenal vessels of the rat after temporary complete renal ischemia (Cain and Fazekas, 1963; Thoenes, 1964; Kaboth, 1962, 1965; Terry et al., 1970) or after injection of the toxins of Amanita phalloides (Cain, 1965) and, in the rabbit, after orthostatic collaps or, more pronounced, following orthostatic collapses in combination with hemorrhages (Cain, 1965). Furthermore, at least on the light microscopic level, similarity seems to exist to histologic findings in the rat kidney after injection of vasopressin (Byrom, 1964), angiotensin II (Byrom, 1964) or methoxamine, a sympathicomimetic agent (Herbertson and Kellaway, 1960). From the histologic findings presented in this study we conclude that severe vasomotor changes occur in the course of acute renal failure induced by MC-injection.

Interestingly enough, the development of structural damage of the arterial wall apparently parallels the progressive obstruction of the terminal proximal tubules by cellular debris, a process assumed to be indicative of a very low glomerular filtration pressure (Flamenbaum et al., 1971). The vascular lesions possibly contribute to the renal insufficiency caused by MC. Communicating our results we hope to present some new morphologic aspects of the pathogenetic mechanism working in the course of MC-induced acute renal failure in the rat.

Materials and Methods

Male Wistar WU rats of the Ivanovas breeding institute (Kisslegg, Allgäu) with a body weight (b.wt.) of 180–270 g were studied in the main experiment. All animals received a standard laboratory diet and tap water ad libitum. Mercury chloride (MC) was dissolved in 0.9% physiologic saline. The rats were injected subcutaneously in the dorsal region with 2.5 mg or 4.7 mg/kg b.wt. of a 1 mg/ml solution of MC. The latter dose was the same as that used by other investigators (Bank et al., 1967; Steinhausen et al., 1969; Flamenbaum et al., 1971; DiBona et al., 1971; Preuss et al., 1975).

The following experimental groups were formed for light microscopic studies: Group Ia (7 rats) was examined 24 h after injection of 2.5 mg MC/kg b.wt., Group Ib (7 rats) 24 h after injection of 4.7 mg MC/kg b.wt. Group IIa (21 rats) was examined 48 h after injection of 2.5 mg MC/kg, Group IIb (24 rats) 48 h after injection of 4.7 mg MC/kg. Previous studies had shown that with very few exceptions, injections of 4.7 mg MC/kg b.wt. resulted in the death of the animals on day 3 or 4 after administration. Therefore the subsequent regenerative phase of the injury was only studied after injection of 2.5 mg MC. Group III (11 rats) was investigated 96 h after injection of 2.5 mg MC/kg and Group IV (8 rats) 7 days after injection of the same dose. After the injection the animals were given free access to food and water. 12 rats each from Groups IIa and IIb were kept in individual metabolic cages. In these animals, diuresis, natriuresis and body weight were determined every 4 h. In an additional group of 10 rats (Group V) injected with 4.7 mg MC/kg, systolic blood pressure was measured under light ether anesthesia by means of a tail plethysmometric method (Byrom and Wilson, 1938). The animals were sacrificed 50 h post-injection. At the time of sacrifice the animals were anesthetized with ether and bled from the carotid artery. Serum creatinine and urea were determined by autoanalyser (Technicon). Na^+ and K^+ were determined by flame photometry.

For light microscopy, both kidneys and adrenals, the pancreas, spleen, mesentery and heart as well as lung and liver tissue – in many animals also brain tissue – were fixed in neutral formaldehyde and embedded in paraffin. The following staining reactions were performed on 5 µ thick sections: hematoxylin and eosin (HE), periodic acid Schiff (PAS) with and without diastase digestion, Elastica-van Gieson, Azan, Goldner's (1938) modification of Massons's trichrome stain, Ladewig's (1938) modification of Mallory's PTAH stain, Fraser-Lendrum's method for fibrin. On frozen sections of fresh kidney tissue, staining with Sudan red and the DMAB-nitrite reaction (Adams, 1957) for tryptophan (which is contained in fibrin in high concentrations) was carried out. Routinely, four longitudinal PAS-stained paraffin sections of each rat kidney were screened for vascular lesions.

Electron microscope studies were performed on an additional group of rats 48 h after injection of 4.7 mg MC (4 animals) and 96 h after injection of 2.5 mg MC/kg b.wt. (4 animals). Controls were injected with 2.5 or 4.7 ml of 0.9% sterile physiologic saline/kg b.wt. The rats used for electron microscopy were not bled before tissue fixation. Immediate fixation of kidney tissue was performed by immersion in phosphate-buffered 6.5% glutaraldehyde for 2 h at 4° C. Tissue specimens were then washed in isoosmotic phosphate buffer (ph 7.6), postfixed in 1% OsO_4 for 1 h and then dehydrated in graded acetone and embedded in Araldite, or dehydrated in graded ethanol and propylene oxide and embedded in Epon (Luft). Semi- and ultra-thin sections were cut on the Reichert OM U_2 microtome. The semi-thin sections were stained with toluidine blue, the

ultra-thin sections were stained with uranyl acetate and lead citrate (Reynolds, 1963) and examined in a Zeiss EM 9 S2 electron microscope.

Previous studies in male Wistar rats from another breeder (Zentralinstitut für Versuchstierzucht, Hannover-Linden) had revealed no significant differences in the histologic findings (to be described below). Vascular injury was also found in female Wistar rats. For comparison, male Ivanovas (Kisslegg/Allgäu) "SIV" rats were examined: In 4 out of 17 rats, no structural damage of the vascular wall was apparent in the routine sections of the kidneys 48 h after injection of 4.7 mg $HgCl_2$/kg b.wt. In some of the remaining 13 rats vascular changes were only slight, in others they were marked.

Results

In this chapter we deal in some detail with histologic changes in the renal and extrarenal blood vessels and, as far as this appears to be important, in the renal tubules. Light microscopy did not reveal any significant abnormalities in the glomerular capillary tufts except that the endothelial cells sometimes appeared to be swollen, particularly 4 and 7 days after MC-injection. The juxtaglomerular cells seemed to be more heavily granulated after 4 and 7 days than in the controls or 2 days after MC-injection. However, no systematic investigations have been performed with regard to this point.

A. 24 h after MC-Injection (Groups 1a and 1b)

1. Vessels

In the kidneys of 2 out of 7 animals injected with 2.5 mg MC/kg, a small segmental PAS-positive homogenization was found in the outer media of one arcuate artery. In only one out of the 7 rats injected with 4.7 mg MC/kg did the outer media of a single interlobular artery show the same damage. The walls of the other renal vessels seemed to be intact in routine sections. No or only slight caliber irregularities were seen, the latter especially in the 4.7 mg group. No thrombus material was found in either the arterial or venous vessels. No fibrinoid changes were observed in the extrarenal arterial vessels.

2. Renal Tubules

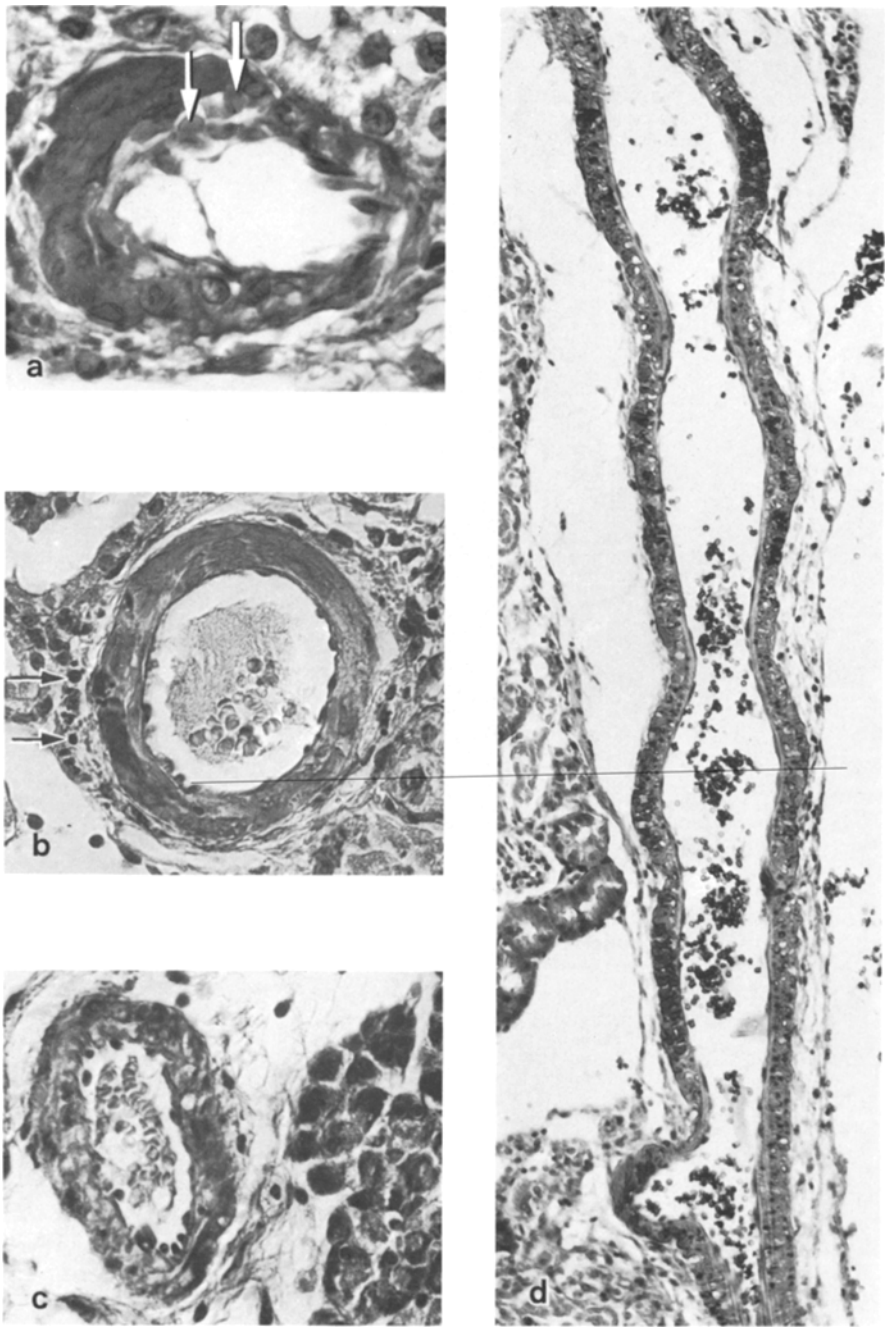
Epithelial necrosis of varying extent was found in the proximal tubules 24 h after injection of 2.5 mg MC/kg. In some rats this was mainly limited to the partes rectae in the outer stripe of the medulla and in the medullary rays of the renal cortex, while in others more than 50% of convoluted proximal tubules were also involved. After injection of 4.7 mg MC/kg, most convoluted cortical segments besides the partes rectae exhibited extensive necrosis. The necrotic cells of the convoluted proximal tubules rested mainly on the basal membrane whereas in the partes rectae they were often detached and loosely accumulated in the lumen. Occasionally they were condensed to amorphous eosinophilic masses which almost completely occluded the tubular lumen. Frequently necrotic epithelia had been washed away into the distal tubules and collecting ducts. Gaps in the tubular basement membrane were not seen.

B. 48 h after MC-Injection (Groups IIa and IIb)

1. Vessels

a) Light Microscopy and Histochemistry. 48 h after injection of 2.5 or 4.7 mg MC/kg, vascular changes of varying degrees were always recognizable in the kidneys, principally in each of the preglomerular vessels from the small hilar arteries to the juxtaglomerular arterioles. However, the lesions were most frequent and most prominent in the arcuate arteries and in the proximal portion of the interlobular arteries. The changes were patchy and irregularly scattered throughout the kidneys in such a way that in the same section many vessels were intact while others were heavily damaged. The relatively mild alterations consisted of edematous swelling of the muscle cells of the media and the frequent occurrence of empty vacuoles within them, often indenting the nucleus (see below). In addition, small vacuoles were occasionally seen at the base of endothelial cells. The most striking change was segmental fibrinoid damage which was restricted to the arterial media (Fig. 1). This fibrinoid damage was strongly PAS-positive with and without diastase pretreatment: it stained greenish or red, or a mixture of both, with Goldner's trichrome stain, red with Ladewig's stain and Fraser-Lendrum's stain, vividly red with the Azan stain, dark blue with Mallory's PTAH stain, yellow with the Elastica-van Gieson stain and occasionally a deep blue of varying intensity with Adams' DMAB stain. The results of these staining reactions suggest the presence of blood plasma proteins and fibrinogen and/or fibrin in the media. The birefringence of damaged muscle cells was increased in polarized light. Small amounts of lipid, especially on the borderlines of homogenized media segments, were demonstrable with Sudan red. In the imbibed media segments, smooth muscle cell nuclei were often not visible. Sometimes they appeared hyperchromatic or pyknotic or, less frequently, enlarged and loosened. Smooth muscle cells that were not degenerated occasionally contained small droplets or rods which stained intensely and homogenously in the PAS-reaction. In many instances, fibrinoid damage was limited to the outer layers of the media while the inner third appeared pale and edematous. Often, however, a whole media segment gave a PAS-positive reaction, especially in small arteries. The intercellular spaces rarely appeared to be slightly dilated (Fig. 2). Occasionally, a few red blood cells lay in the damaged vascular wall. Perivascular hemorrhages were not seen.

Fig. 1 a–d. Vascular changes 48 h after injection of 4.7 mg MC/kg b.wt. **a** Interlobular artery with segmental fibrinoid degeneration of the media. Single red blood cells in the inner third of the damaged media segment (arrows). Ladewig's stain × 560. **b** Arcuate artery. Nearly the whole circumference of the media shows fibrinoid degeneration. Nuclei of smooth muscle cells have disappeared or seem to be pycnotic. The lamina elastica interna is stretched, the endothelial cells are extremely flattened. A few PAS-positive droplets are found in the adventitia (arrows). PAS-stain × 304. **c** Small pancreatic artery. Swollen, partially vacuolized media with segmental homogenization of the outer two thirds. PAS-stain × 313. **d** Interlobar artery, longitudinal section. Note the bead-like appearance of the vessel and the segmental fibrinoid degeneration. The damaged segments are usually dilated. Goldner's stain. × 106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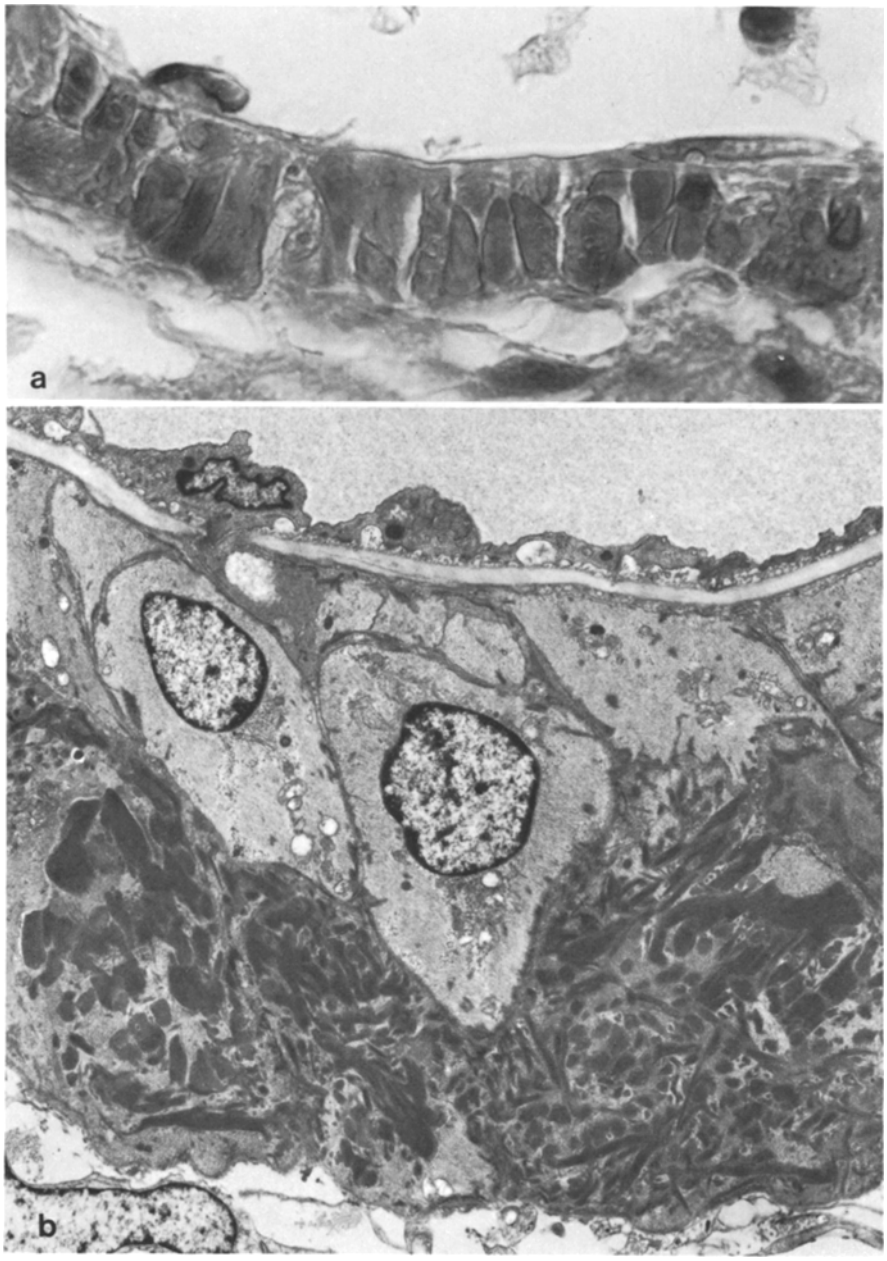


Fig. 2. **a** Arcuate artery, longitudinal section. Endothelial cells are flattened. The internal elastic membrane is stretched. Nearly all smooth muscle cells of the media appear to be degenerated, staining an almost homogeneous red with Goldner's stain. The single nucleus-bearing light smooth muscle cell in the left half of the picture seems hydropic. In the right half of the picture the interstitial spaces seems slightly enlarged sometimes. Goldner's stain. ×993. **b** Small intrarenal artery. The outer media is imbibed by a finely granular material, the muscle cells are degenerated and there is extensive fibrin precipitation. Usually this is the electron microscopic equivalent to the more or less homogeneous appearance of the same area under the light microscope. Note the myoendothelial junction (upper left) and the straightened internal elastic membrane. ×5850

Fibrinoid changes in the media of the juxtaglomerular arterioles were rare and in most cases limited to the proximal portions. However, the juxtaglomerular arterioles often seemed constricted with a strong undulation of the elastic membrane, endothelial stratification and a pale-stained, somewhat thickened muscular wall.

Pronounced caliber irregularities were frequently seen in preglomerular arterial vessels. The heavily damaged arterial segments were mostly dilated and showed stretched elastic membranes and extremely flattened endothelial cells. Dilatations of this kind were most prominent in the proximal portion of the interlobular arteries but were also demonstrable in larger arteries, for instance in interlobar arteries (Fig. 1). As a result of segmental dilatation, bead-like contours of the vascular wall were occasionally seen.

To obtain an approximate criterion for the respective extent of the vascular alterations, the fibrinoid changes were counted on 4 PAS-stained longitudinal renal sections and then listed, differentiating between small hilar arteries, interlobar, arcuate and interlobular arteries, and juxtaglomerular arterioles. Cross-sections of the damaged vessels were also counted if there was the possibility that one and the same vessel had been cut more than once in the section. Thus counted, the total number of vessels cuts revealing fibrinoid changes ranged from 6 to 74 (mean value per slide: 7.9) in the group who had received 4.7 mg MC/kg (Group IIb) and from 1 to 70 (mean value per slide: 5.4) in the group injected with 2.5 mg MC/kg. Two of the 21 animals of the 2.5 mg group had only minimal vascular lesions.

Thrombus material was found in small and medium-sized renal veins in 7 of the 21 rats injected with 2.5 mg MC/kg. Most of the thrombi were small and deposited at the vascular wall without occluding the vascular lumen. No thrombus material was seen in the 4.7 mg group.

Outside the kidneys, patchy fibrinoid vascular changes were particularly observed in mesenterial and pancreatic arterial vessels (Fig. 1a).

This was the case in about 30% of the rats examined 48 h after the injection of 2.5 mg MC. In one of these rats, a coronary artery was also injured in the same way. Two thirds of the Group IIb rats, injected with 4.7 mg MC/kg, had fibrinoid changes in mesenterial arterial vessels, about 55% in the pancreas, and in one rat a coronary artery was again affected. In Group V, focal fibrinoid vascular changes in varying degrees were observed in the mesentery of all rats, in the pancreas of 9 (including 2 with minimal changes) and in the spleen and periadrenal vessels of 2 rats.

b) Electron Microscopy. For these studies, tissue blocks of interlobular and arcuate arteries were chosen in which vascular lesions had been recognizable by light microscopy in semi-thin sections.

The endothelial cells of the arteries showed, if any, only moderate hydropic cytoplasmic alterations and slight mitochondrial swelling. At the level of heavily damaged and dilated media segments, the endothelial cells were commonly considerably flattened. However, the nuclei were sometimes bulging into the lumina. Small bundles of filaments were sometimes prominent in the endothelial cytoplasm. In some places (particularly at the height of the media changes) the subendothelial space was enlarged and contained amorphous, flaky, faintly osmiophilic material, presenting the aspect of blood plasma in the lumen of the vessel (Fig. 3). Although the intercellular spaces sometimes showed a ten-

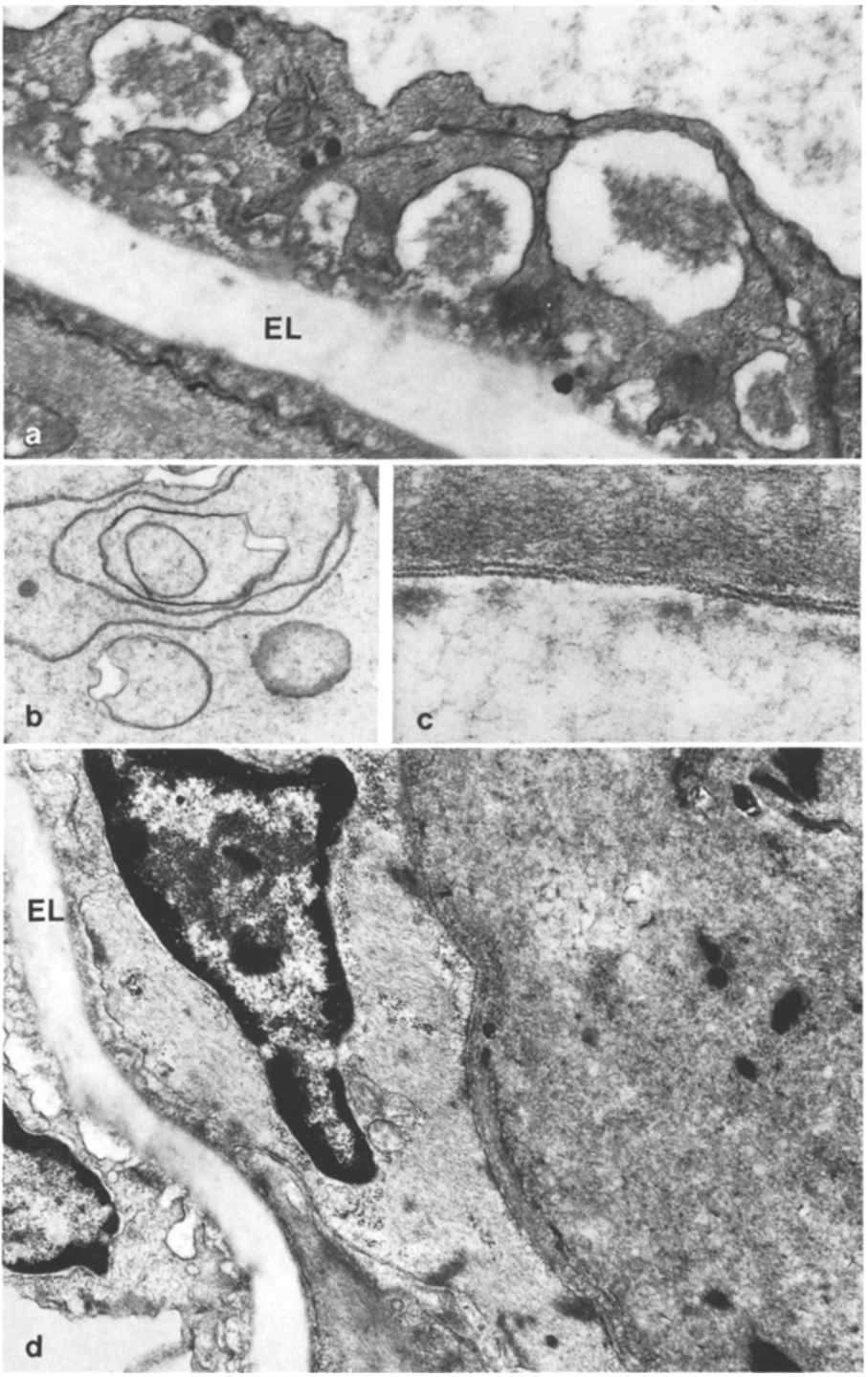
dency to dilate, no actual endothelial gaps were found, neither from separation of endothelial junction nor from destruction of endothelilal cells. Adherence of platelets or filamentous fibrin were not detectable at the luminal side. In the dilated vascular segments, the lamina elastica interna was straightened and commonly appeared as a broad light band. Strongly osmiophilic streaks were occasionally embedded in the otherwise homogenous elastic membrane. However, similar but shorter streaks also occurred in the controls (see also Ooneda et al., 1965). Myoendothelial junctions seemed to appear somewhat more frequent than in the controls, and the fenestrae of elastic membrane appeared to be slightly enlarged.

In the muscular layer of the vascular wall a broad spectrum of pathologic changes was observed. Although occasional muscle cells exhibited increased osmiophilia, the basic type of cell damage was a hydropic alteration of the smooth muscle cells.

In what appeared to be the first stages of intracellular edema, the muscle cells showed dilated cisternae of the endoplasmic reticulum, a diminution of ribosomes, a swelling of the mitochondria, mostly with some disorganization of the cristae and an electron-lucent matrix, and a diffuse dispersion of myofilaments. Other edematous cells had developed an increased transparency, and in further stages, diminution and flaky disintegration of the myofilaments was found. Irregular systems of vacuoles with partly ruptured membranes occurred, apparently derived from endoplasmic reticulum (Fig. 3b). In severe edematous damage, the swollen cytoplasm was optically almost empty, containing only minute amounts of light granular or more amorphous material. Protrusions of such extremely hydropic cells without caveolae at their cell membranes into adjacent, more intact muscle cells were found, indenting the nuclei of these cells. In sections where such hydropic cellular protrusions were surrounded by nearly normal muscle cells, the aspect of large intracytoplasmic vacuoles was given at low-grade magnification (corresponding to the light microscopic appearance). The detection of two adjacent unit membranes at high magnificaton then demonstrated that there were two different cells (Fig. 3a). This phenomenon has been described for vascular smooth muscle cells in the condition of ischemia (Thoenes, 1964; Tapp, 1969) and in acute renal failure of the rat after s.c. injection of glycerine (Suzuki and Mostofi, 1970). However, edematous changes in occasional smooth muscle cells were also found in renal vessels of our control group. The occurrence of single vacuoles in the vascular wall of untreated animals has already been pointed out by Knauff and Schramm (1956) in the rabbit and by Veltmann et al. (1975) in the dog.

A further type of damage which seemed to develop on the basis of cell hydrops is characterized by diffuse imbibition of the cytoplasm of smooth muscle cells by a finely granular, homogenous and occasionally finely flaked material of medium density (Fig. 3). Mitochondria were visualized which appeared to be infiltrated by the same material. Often the cells were imbibed to such an extent that only a narrow peripheral rim of condensed cytoplasm without caveolae or dense areas at the cell membrane were visible. However, even this poorly

Fig. 3. a Amorphous, flaky material looking like blood plasma is seen in the subendothelial and sometimes in the interendothelial space. *EL*=internal elastic membrane. ×23,825. **b** Extreme intracellular edema of a vascular smooth muscle cell with irregular systems of vacuoles and membranes. ×14,625. **c** Extremely hydropic smooth muscle cell (lower half of the picture) adjacent to a relatively intact smooth muscle cell (upper half). Note the two adjacent unit membranes. ×70,800. **d** A peculiar type of cellular damage is seen in the arterial media. The swollen smooth muscle cell in the right half of the picture is extremely imbibed by a finely granular homogeneous and occasionally flaky material, which is probably blood plasma material. *EL* internal elastic membrane. ×19,800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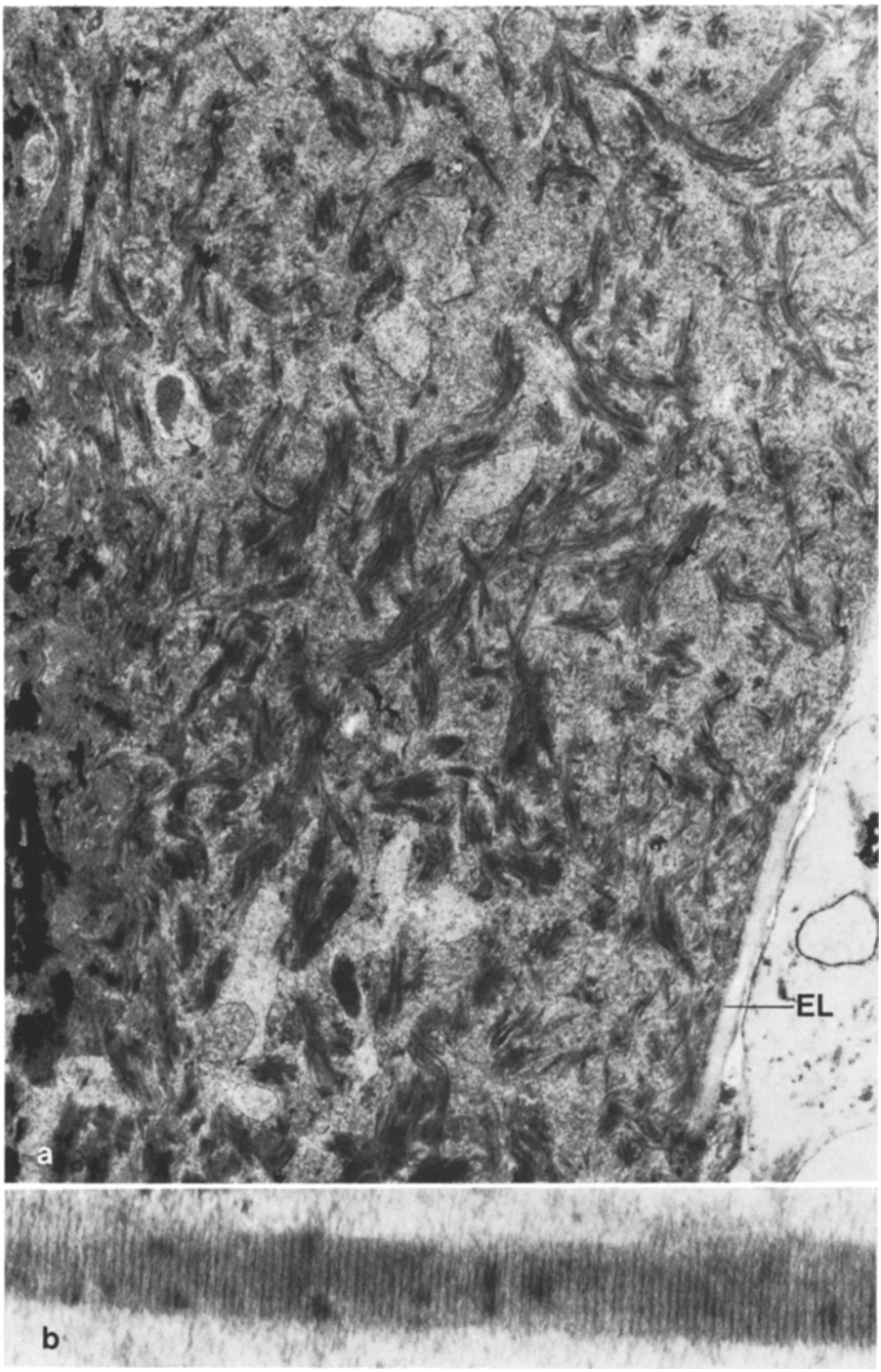


Fig. 4. **a** Swollen smooth muscle cell of the outer media intensely imbibed by a finely granular material and showing many irregularly scattered intracytoplasmic bundles of filamentous fibrin. *EL*=external elastic membrane. ×29,220. **b** Fibrin precipitate with typical cross striation showing a periodicity of approx. 170 Å, ×81,500

differentiated peripheral cytoplasmic rim might be absent. Occasionally fine granular material could also be observed in the interstitial space between smooth muscle cells.

Intracellular fibrin precipitates were found within the swollen and imbibed muscle cells, mostly irregular bundles of thin filamentous material (Fig. 4), some of them exhibiting an axial periodicity of approximately 170 Å (160–200 Å). Further types of fibrin formation were recognized in the media: Sharply contoured, crystal-like formations arranged in hexagonal or polygonal forms with cross striations of the above mentioned or of a 95–115 Å periodicity and polygonal, often large, crystal-like formations which in addition to a basal periodicity of 95–115 Å presented an apparently superimposed broader striation of variable width and periodicity enclosing a broad angle to the basal striation (Figs. 2, 5, 6). The most extensive fibrin deposits were found in combination with various degrees of necrobiosis, which in some places even had progressed to cellular necrosis (Fig. 5). Some paracrystalline structures might be built up from fibrinogen (see Cohen et al., 1963). A more detailed study of the substructure and periodicity of the crystal-like formations is planned by means of image analysis. It must be stressed that no fibrin material was found in the space between the endothelial cells and the internal elastic membrane.

The more intact muscle cells adjacent to the severely damaged areas sometimes contained accumulations of lysosomal bodies or few lipid droplets. In the outermost media, protrusions of damaged muscle cells imbibed with fine granular material with and without fibrin precipitations were sometimes in close contact (through stomata of the external elastic membrane) with an adventitial cell. Here and there, some small bundles of filamentous fibrin were found outside the muscular layer in the adventitial space (Fig. 7).

2. Renal Tubules

48 h after injection of 2.5 or 4.7 mg MC/kg b.wt. extensive epithelial necrosis was found in the partes rectae of the proximal tubules of both cortex and medulla, and in varying extent in convoluted cortical segments. Necrosis was of much greater extent in the 4.7 mg group than in the 2.5 mg group (Table 1). In both experimental groups (IIa and IIb) condensed cellular debris was found, occluding the lumina of the terminal partes rectae in the outer stripe of the renal medulla and sometimes continuing into the subsequent segments of Henle's loop. Usually, the amounts of cellular debris or casts in the lumina of the collecting ducts and distal tubules were not as great as 24 h after MC-injection. Here and there, single squamoid regenerating cells lined the otherwise nude basement membranes of destroyed proximal tubules. The epithelial cells of the distal tubules (convoluted and straight portions) frequently showed cytoplasmic swelling and nuclear enlargement. Necrobiosis of single cells or increased numbers of mitosis were also occasionally seen, especially in the 4.7 mg group. Extent and spectrum of tubular damage varied considerably between individual rats, especially in the 2.5 mg group.

C. 96 h after Injection of 2.5 mg MC/kg

1. Vessels

a) Light Microscopy. 96 h after MC-injection, PAS-positive media lesions were found in 3 of 11 rats. In the routine sections, two of them were found to

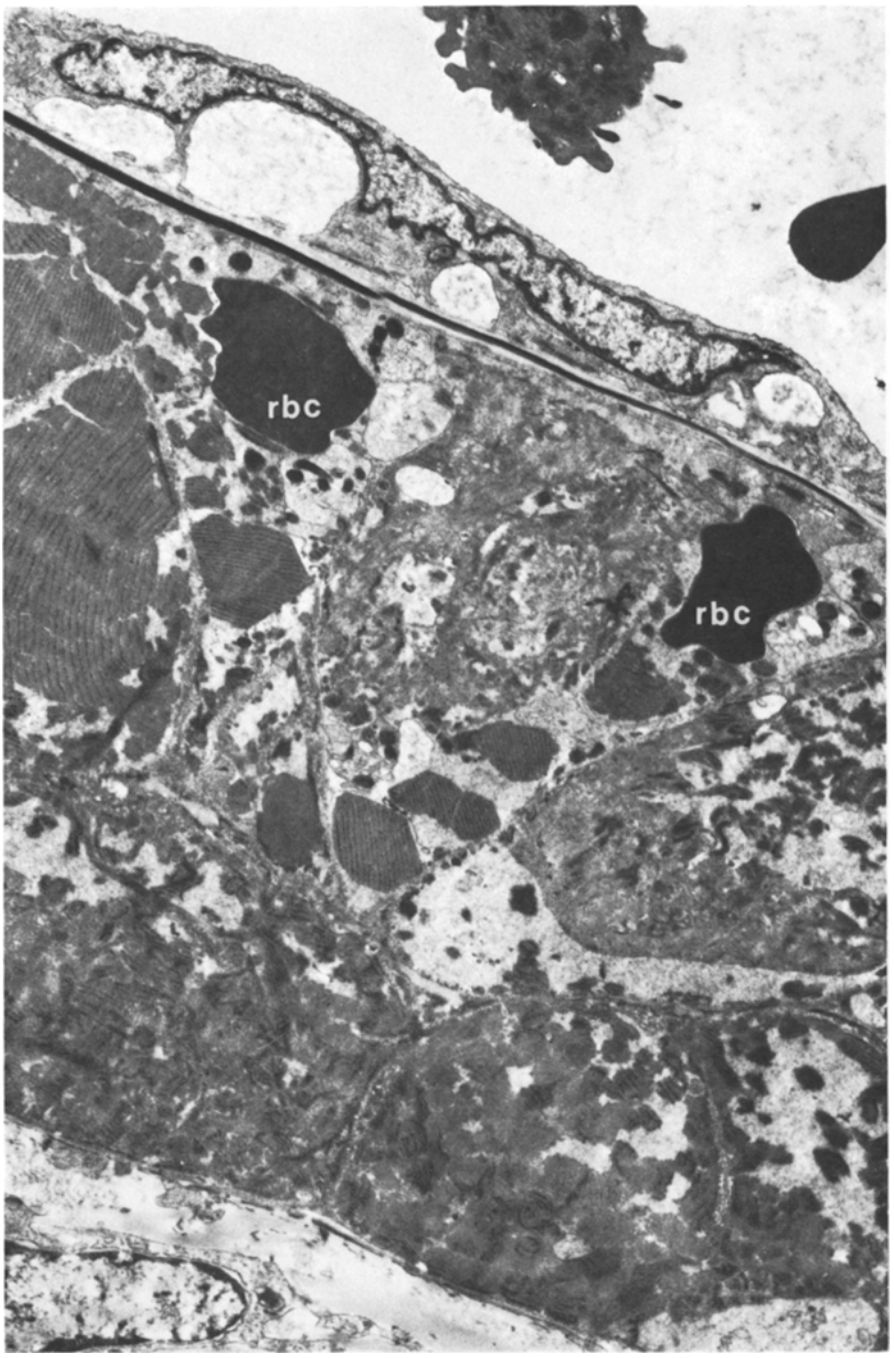


Fig. 5. Maximum arterial damage with necrosis of the smooth muscle cells and deposition of fibrin masses, often in the form of crystal-like bodies. Note also extravasated red blood cells (*rbc*). ×9510

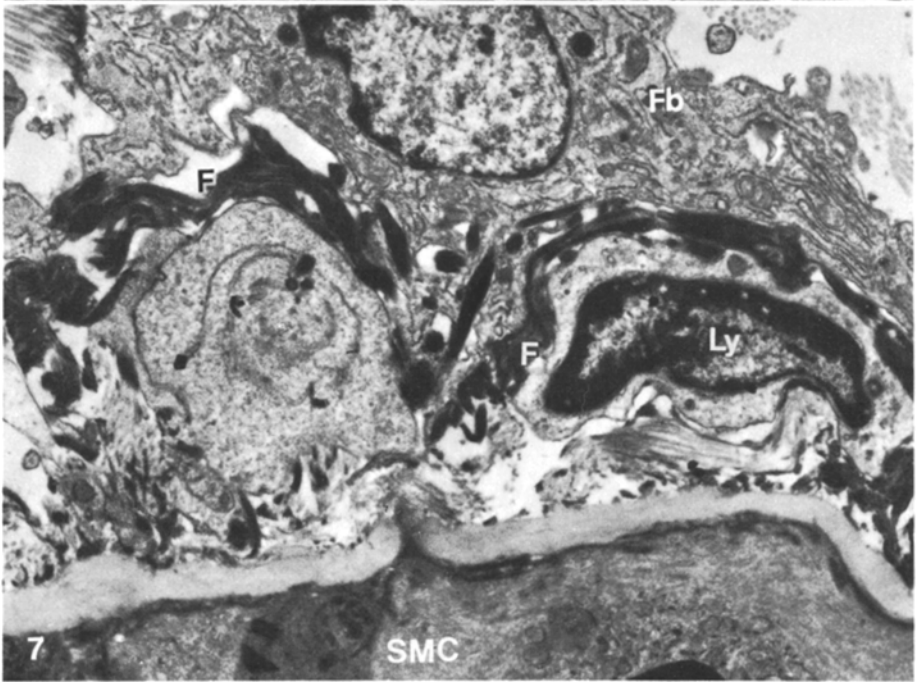
have this type of lesion in only one artery. In the third animal which had exceptionally high serum levels of creatinine and urea as well as extensive tubular necrosis, several preglomerular arterial vessels exhibited fibrinoid damage of the media. In the kidneys of all rats, the arteries often showed a thickened wall with swollen, pale-stained and frequently vacuolized smooth muscle cells. The nuclei of both endothelial and smooth muscle cells were often enlarged and clear, but in some areas small and hyperchromatic. A mitosis could be detected occasionally (Fig. 8). Subendothelial vacuoles were often seen. Constriction of arterial vessels was at least as frequent, and sometimes even more so, as in the animals examined after 48 h, while segmental dilation seemed to occur less frequently. A moderate number of mononuclear inflammatory cells had accumulated around some vessels, and the normally present periadventitial cells were enlarged and rounded. Small PAS-positive droplets were occasionally observed in perivascular cells. Three rats had mural thrombi in small and medium-sized renal veins (Vv. interlobulares et arcuatae), sometimes with incomplete endothelization. Outside the kidneys, segmental fibrinoid changes of the outer media were found in two animals. In each of them, the lesions were restricted to one pancreatic artery.

b) Electron Microscopy. Dilated vessel segments did not occur in the tissue specimens embedded for electron microscopy. The arteries appeared to be contracted (possibly due to fixation) and showed heavily undulated elastic membranes and pronounced infoldings of the nuclei of endothelial and smooth muscle cells. As before, hydropic cell changes were often seen in the arterial media, mostly in the outer cell layer. Some extremely hydropic cells bulged outwards, projecting above the outer contour of the vascular wall. Hydropic alterations of endothelial cells were slightly better visible than after 48 h. In the endothelial cells, and more pronounced in some smooth muscle cells, smooth and rough endoplasmic reticulum or free ribosomes appeared to be increased as well as rounded mitochondria, which in some muscle cells were arranged in large groups in a paranuclear position. While some muscle cells showed increased cytoplasmic transparency, others exhibited an increased density and a certain blurring of their ultrastructure, sometimes with accumulation of some secondary lysosomes. In one rat with PAS-positive vascular changes, the cytoplasm of a few dark muscle cells was impregnated with a fine granular material as described in the 48-h group. Intracellular fibrin formations arranged in clumps were seen in the same rat.
Fibrin material could not be detected in the adventitial space.

2. Renal Tubules

Medullary and varying numbers of cortical proximal tubules were relined with low, regenerated epithelial cells. The straight portions were often considerably dilated. While the more proximal segments contained little cellular debris, casts of condensed necrotic cells were still present in some terminal proximal tubules, although the number of these cells was distinctly lower than 48 h after MC-injection. In the interstitial space, focal mixed lymphomonocytic and granulocytic infiltrates were observed which were frequently located in a perivascular position. So-called hyaline droplets were very occasionally seen in the proximal tubules.


6


Fb
F
F
Ly
7
SMC

D. 7 Days after Injection of 2.5 mg MC/kg

1. Vessels

The media of some intrarenal arterial vessels seemed to be thickened by swollen muscle cells with enlarged and loosened nuclei. Altogether, the vascular changes were less marked than 4 days after MC-injection. Vasoconstriction occurred less frequently. No PAS-positive lesions were seen. A moderate number of mononuclear inflammatory cells and a few granulocytes were found around some vessels. In two rats a mural thrombus with invading fibroblasts was observed in an intrarenal vein. There were no clearly evident vascular changes in the extrarenal arterial vessels.

2. Renal Tubules

Proximal tubules with regenerated epithelia of various height, especially straight portions, were widely patent, containing remnants of necrotic cells and occasional casts. In the kidneys of 3 animals, occasional calcified or uncalcified clots of necrotic material were attached to the (previously nude) basement membrane and covered by regenerated epithelial cells. Cellular debris was absent in the collecting ducts and distal tubules. Only a few or a moderate number of focal mononuclear inflammatory infiltrates were found in the interstitial space.

E. Relationship between Vascular Damage and Functional Data

After injection of 2.5 mg MC/kg as well as after the 4.7 mg dose, considerable variations were found when the individual rats within a single group were compared with regard to the degree of renal insufficiency, tubular damage and vascular lesions. Usually, rats with little fibrinoid changes exhibited a smaller extent of tubular necrosis and lower levels of blood urea and creatinine than the mean of the experimental group and vice versa. However, there were always exceptions, for instance pronounced fibrinoid changes with relatively little tubular necrosis, and also little fibrinoid changes in the presence of extensive tubular damage.

It is remarkable that vascular lesions fully developed at a time (i.e. between 24 and 48 h after MC-injection) when renal insufficiency also approached its maximum and tubular obstruction by cellular debris became increasingly severe. On the other hand, 96 h after injection of 2.5 mg MC/kg, when regeneration of tubular epithelia is under progress while serum levels of creatinine and urea have already markedly decreased and serum potassium has returned to normal, the vascular alterations are regressing. However, it has to be pointed out that

Fig. 6. Different types of crystal-like fibrin formations in the media. In addition to a fine basal striation of approx. 110 Å most bodies show a superimposed broader striation. × 50,400

Fig. 7. Fibrin deposits (*F*) in the adventitial space between lymphocytes (Ly) and a fibroblast (*Fb*). *EL* external elastic membrane. *SMC* smooth muscle cell of the media. × 9480

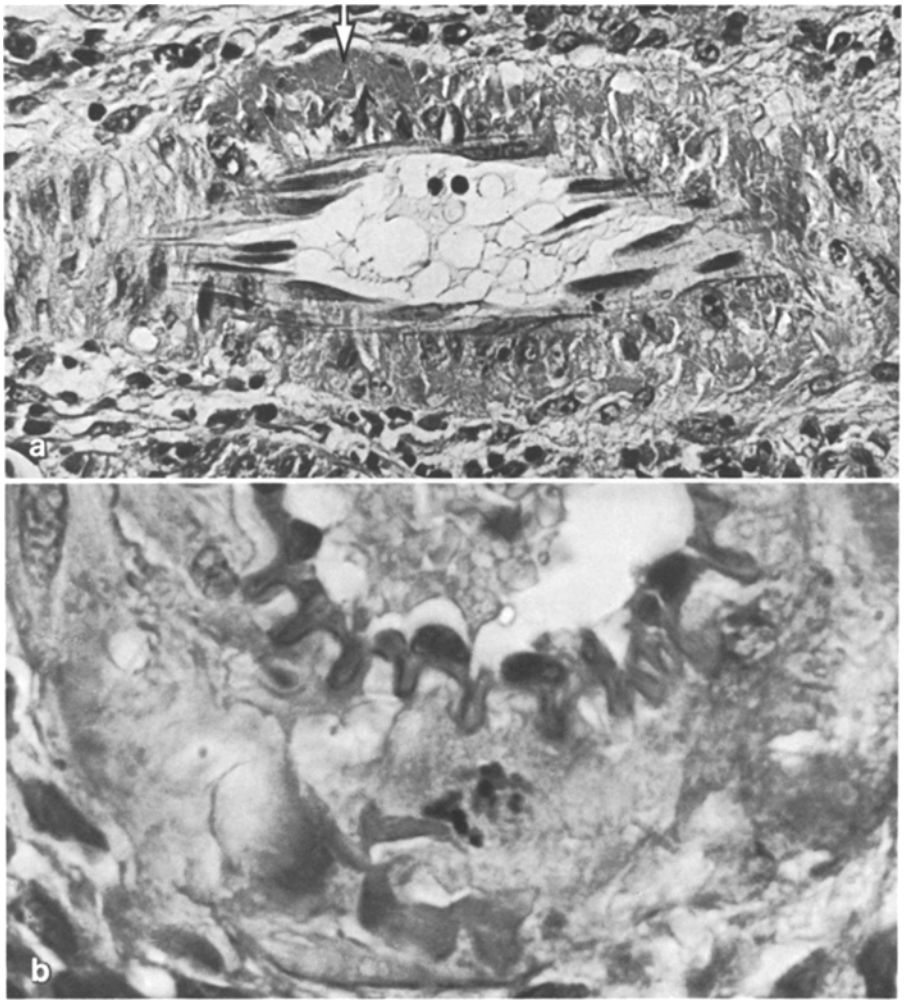


Fig. 8a and b. Intrarenal vessels 96 h after injection of 2.5 mg MC/kg b.wt. **a** Smooth muscle cells as well as endothelial cells of this artery seem to be swollen and their nuclei enlarged. Segmental fibrinoid degeneration of the media is still found (arrow). Note perivascular inflammatory cells. PAS-stain, ×322. **b** Arcuate artery with intensely swollen and hydropic smooth muscle cells of the media and patchy PAS-positivity. Note the large mitotic figure. PAS-stain, ×1136

the state of complete anuria is already reached in some rats at a time when structural damage of the arterial wall is usually not yet detectable (Table 1).

Systolic blood pressure when measured by tail plethysmography showed a slight initial drop in 5 of 10 rats 10 h after injection of 4.7 mg MC. When measured 50 h after injection before sacrifice, the values were elevated for the first time above 130 mm Hg in 4 rats (145, 140, 135 and 135 mm Hg). However, we only assume moderate hypertension if values of 140 mm Hg have been

Table 1. Comparison of some functional data with morphologic findings on the kidneys of individual rats 48 h after injection of 4.7 mg MC/kg b.wt.

Rat No.	Diuresis (ml/100 g b.wt.)	Time of anuria post-injection (h)	Creatinine (mg%)	Fibrinoid vasc. lesions (mean value per slide)	Estimated % of necrotic loops of convoluted prox. tubules
1	2.4	40	5.5	1.5	75
2	2.8	40	6.6	5	80
3	2.4	16	6.5	4.5	80
4	9.8	–	4.5	5	80
5	8.3	–	5.3	6	80
6	3.8	–	5.2	8	65
7	14.2	–	4.3	3.5	40
8	1.7	16	6.1	2.25	75
9	4.8	–	5.9	17.5	75
10	6.0	–	5.6	10.75	75
11	3.8	–	6.1	5.75	80
12	1.1	12	6.6	5.25	75

repeatedly found. In further experiments blood pressure values above 130 mm Hg were not measured.

Discussion

Marked structural changes of renal and certain extrarenal arterial vessels of the rat have been found in the present study in the course of acute renal failure caused by injection of 2.5 or 4.7 mg MC/kg b.wt. One may wonder why comparable changes, the most impressive of which is fibrinoid necrosis of the vascular media, have not been reported by the many other investigators. working with MC. We suppose that essential reasons for the detection of the lesions were the dosage of MC utilized in this study and the time of examination after MC-administration.

In previous studies (unpublished) only rare and slight fibrinoid changes could be observed in renal vessels after injection of 1.75 mg MC/kg, while after injection of 8 mg MC/kg they were found in 5 out of 6 rats. With regard to time we found only rare and slight fibrinoid changes in individual rats 24 h after MC-injection. In contrast, 48 h after injection of 2.5 or 4.7 mg MC/kg vascular injury had regularly developed in the preglomerular renal arterial system and to a high percentage in certain extrarenal circulatory areas (mesentery, pancreas). 96 h after injection of the 2.5 mg dose fibrinoid damage of the vascular wall had largely redeveloped. Reviewing the literature we found that most workers had chosen other dosages of MC, or other times of examination. Where the time of examination was the same, perhaps more attention was paid to functional data than to the morphology of renal vessels. However, we cannot exclude that special characteristics of the Wistar rats used in this study might play a role. As mentioned in the chapter "Material and Methods" fibrinoid vascular lesions were not found as regularly and as richly in the so-called SIV-rats from the same breeding institute. Compared to the more robust "SIV"-rats the Wistar rats utilized in this study are dainty and sensitive animals (communicated by Dr. K. Albus, scientific head of the breeding institute).

Morphology of Renal Vessels in Other Models of Acute Renal Failure

Structural changes comparable to those found after MC-poisoning have been noted in preglomerular arterial vessels of rat kidney in the course of acute renal failure following temporary complete renal ischemia (Cain and Fazekas, 1963; Thoenes, 1964; Kaboth, 1962, 1965; Terry et al., 1970). As early as 4 resp. 8 h after recirculation of the blood through the kidneys, the first lesions were seen. They regressed after a few days.

Cain (1965), conducting light microscopic studies, described similar, always transient changes in the Vasa afferentia of the rabbit kidney after orthostatic collapses and, more pronounced, after a combination of hemorrhages and collapses. In addition evidence of vasospasm was found. Comparable changes could also be produced in arteries and arterioles of the rat kidney by the injection of toxins of Amanita phalloides (Cain, 1965).

Morphologic evidence of severe vasomotor disturbances with initial complete renal ischemia and long-term recurrent focal vasospasm in the later course has been pointed out in studies on the "crush kidney" of the guinea pig (Donner and Holle, 1958; Holle, 1959). In these experiments the frequent vacuolization of smooth muscle cells of renal arterial vessels has been interpreted as a degenerative change (Holle, 1959).

More recently, using rapid frozen tissue, Suzuki and Mostofi (1970) reported on transient spasm of the renal arterioles and small arteries in glycerine-induced acute renal failure in Sprague-Dawley rats. The vascular changes, which began a few minutes after the injection and reached their maximum within 2 h, were said to be similar to those of epinephrine-injected animals. Vacuolization of vascular smooth muscle cells was also observed. Histologic findings on extrarenal vessels have not been mentioned in any of the studies mentioned above.

Progression of Vascular Changes Following MC-Injection

Following MC-injection, two different phenomena which often seem to overlap can be observed in the morphogenesis of damage to the vascular wall. The first, probably the consequence of hypoxia and impairment of the sodium pump, manifests itself in the form of severe intracellular edema of the smooth muscle cells. The second phenomenon is a peculiar form of cellular damage and apparently the next step in the development of vascular wall lesions: Swollen muscle cells are imbibed with blood plasma constituents which consist of fibrinogen and proteins, giving the muscle cells the appearance of a nearly homogenous or finely granular mass. That plasma substances do indeed imbibe the vascular wall is clearly evidenced by the demonstration of fibrin formations, in many of the damaged muscle cells. This type of cellular damage has already been found by Thoenes (1964) in renal vessels, and by Kerenyi and Jellinek (1972) in mesenterial vessels, in each case after temporary complete ischemia and subsequent recirculation. The fact that the lesions usually first appear in the outer third of the media and apparently progress in the direction of the internal elastic membrane also indicates that preexisting cellular damage paves the way

for the plasmatic imbibition of the cells. In accordance with the view of Thoenes (1964) we assume that more than only increased endothelial permeability (dysoria according to Schürmann and MacMahon, 1933) was present under our experimental conditions.

A peculiar finding, probably the electron microscopic counterpart of the birefringent structures, was the occurrence of crystal-like fibrin formations in heavily damaged media segments. Similar structures have been reported in experimental hypertension in the rat, lying in the space between endothelial cells and the internal elastic membrane (Ooneda et al., 1965; Wiener et al., 1965; Sawatari, 1966; Kerenyi et al., 1966; Jellinek et al., 1967; Gardner and Matthews, 1969; Jellinek, 1971; Kojimahara et al., 1971; Hatt et al., 1972) or within intimal cells (Hatt et al., 1972). In addition to the subendothelilal position, Wiener et al. (1965), Hüttner et al. (1968) and Aikawa and Koletsky (1970) also found them in the intercellular space of the media. In contrast to the report in hypertensive rats, the crystal-like fibrin formations that we observed were exclusively located in the muscular layer of the vascular wall.

After injection of 2.5 mg MC/kg – similar to the findings following temporary complete renal ischemia (Cain and Fazekas, 1963; Kaboth, 1962, 1965; Cain, 1965; Terry et al., 1970) – the fibrinoid damage of the vascular wall soon disappeared after its development. With few exceptions it was no longer demonstrable 96 h post-injection. Apparently, most of the muscle cells which gave a PAS-positive reaction 48 h after MC-administration only reached a prenecrotic stage and survived. Where necrosis occurred, it was apparently compensated by mitosis and perhaps (transiently?) also by the remaining swollen muscle cells which closed the gap. Serum proteins and fibrin were quickly resolved in a way of which we know too little yet. Unexpected was the speed with which resolution developed. Adventitial cells, especially lymphomonocytoid cells, probably play a certain role in resolution.

Possible Role of Vasomotor Changes

Although it is difficult to extrapolate from the appearance of fixed tissue to the state of contraction in vivo, we suppose that vasomotor changes also occur in the course of acute renal failure caused by the injection of 2.5 or 4.7 mg MC/kg b.wt. This is in accordance with the measurements of Cirla et al. (1970) who emphasized a significant dose-dependent vasospastic effect of MC which was slight in the systemic circulation, clearly evident in the circulatory area of the femoral artery, and very marked in the renal area. Furthermore, we suppose that an intense prolonged or recurring vasospasm or alternating spasm and vasodilatation might play a decisive role in the development of fibrinoid damage of the arterial wall.

In discussing this point we have to remember that metabolic exchanges in small and medium-sized arteries exclusively take place by diffusion from the vascular lumen. It is imaginable that vascular muscle cells succumb to an oxygenic and nutritional deficiency in a persistent vasospastic state due to a diminution of the filtration area, a narrowing of the fenestrae of the elastic membrane, and increased overall tension in the vascular wall (Holle, 1959; Staubesand, 1959; Vancov, 1973). However, it is remarkable that segments with the strongest fibrinoid damage are usually not contracted but, on the contrary, dilated. Perhaps previously intensely contracted vascular walls lose their contractile force under persistent hypoxia and terminally dilate, but it appears to us more probable that post- or prestenotic dilated vessel segments are damaged to a greater extent.

This is understandable if Laplace's equation is applied to biological structures (Wolf, 1952). Laplace's equation for the internal pressure P in a cylinder (vessel) is $P=T/r$, T being the tangential tension in the wall and r the radius. If so, a dilated vascular segment would have to suffer a mural tension which increases in proportion to the radius of the vessel. In addition, though this is not a dominant factor (Wolf, 1952), the lateral pressure of blood flow increases in a dilated vascular segment where the velocity of flow is reduced. Following MC-injection reduction of blood flow might have reached in some areas the extent of prestasis or stasis and damaged the vascular smooth muscle cells and increased the permeability of the vascular wall through oxygenic and nutritional deficiency and diminished transportation of the metabolic products. This effect of prestasis in the case of MC-poisoning has already been discussed by Elbe (1905) and Weiler (1913). It must be stressed that the factors mentioned above, and in particular their combination, would be able to damage the vascular wall, possibly in synergism with other agents (see below), and that an additional increase of systemic blood pressure would not necessarily have to be assumed.

Role of Blood Pressure

We have no evidence that the elevation of *systemic* blood pressure was an important or necessary factor in the development of the fibrinoid vascular changes after MC-injection,because only some of the rats showed a slight elevation of systolic blood pressure towards the end of the experiment. This elevation was not indicative of systemic hypertension. The fact that we found fibrinoid vascular lesions not only in renal vessels but also in certain extrarenal vascular areas (particularly in the mesentery and the pancreas) after MC-injection might favor the idea of a transient rapid rise of systemic blood pressure which was not detected in our blood pressure measurements. However, these lesions might also have been caused by prolonged vasomotor changes with the consequences discussed above, or perhaps by a certain local sensitivity to vasoactive agents released under the given experimental conditions.

Role of Vasoactive Agents

Fibrinoid vascular changes have also been found in light microscopic investigations of the rat kidney after injection of vasopressin (Byrom, 1964), angiotensin II (Byrom, 1964) and the sympathicomimetic agent methoxamine (Herbertson and Kellaway, 1960), each of which induced a transient rapid rise of systemic arterial blood pressure. However, although some differences might exist in the fine structure between the angiotensin II induced lesions (examined in gut arterioles of the rat by Goldby and Beilin, 1972 and Thorball and Olsen, 1974) compared to those presented in this study, we have to discuss a possible release of vasoactive substances of renal origin in the course of MC-poisoning, especially since renin has been hypothesized to be a factor which increases vascular permeability to plasma proteins (Cuthbert and Peart, 1970). Circulating and local angiotensin II levels are known to play also a physiological role in the control of renal vascular tone (Hollenberg et al., 1972; Osborn et al., 1974; Waugh, 1972).

Hirasawa (1969) has reported a significant increase of renal renin content and plasma renin activity in rats 24 h after i.m. injection of an extremely high

dose of MC (100 mg/kg) and a slight, not significant increase after a lower dose (5 mg/kg). Plasma angiotensinogen was elevated after both doses (Hirasawa et al., 1968). Matthews et al. (1974) found an acute rise of plasma renin activity and angiotensin II levels in rats one hour after i.p. injection of 2.5 mg MC/kg, and still elevated levels at 4 h. 48 h post-injection, plasma renin activity was in the normal range (no measurements were taken between 4 and 48 h). Rojo-Ortega et al. (1974) observed changes in the ultrastructure of juxtaglomerular cells which indicated a state of hypersecretory activity 16 h after i.m. injection of 12 mg MC/kg b.wt. into rats. A similar state, but with more abundant mature secretory granules, was seen after 72 h when hypergranularity was visible by light microscopy. However, as already stated by Oken (1972), one cannot simply compare the findings obtained with high-dose MC administration with those resulting from experiments with a lower dose. Furthermore the question remains if an activation of the renin-angiotensin axis might be the cause of altered renal blood flow or simply reflect a secondary response to renal ischemia (see Flamenbaum, 1973).

Recently, Bulger and Siegel (1975) have reported MC-induced alterations of the interstitial cells of the renal papilla which indicated increased synthetic activity. These cells are thought to produce the hypotensive substance prostaglandin which is a renal vasodilator and according to Aiken and Vane (1973) attenuates the renal vasoconstrictor activity of angiotensin.

On the other hand we want to emphasize the possible role of catecholamine excess. Although we found only a slight fall of systemic blood pressure in some rats in the early phase of the experiment, at least some of them might have been in a state of shock. Gessler and Schroeder (1965) have reported a more striking drop of blood pressure 24 h after injection of 5 mg MC (measured directly in the carotid artery by manometric method). An excessive release of catecholamine and peripheral vasoconstriction have been well established in shock states (Spink et al., 1966; Hardaway, 1968). As has been reported by many investigators (for review see Carrière, 1975) intrarenal or intravenous administration of norepinephrine or epinephrine causes vasoconstriction in the outer renal cortex independently of its effect on blood pressure. Angiotensin II has also been reported to stimulate not only the adrenal cortex but also the adrenal medulla (Peach et al., 1966, 1970) resp. to potentiate the response of the vascular wall to noradrenaline and to other spasmogens (Day and Moore, 1976). Furthermore, a high catecholamine releasing ability of divalent ions of mercury has been found (Hart and Borowitz, 1974). From a histological point of view, the development of fibrinoid necrosis of the small blood vessels of the heart induced by noradrenaline infusion in the dog (Jellinek et al., 1966) seems to have very much in common with the vascular lesions found after MC-poisoning. This again favors the idea that vasoconstriction or alternating spasm and overdistension of the renal and some extrarenal arterial vessels and, related thereto, increased renal nerve vasoconstrictor activity following increased catecholamine secretion might operate in the course of MC-induced renal failure and create the conditions for later hypoxemic lesions of vascular smooth muscle cells and imbibition of the vascular wall by blood plasma constituents. Azotemia

may facilitate their production in synergism with electrolyte disturbances (hypocalcemia, hyperkaliemia) and other biochemical and nerval mechanisms.

Correlation of Structure and Function

It is remakable that the development of fibrinoid vascular changes paralleled, with regard to time, the progressive impairment of renal function and the more or less extensive obstruction of tubules by cellular debris and cast formation, and that there was a redevelopment when the rats entered the early recovery phase of acute renal failure (2.5 mg group). Obstruction of renal tubules has been confirmed in micropuncture studies of rat kidneys 22–26 h after injection of 4.7 mg MC/kg b.wt. and interpreted as indicating low glomerular filtration pressure (Flamenbaum et al., 1971). Systemic hypotension as a cause of the decreased glomerular filtration rate has been ruled out (Oken, 1972) and a glomerular vasomotor phenomenon has therefore been incriminated. After tubular obstruction had been relieved by fluid injection, single nephron glomerular filtration resumed spontaneously and achieved an essentially normal rate (Flamenbaum et al., 1971). Oken (1972) has raised the question whether there might be some direct feedback mechanism which modifies the effective filtration pressure in the obstructed, mercury-poisoned nephron. Therefore the demonstration of structural abnormalities in the walls of preglomerular arterial vessels at the time of tubular obstruction might provide some interesting new information. Numerous sudies of acute renal failure of different etiology have assumed a tubulo-glomerular feedback between the macula densa segment and the glomerular vas afferens (Thurau and Schnermann, 1965; Mason and Thurau, 1975). The observation of vascular lesions at a great distance from the juxtaglomerular apparatus suggests the existence of additional mechanisms which might explain the unsatisfactory results obtained from the sole consideration of a narrowly limited local renin-angiotensin effect (Oken, 1975).

Some of the rats reached the state of complete anuria as early as 12–16 h after injection of 4.7 mg MC/kg (Table 1). This is a time when fibrinoid vascular changes apparently do not occur. We therefore suggest that in the early phase of MC-induced acute renal failure, purely functional vasomotor changes may operate while in a later phase (24–48 h post-injection), additional structural changes develop in the vascular wall as a consequence of prolonged intense vasospasm or vasodilatation, or alternating spasm and vasodilatation. While intense vasomotor reactions occur, the perfusion of the tissues will be disturbed. In the kidneys this factor might, to a certain degree, contribute to the tubular damage caused by MC. The fact that mild vascular changes occurred several days after MC-injection indicates that imbalance in the vascular tone persists into the recovery phase of acute renal failure. Further studies are in progress to elucidate the mechanism and significance of the vascular changes.

Acknowledgements. Grateful acknowledgment is made to Miss B. Thoms, Mr. W. Dechert and Miss M. Glebe for their valuable technical help and to Mrs. J. Kemmer for her assistance in translating the manuscript.

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Received August 9, 1976