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Intra- and Extrarenal Vascular Changes in the Acute Renal Failure of the Rat Caused by High-Dose Folic Acid Injection*

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Summary. Male Wistar rats were investigated 9, 24 and 48 h and 4 and 8 days after a single s.c. injection of 500 mg folic acid (FA)/kg b.wt. dissolved in 0.3 M $NaHCO_3$. Temporary acute renal failure (ARF) developed after the injection. In the early stage of ARF most renal tubules appeared to be obstructed mainly by intratubular FA-precipitates. Necrosis of tubular epithelium developed at the same time. Its maximum extent was reached 48 h post-injection, particularly in the partes rectae of the proximal tubules. By this time the FA-precipitates had already diminished and were predominantly localized in the ascending thick limb of Henle's loop. Signs of intrarenal vasomotor changes and structural lesions of the vascular wall were also found. The most impressive finding was the development of fibrinoid medial lesions, mostly in the arcuate and interlobar arteries. The smooth muscle cells (SMC) of these generally dilated vascular segments appeared to be edematous and had imbibed blood plasma material, some had become necrotic. In many of these damaged cells intracellular fibrin (or fibrinogen) precipitates were seen. Subendothelial fibrin deposits were not detected. The vascular lesions were patchy and irregularly scattered throughout the kidneys but were also found in the pancreas, mesentery, heart, occasionally in the brain, and, in one rat, also in the liver. They occurred as early as 9 h post-injection but reached their greatest renal and extrarenal extent 48 h post-injection when azotemia, electrolyte disturbances and tubular damage were likewise at a maximum. Systemic arterial blood pressure was not elevated. The fibrinoid lesions were decreased 4 days post-injection and had completely disappeared (with one exception) 8 days post-injection. Residual damage and reactive changes, however, seemed to persist for some time.

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The regression of the vascular lesions was accompanied by regeneration of the tubular epithelium and marked improvement of renal function. FA-precipitation, tubular necrosis, vascular lesions and renal insufficiency were largely prevented or at least diminished by further alkalinization of the injection solution (using 1 M $NaHCO_3$ as solvent). It is concluded that the intratubular precipitation of FA in the main experiment resulted in functional tubular obstruction which induced considerable vasomotor changes, and thereby vascular lesions and circulatory disturbances – probably independent from the juxtaglomerular apparatus. The circulatory disturbances might be of special importance in the maintenance of FA-induced ARF. A temporary impairment of renal autoregulation might be considered since this autoregulation depends on the functional integrity of the renal vessels.

Key words: Kidney failure acute – Folic acid – Vasomotor system – Vascular disease – Arterial permeability.

Zusammenfassung. Männliche Wistarratten wurden 9, 24 und 48 h sowie 4 und 8 Tage nach einer einmaligen s.c. Injektion von 500 mg Folsäure (FS)/kg KG gelöst in 0,3 M $NaHCO_3$ untersucht. Nach der Injektion entwickelte sich temporär ein akutes Nierenversagen (ANV). Im frühen Verlauf des ANV erschienen die meisten Harnkanälchen vorwiegend durch FS-Präzipitate verstopft. Zugleich entwickelten sich Tubulusepithelnekrosen. Sie erreichen ihr größtes Ausmaß 48 h nach der Injektion mit punctum maximum in den partes rectae der proximalen Tubuli. Zu diesem Zeitpunkt waren die FS-Präzipitate bereits teilweise zurückgebildet und überwiegend in den partes rectae der Mittelstücke lokalisiert. Darüber hinaus fanden sich Zeichen einer gestörten intrarenalen Vasomotorik und Gefäßwandschäden. Eindrucksvollster Befund war die Entwicklung fibrinoider Medialäsionen, am häufigsten in Arcuata- und Interlobulararterien. In diesen meist dilatierten Gefäßsegmenten erschienen die glatten Muskelzellen ödematös und mit Blutplasmasubstanzen imbibiert. Muskelzelluntergänge kamen ebenfalls vor. Viele geschädigte Muskelzellen wiesen intrazelluläre Fibrin-(oder Fibrinogen) präzipitate auf, keine subendothelialen Fibrinablagerungen. Die Gefäßveränderungen waren fleckförmig und unregelmäßig über die Nieren verteilt, aber auch im Pankreas, im Mesenterium, im Herzen, manchmal im Gehirn und (bei einer Ratte) in der Leber nachweisbar. Sie waren bereits 9 h nach der Injektion vorhanden, jedoch am stärksten 48 h nach der Injektion entwickelt. Zum gleichen Zeitpunkt hatten auch die Azotämie, die Elektrolytstörungen und die tubulären Schäden ihr Maximum erreicht. Der arterielle Blutdruck war nicht erhöht. Die fibrinoiden Läsionen waren 4 Tage nach der Injektion wieder seltener und nach 8 Tagen fast vollständig verschwunden. Restschäden und reaktive Veränderungen persistierten jedoch für einige Zeit. Mit der Rückbildung der Gefäßläsionen ging zeitlich die Regeneration der Tubulusepithelien und eine deutliche Besserung der Nierenfunktion einher. Durch stärkere Alkalisierung der Injektionslösung (Verwendung von 1 M $NaHCO_3$) wurden die FS-Präzipitation, die Tubuluszellnekrosen, die vasku-

lären Läsionen sowie die Niereninsuffizienz weitgehend verhindert oder zumindest gemildert. Es wird angenommen, daß die FS-Präzipitation eine funktionell wirksame tubuläre Obstruktion verursachte, die – wahrscheinlich unabhängig vom juxtaglomerulären Apparat – erhebliche vasomotorische Störungen und auf diesem Wege Gefäßwandschäden und Zirkulationsstörungen induzierte. Letztere könnten von besonderer Bedeutung für die Aufrechterhaltung des FS-induzierten ANV sein. Es könnte auch eine temporäre Störung der renalen Autoregulation vorliegen, da ihr Funktionieren von der Integrität der Nierengefäße abhängt.

Introduction

Considerable disturbances of intrarenal hemodynamics in different types of acute renal failure (ARF) have been widely assumed for man as well as for experimental animals (Hollenberg et al., 1970; Truniger and Grandchamp, 1971; for review see Flamenbaum, 1973; Venkatachalam et al., 1976; Oken, 1976). Since the circulatory abnormalities were clearly associated with a decrease of the glomerular filtration rate (GFR), vascular mechanisms have been supposed to play an important role in the pathogenesis of ARF. The nature of the intrarenal circulatory disturbances in ARF seemed to be at least partially elucidated by the tubuloglomerular feedback hypothesis propagated by Thurau and coworkers (Thurau, 1964; Thurau and Schnermann, 1965; Mason and Thurau, 1975; Thurau et al., 1976; Thurau and Boylan, 1976). According to these authors, oligoanuria in ARF is initiated by a damage of the tubular epithelia leading to a decrease of tubular reabsorption and consequently to a rise of sodium chloride concentration in the macula densa segment of distal tubules. The increased sodium chloride concentration would then signalize the juxtaglomerular apparatus (JGA) to locally activate the renin-angiotensin system in order to reduce GFR. In this way, glomerular filtration and tubular reabsorption would be kept in balance so that excessive filtrate is not delivered to an epithelium which is unable to reabsorb it. This hypothesis seemed also capable of explaining the discrepancy between the massive loss of renal function and the rather discrete morphologic signs of renal damage present in many cases of ARF, at lest in man (Finckh, 1962; Bohle and Thurau, 1974). However, the functioning of the tubuloglomerular feedback mechanism depends on the functional integrity of the preglomerular arterial vessels (Thurau, 1966) and on an uninterrupted delivery of fluid to the distal tubules (Ploth and Schnermann, 1975).

In previous studies on ARF in the rat induced by mercury chloride (MC) we were impressed by the occurrence of considerable structural alterations in the intrarenal and even in certain extrarenal arterial vessels. The spectrum of changes ranged from edematous swelling of arterial smooth muscle cells (SMC) to segmental fibrinoid necrosis of the media (Zimmermann et al., 1977). In the same experiments, a parallelism was found between the maximum of the vascular changes, the maximum of azotemia and the development of debris cylinders which more or less filled up the lumina of most renal tubules. The

present study was undertaken to see whether lesions of the same type occur also in any of the other known models of ARF. Highdose folic acid (FA) injection was chosen as a model in which temporary tubular obstruction seems to be an important pathophysiological event (see below). Studying renal and extrarenal vessels between 9 h and 8 days after the injection of 500 mg FA/kg b.wt., vascular changes very similar to those found in MC-induced ARF were seen. These changes seemed also comparable to the lesions described in the rat kidney after temporary complete ischemia (Cain and Fazekas, 1963; Thoenes, 1964; Cain, 1965; Kaboth, 1965; Terry et al., 1970; Kashgarian et al., 1976). The vascular changes were compared with the extent of tubular damage as well as with renal function. Evidence ist presented that in addition to tubular obstruction, hemodynamic disturbances might play an important role, at least in the maintenance of FA-induced ARF in the rat, and that renal autoregulation, including the tubuloglomerular feedback system, might be temporarily impaired.

Materials and Methods

Male Wistar WU rats from Ivanovas breeding institute, Kisslegg/Allgäu, weighing between 220 and 290 g were used in the experiments. All animals were fed a standard laboratory chow (Hope Farms) and tap water ad libitum. Folic acid (E. Merck, Darmstadt) was dissolved in 0.3 M or 1 M sodium bicarbonate (pH 7.4) and injected in a 50 mg/ml solution, either s.c. into the dorsal region at two different sites or i.v. into the tail vein. The injections were given between 9 and 11 o'clock a.m. The 8 experimental groups are listed in Table 1.

After the injection the animals were given free access to food and water. The rats of Group 2–8 were kept in individual metabolic cages. Urinary volume and urinary electrolytes were determined in these animals. Systolic blood pressure was measured under light ether anesthesia by means of a tail plethysmometric method (Byrom and Wilson, 1938). In 10 rats of Group 3 this was done 3, $9^1/_2$, 25, and 48 h post-injection. Occasionally, however, reliable values could not by obtained because of tail edema. At the time of sacrifice the animals were anesthetized with ether and bled from the carotid artery. In all animals serum creatinine and urea were determined by autoanalyser (Technicon), and Na, K and Ca by flame photometry.

One half of both kidneys, the pancreas, the spleen, the mesentery with segments of the small intestine, the heart, the brain as well as lung and liver tissue, and in several animals also the adrenals, were fixed in 4% neutral formaldehyde for light microscopy. The second half of the kidneys was fixed in absolute alcohol or Carnoy's solution (absolute alcohol-chloroform-glacial acetic acid 6:3:1). Among several other tested fixation solution, Carnoy's solution proved to be best for studying renal morphology and for demonstrating intratubular FA precipitations. FA are eluted from the renal tissue by water-containing fixation solutions and, in our experience, also from paraffin sections if they are in contact with water for some time. For optimal preservation, paraffin sections should therefore not be equalized in a water-bath but in absolute alcohol, and contact with water during the staining procedures should be reduced to a minimum. Routine staining of 5 µm thick paraffin sections was performed with hematoxylin-eosin (H&E) and the periodic acid Schiff (PAS) method. In addition the following staining reactions were done: Elastica-van Gieson, Goldner's (1938) modification of Masson's trichrome stain, Ladewig's (1938) modification of Mallory's PTAH stain and Fraser-Lendrum's method for fibrin. Four longitudinal PAS-stained sections of each rat kidney were routinely screened for vascular lesions.

For electron microscope studies, kidney tissue of rats from Groups 3–5 and untreated controls were immediately fixed by immersion in phosphate-buffered. 6.5% glutaraldehyde for 2 h at 4°C. The 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. Semithin and ultrathin sections were cut on the Reichert OM U_2 microtome. The semithin sections were stained with toluidine blue, the ultrathin sections with uranyl acetate and lead citrate (Reynolds, 1963) and then examined in a Zeiss EM 9 S 2 electron microscope.

Table 1. Experimental groups

Group	*n*	Route of injection	Injection solution	Time of sacrifice post-injection
1	12	s.c.	500 mg FA/kg b.wt. dissolved in 0.3 M sodium bicarbonate	9 h
2	12	s.c.	idem	24 h
3	24	s.c.	idem	48 h
4	10	s.c.	idem	4 days
5	8	s.c.	idem	8 days
6	12	i.v.	idem	48 h
7	10	s.c.	500 mg FA/kg b.wt. dissolved in 1 M sodium bicarbonate	48 h
8[a]	10	s.c.	0.3 M sodium bicarbonate	48 h

[a] Controls

Results

I. GENERAL FINDINGS

Within the first 9 h after the FA injection, approximately 10% of the experimental animals of Groups 1–6 showed convulsions. 7 animals (=7.1%) of different groups died on the first or second day after the injection. The other rats were relatively apathetic during the first two days but became again interested in food from the third day on. The body weight was reduced by a mean of 7% (maximum 15%) during the first 48 h, of 13.8% (maximum 16.1%) after 4 days, of 7% (maximum 14.6%) after 8 days. The animals from Groups 7 and 8 showed a normal behaviour. Body weight was slightly reduced in Group 7 and unchanged in controls.

II. RENAL FUNCTION

1. Urinary Volume

The urinary volume of the controls was 4.9 ± 1.1 ml on the first and 4.4 ± 1.9 ml on the second day. The values of Groups 3–5 were similar: Some rats had polyuria on the first day whereas others entered the polyuric phase after two days of oligoanuria (3.3 ± 4.0 ml on the first, 7.95 ± 5.2 ml on the second, 20.3 ± 6.47 ml on the third, 20.7 ± 4.7 ml on the fourth and 30.3 ± 6.3 ml (values of 4 rats only) on the 8 day). Rats in Group 2 differed in so far as most of them were polyuric on the first day (14.0 ± 13.9 ml). With the exception that this group was included later into this study a cause for this variation was not detectable. In Group 6 the urinary volume was 6.48 ± 7.1 ml on the first, and 12.3 ± 10.8 on the second day and in Group 7 13.6 ± 4.3 ml on the first and 12.2 ± 7.2 ml on the second day ($\pm$ = standard deviation).

2. Serum Urea and Creatinine

In the controls serum urea was 45.7 ± 4.5 mg% and serum creatinine 0.42 ± 0.09 mg%. The values obtained from Group 1–5 are shown in Figure 11. All rats had considerably increased values,

but they were lower in animals which showed polyuria on the first day instead of oligoanuria. In Group 6 serum urea was $457 \pm 171{,}4\%$ and serum creatinine 5.56 ± 2.5 mg%. In Group 7 serum urea was 129 ± 104 mg% and serum creatinine 1.38 ± 0.78 mg%.

3. Serum Electrolytes

The Na^+ values were within the normal range except for elevated values 9 h after FA-injection. K^+ was strongly increased during the first two days and returned to the normal range after the fourth day (Fig. 11). Ca^{++} was initially increased (5.72 ± 0.51 mVal) after 9 h but then droped to low levels (4.2 ± 0.98 mVal after 48 h) and returned to almost the normal range after the fourth day (4.78 ± 0.64 mVal). No pathological Ca^{++} values were found in Groups 7 and 8.

4. Urinary Electrolytes (Group 3, 4, 7, 8)

Sodium and potassium excretion was increased on the first day in Group 8 (controls). After FA-injection total sodium excretion was slightly diminished on the first, and in the normal range on the following days. Potassium excretion was considerably reduced during the first two days and had returned to nearly normal after the fourth day. Calcium excretion was always in the normal range. Total sodium excretion in Group 7 was considerable increased on the first and normal on the second day, whereas potassium excretion was slightly but not significantly reduced on the first day and calcium excretion was within the normal range.

III. NET RENAL WEIGHT

Compared to controls, net renal weight per 100 g b.wt. increased considerably after FA-injection, the increase being 39.7% after 9 h, 48.3% after 24 h and 74.7% after 48 h. The maximal increase was reached 4 days post-injection when the net weight had doubled. There was a decrease after 8 days (54%). Weight increase in Group 6 was 67.2%, and in Group 7 31.2%.

IV. RENAL MORPHOLOGY

1. Gross Findings

The kidneys of the controls were normal. 9 h after FA-injection the surface of the enlarged kidneys showed a dense yellow-orange mottling on a pale brown ground color. On the cut surface a marked yellow-orange striation of the outer medulla was seen which continued into the inner medulla with lessening color intensity. A coarse radial yellow-orange striation of the cortex seemed to correspond to the medullary rays. 24 h post-injection the mottling of the renal surface was slight, and after 48 h it was only recognizable in few animals. The intensity of the striation of the cut surface had lessened after 24 h but was usually still distinct after 48 h, especially in the inner zone of the outer medulla. After 4 days, the intensity of the medullary striation had further diminished or was completely absent. After 8 days the kidneys were still pale. Punctiform grey-white areas were usually seen on the surface and fine grey-white stripes in the cortex. The yellow-orange striation typical for FA was not present in Group 7.

2. Histologic Findings

a) Renal Tubules

Controls: No pathologic changes were seen.

9 h after FA-Injection. Most of the tubules and collecting ducts contained yellow birefringent crystal-like FA-precipitates (Fig. 1); most of them were found in

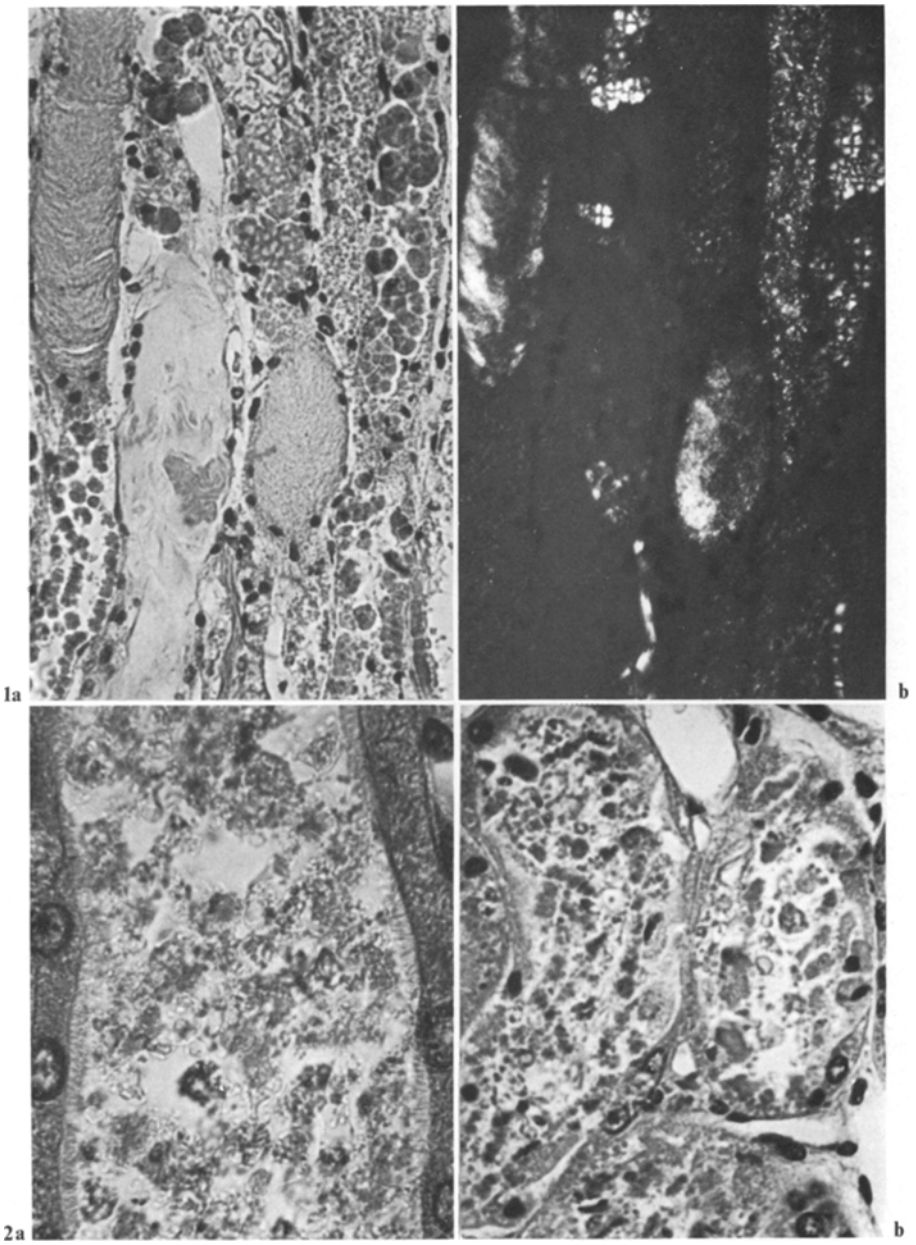

Fig. 1. a Tubules of the renal medulla apparently obstructed and partly distended by crystal-like FA-precipitates or protein-like material or a mixture of both (9 h post-injection). H. & E., ×213. **b** Distinct birefringence of the crystal-like FA-material in the polarized light

Fig. 2. a Intraluminal accumulation of cellular debris in the straight portion of a proximal tubule 24 h after FA-injection. Brush borders of the tubular epithelia are relatively well preserved in this part of the tubule. H. & E. ×714. **b** Extensive epithelial necrosis and intraluminal cellular debris in straight portions of proximal tubules 24 h after FA-injection. H. & E. ×302

Henle's loop. In the proximal tubules they were usually finely crystalline and often in contact with the brush borders. FA-material seemed occasionally to be included in the cytoplasm of the tubular epithelia. In the more distal segments of the nephron, FA-precipitates had formed coarse clods, clumps or structures resembling spiked clubs. Furthermore, many thin segments of Henle's loop, distal tubules (mainly the partes rectae) and collecting ducts were filled and sometimes distended by hyaline, protein-like material ("casts") (Fig. 1). In addition, most proximal tubules contained granular or vesicular detritus of cells or cell compartments (Fig. 2a). Hydrops, cytolysis or coagulation necrosis of tubular epithelia (Fig. 2b) could easily be detected, particularly in the straight portions. Scattered necrosis was also found in the distal tubules. Usually, the proximal and distal convoluted tubules were diffusely dilated. A mild or moderate widening of the interstitium was sometimes seen.

24 h after FA-Injection. The FA-precipitates had significantly diminished; although some deposits were still found in the straight portions of the proximal tubules, most of them were located more distal especially in the ascending thick limb of Henle's loop. Hyaline casts were again found in distended tubules of the medulla and in collecting ducts. Focal dilatation of the cortical tubules was frequently observed. Epithelial necrosis was more frequent and mainly found in the partes rectae of the proximal tubules. Necrotic cells were generally sloughed into the tubular lumen. Sometimes they seemed calcified like the above mentioned granular detritus, which was detected in all portions of the proximal tubules. In the distal tubules necrosis was rarer.

48 h after FA-Injection. Although the FA-precipitates had further diminished many tubules and collecting ducts still contained considerable quantities of FA. The partes rectae of the proximal tubules were often dilated. In these segments, particularly in the cortical medullary rays, cellular necrosis seemed more extensive than 24 h post-injection. Calcification of necrotic epithelia or of cellular fragments was present in 16 out of 22 rats. In addition, freshly degenerated epithelia or necrobiotic changes were detected in some animals. On the other hand, flat regenerating cells occurred occasionally. All portions of the proximal tubules generally revealed swollen epithelia with hydropic vacuolization of the cytoplasm and enlarged nuclei. Mitoses were found ubiquitously, their rate seeming highest at this time of examination. The distal tubules were often dilated and cytoplasmic swelling and nuclear enlargement were also present, but cellular necrosis was far less frequent than in the proximal tubules.

Similar results were obtained in Group 6. Generally, however, the FA-precipitates were slightly less extensive and epithelial necrosis seemed to occur slightly less frequent. No calcifications were observed. In Group 7 no FA-precipitates and casts, or only moderate quantities, were found. Epithelial necrosis was relatively frequent in some rats, mild in most and absent in one animal. Single necrotic cells were calcified in 5 rats.

4 Days after FA-Injection. FA-precipitates and casts were still found but had diminished in number. Cortical tubular dilatation was frequent and particularly

pronounced in the distal tubules. The number of proximal tubules with swollen and vacuolized epithelia varied. Some necrotic cells were still found in the lumina of proximal tubules, and freshly degenerating epithelia could be detected exceptionally. Epithelial regeneration had usually progressed but seemed disturbed where calcified masses had been deposited. Focal interstitial inflammatory infiltrates and occasional multinucleated giant cells were seen in these sites.

8 Days after FA-Injection. Intratubular FA-precipitates or protein-like material as well as cellular detritus were almost absent. The proximal und distal tubules were focally dilated, the latter more often than the former. The epithelia of the proximal tubules were swollen und heavily vacuolized in some areas and small, light-staining or basophilic in others. The interstitial infiltrates were slightly more disseminated, but had focally accumulated together with proliferated fibroblasts at sites where clots of calcified necrotic material lay in the tubular lumina. This material was sometimes covered by regenerated epithelia or surrounded by multinucleated giant cells.

b) Intrarenal Vessels

Light Microscopy. Structural changes in the preglomerular arterial vessels were found in most animals of Group 1–6. Relatively mild changes consisted of edematous swelling of SMCs. The most striking change was segmental fibrinoid damage of the media (Fig. 4). The results obtained with the PAS-reaction, Goldner's stain, Ladewig's stain, the Fraser-Lendrum stain and the Elastica-van Gieson stain suggested the presence of blood plasma and fibrinogen and/or fibrin in the media, but not in the subendothelial space. Rarely, nuclear hyperchromasia was found in endothelial cells. Occasionally, red blood cells had invaded the damaged media and mild perivascular edema was apparent. The fibrinoid changes were patchy and irregularly scattered throughout the kidneys. The damaged arterial segments were usually dilated showing stretched elastic membranes and flattened endothelial cells. Considerable caliber irregularities were observed and bead-like contours were sometimes indicated in longitudinal sections. 9 h after FA-injection, fibrinoid changes were found in 9 out of 11 rats, after 24 h in 8 of 11 rats, after 48 h in 19 of 22 rats, after 4 days in 7 of 10 rats, after 8 days in none of the rats, in Group 6 in 10 of 12 rats, in Group 7 in 5 of 10 rats and in none of the controls. While 9 h post-injection the fibrinoid changes were mainly found in the arcuate arteries, they subsequently increased in number, especially in the interlobular arteries. They often progressed with time from the outer third of the media to its inner portions. Structural damage of afferent arteroles was rare and, if present, limited to their proximal portions next to the branch-off from the interlobular artery. Occasional fibrinoid lesions were found in small hilar arteries.

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. Cross-sections of the vessels were also counted if there was the possibility that one and the same vessel had been

cut more than once in the sections. Thus counted, the total number of vessel cuts per slide was in the mean 22. The mean number of vessel cuts revealing fibrinoid damage was 2.5 per slide after 9 h, 4.5 after 24 h, 6.9 after 48 h and 2.2 after 4 days. The mean number in Group 6 was 5.9 and in Group 7 0.8.

4 days post-injection the nuclei and cytoplasm of endothelial cells and medial SMCs were often enlarged. Mitosis was observed occasionally in SMCs, and exceptionally in endothelial cells. 8 days post-injection the SMCs still appeared enlarged and slightly edematous in single arteries. Moderate numbers of mononuclear inflammatory cells and adventitial cells with large nuclei were seen around some vessels 4 and 8 days post-injection. No thrombi were found in the arteries or capillaries (glomeruli see below), but parietal thrombi which did not occlude the vascular lumen were found in medium-sized renal veins in 3 rats 48 h post-injection.

Apart from occasional hydropic epithelial alterations the *glomeruli* generally appeared unchanged, but 9 h after FA-injection protuberances of infraglomerular proximal tubular epithelium into Bowman's space were found in some kidneys (Fig. 3) as previously described in detail by Helmchen (1967) during temporary complete renal ischemia. 24 h post-injection this was a very exceptional finding. In 2 rats glomerular aneurysms occluded by thrombi were found 4 days post-injection (one resp. two glomeruli only in the routine sections).

Electron Microscopy. 48 h after FA-injection moderate hydropic changes occurred in endothelial cells of preglomerular vessels. Bundles of thin filaments were sometimes prominent in the cytoplasm. The subendothelial spaces were often enlarged and contained amorphous material resembling blood plasma, but no fibrin could be detected. Destruction of endothelial cells was not observed. No adherence of platelets or filamentous fibrin was found at the luminal side.

A 38 resp. 200 mμ wide interendothelial cleft resulting from the separation of interendothelial junctions was seen twice in a dilated vasular segment. In one of these cases (Fig. 5) one adjacent endothelial cell showed nuclear infoldings and a bulging towards the lumen. This finding might indicate active cellular contraction (Majno et al., 1969; Constantinides and Robinson, 1969; Joris et al., 1972; Shimamoto and Sunaga, 1972) but does not prove it, particularly in tissues fixed by immersion (Haudenschild et al., 1972; seen also Hammersen, 1976). On the other hand, the gaps which were found in *dilated* vascular segments might not be artefacts, since similar changes were not observed in untreated controls.

The lamina elastica interna usually appeared as a broad light band and was stretched in the arterial segments with media changes. Varying degrees of intracellular edema were present in the muscular layer (Fig. 7). This was accompanied by diminution and disintegration of myofilaments and often by degeneration of the mitochondria and endoplasmatic reticulum. In the case of extreme cellular hydrops, the swollen cytoplasm seemed to be almost empty. Hydropic parts of SMCs often protruded into adjacent intact muscle cells. The intercellular space was usually considerably narrowed as a consequence of the cellular swelling. Extremely swollen muscle cells very often showed a finely granular, homogenous or finely flaky matrix of medium density, probably representing a mixture of edematous cytoplasma and insudated blood plasma material (Fig. 6, 7). Within the cytoplasm of these imbibed muscle cells, irregular

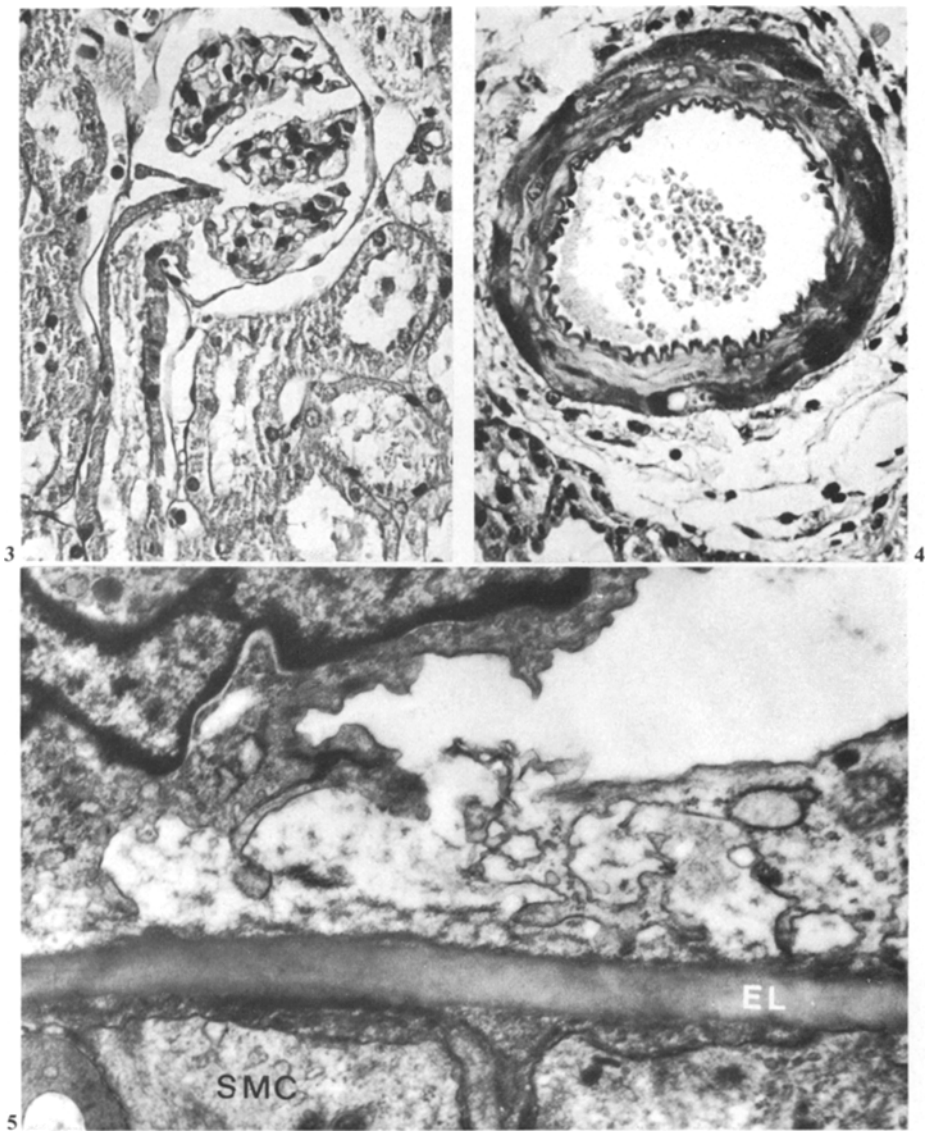

Fig. 3. Funnel-like protrusion of the infraglomerular proximal tubular epithelium, which is detached from the basement membrane, into Bowman's space. H. & E. ×247

Fig. 4. Intrarenal artery with small "vacuoles" and fibrinoid changes in the muscular layer, predominantly in the outer third of the media. PAS-stain, ×340

Fig. 5. Interendothelial cleft. The adjacent endothelial cell on the left is bulged towards the lumen. *EL* internal elastic membrane. Media SMC's show edema of the cytoplasm. ×70,640

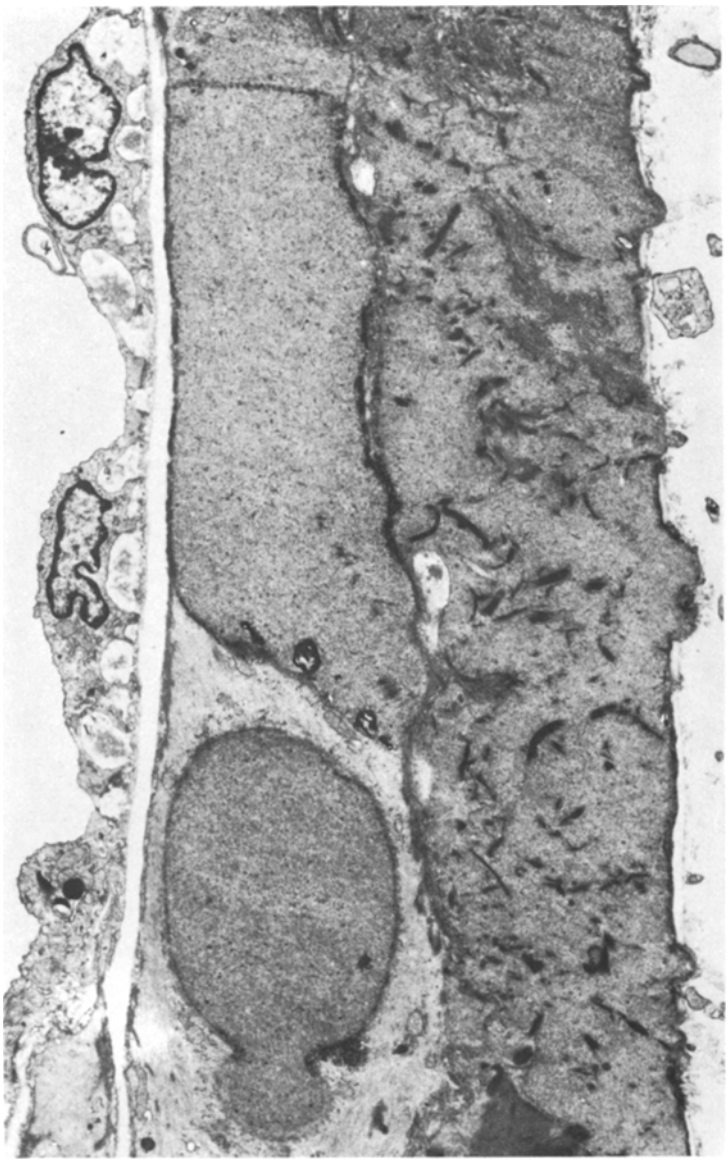

Fig. 6. Intrarenal artery: SMC's of the media are swollen and show a finely granular matrix, probably representing a mixture of edematous cytoplasm and insudated plasma material. Intracytoplasmic precipitates of a partly cross-striated material (see Fig. 8) occur in the outer cell layer. Intercellular spaces of the media are markedly narrowed. A finely granular material is also found in the subendothelial space. ×7260

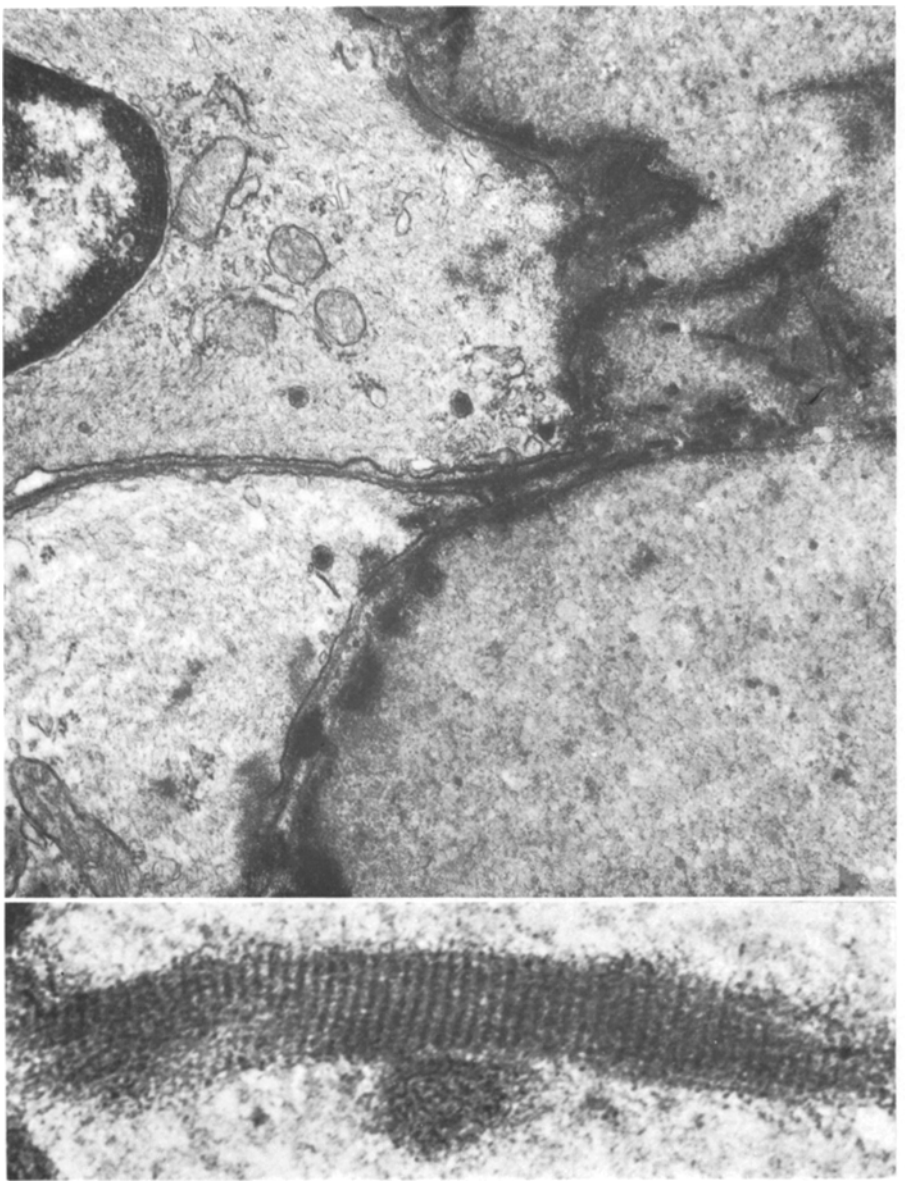

Fig. 7. Swollen muscle cells of the arterial media. Those on the left side show various degrees of intracellular edema, those on the right are diffusely imbibed by a finely granular, homogeneous or flaky material, probably blood plasma material. × 27,110

Fig. 8. Cross-striated intracytoplasmic precipitate with an axial periodicity of 17.2 mμ thought to represent fibrin (or fibrinogen). × 113,000

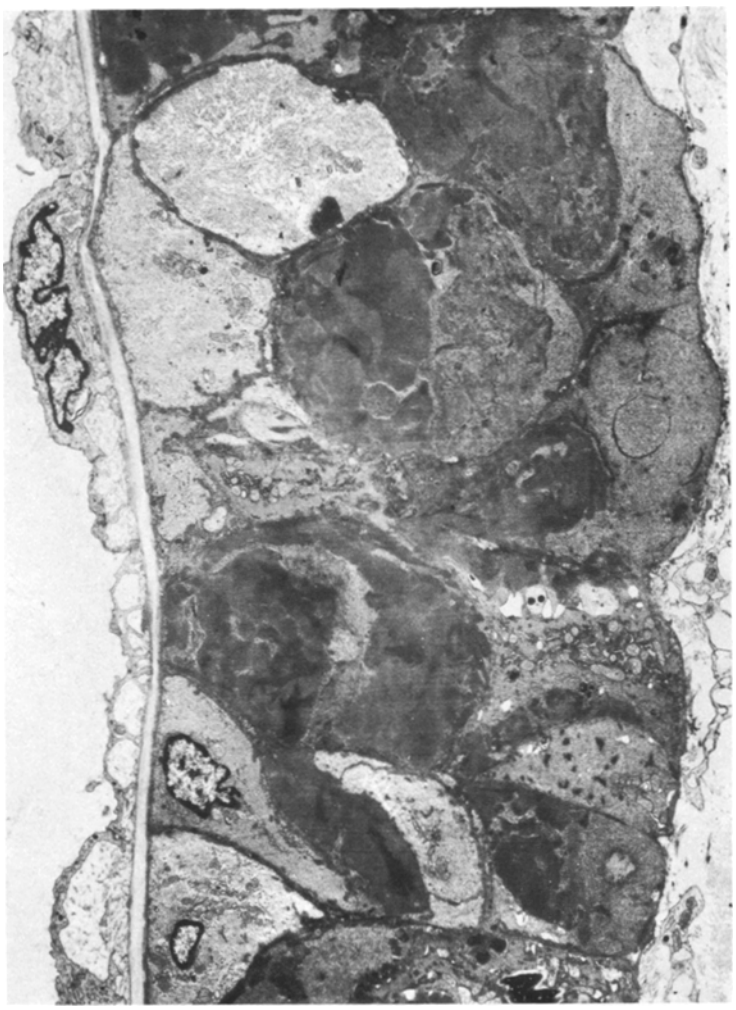

Fig. 9. More progressed stage of fibrinoid degeneration. Large fibrin masses are deposited in the arterial media in the form of clumps or crystal-like bodies. × 5030

bundles of thin filamentous material were often observed and sometimes there was a fibrous cross-striated material with an axial periodicity of approximately 17.2 mμ (Fig. 8). Thus, apparently fibrin or fibrinogen (Stryer et al., 1963) was precipitated intracellularly. Furthermore large crystal-like striated bodies, probably also fibrin or fibrinogen precipitates, were found as described in our MC-experiments, and again they often occurred in combination with necrobiosis or even necrosis (cytolysis) of SMC (Fig. 9). The regression of fibrinoid damage and the reactive changes in the arterial media will be described in a separate publication.

In the few instances that the juxtaglomerular apparatus (JGA) was examined 48 h post-injection, the epitheloid cells of the afferent arterioles showed no obvious signs of secretory hyperactivity.

However, 4 and, to a minor degree, 8 days post-injection, some hyperactivity was indicated not only by the occurrence of dilated endoplasmic reticulum and a moderate augmentation of ribosomes and mitochondria but also by an apparent increase in the number of secretory granules, which sometimes had a striated interior structure. In preliminary studies on the interstitial cells of the renal papilla, which are commonly believed to synthesize prostaglandin (s), the ultrastructure was essentially unchanged with a nearly normal content of lipid droplets 48 h post-injection, but after 4 days the cells appeared to be heavily stimulated and almost free of lipid droplets. These changes were even more pronounced 8 days post-injection. Cortical interstitial cells appeared to be stimulated too.

V. CORRELATION OF STRUCTURE AND FUNCTION

Considerable variations were found when individual rats within a single group were compared with regard to degree of renal insufficiency, tubular damage and vascular lesions. But the following gross correlation commonly seemed to exist: a relatively long phase of oliguria, a high loss of body weight, a high increase in net kidney weight, a relatively high degree of azotemia and hyperkaliemia and a relatively extensive tubular damage in the rats with many vascular lesions and vice versa. The maximum of functional and vascular changes was reached at a time when intratubular FA-deposits had already diminished but were still present in considerable quantities and when cellular debris had accumulated in many tubular lumina. Between 4 and 8 days post-injection the vascular lesions regressed, FA-precipitates were strongly diminished, tubular cells were regenerating and renal function was normalizing.

VI. PATHOHISTOLOGY OF EXTRARENAL TISSUES

Between 9 h and 4 days after FA-injection, segmental fibrinoid changes of small or medium-sized arterial vessels were also detectable outside the kidneys in most rats (Fig. 10). The frequency of these changes was related to the extent of the renal vascular lesions. When fibrinoid changes were absent in the renal vessels, none or almost none of these changes occurred in the extrarenal vessels. The maximum of extrarenal vascular damage was also reached 48 h after FA-injection and distributed as follows: in 95% in the pancreas, in 75% in the mesentery, in 50% in the heart, especially in the intramural vessels of the right ventricle, in 15% in cerebral arteries or arterioles, and in one rat in the liver. 8 days post-injection, only one rat had fibrinoid damage of a single pancreatic artery.

Parenchymatous lesions were also found in some organs: Interstitial edema of the pancreas was observed in several rats (especially 48 h post-injection) but was massive (and rich in fibrin) in only two. Focal necrobiotic changes or necrosis of acinar cells, mild focal acinar dilatation and focal vacuolization of acinar epithelia was also detected in some animals (Fig. 10d). In the heart, necrosis of single cells or of small groups of muscle fibers was frequent (Fig. 10a). Mononuclear infiltrates and proliferation of mesenchymal cells were found in the later course but were sometimes combined with fresh lesions. Scattered necrosis and in some areas swollen epithelia with a PAS-positive granularity

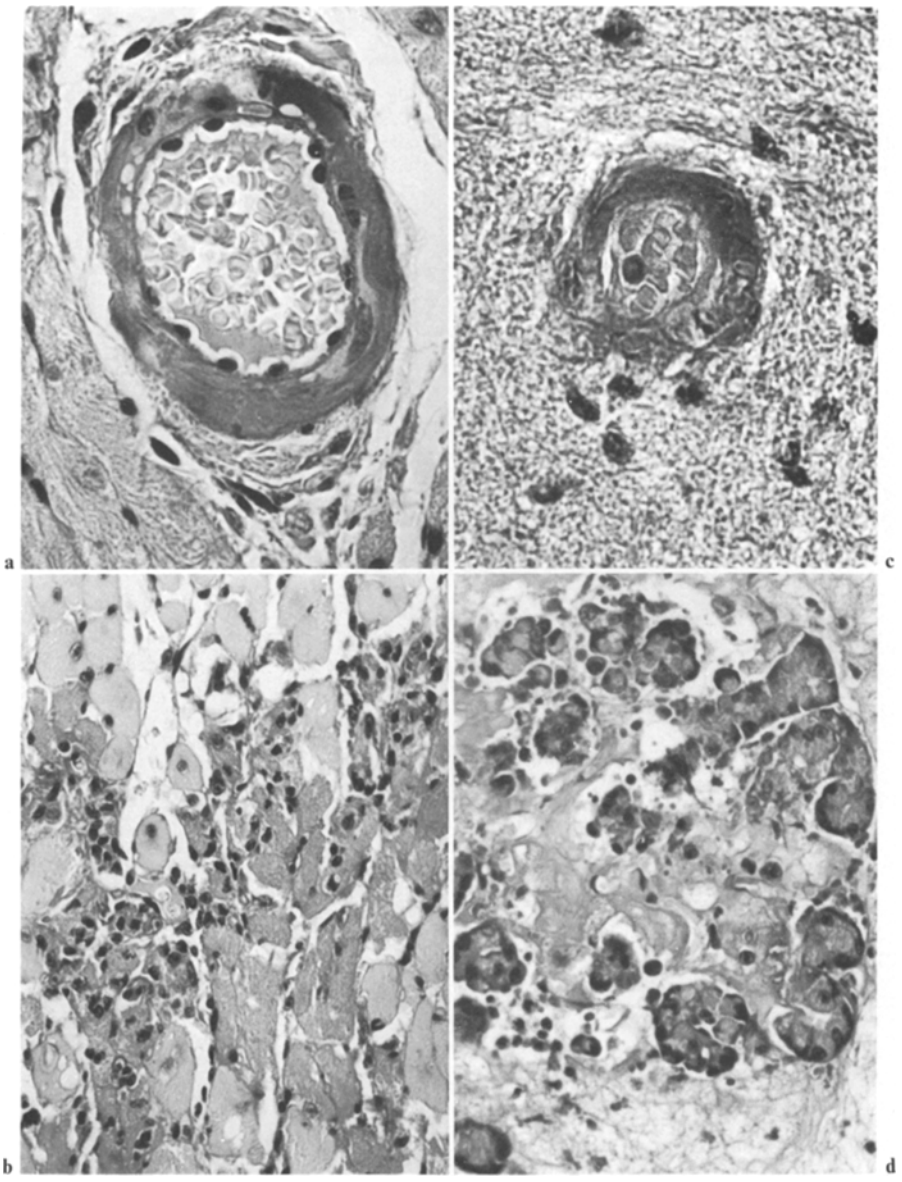

Fig. 10a–d. Changes in extrarenal tissues. **a** Extensive fibrinoid media damage of an intramural coronary artery of the heart (48 h post-injection). PAS-stain, ×417. **b** Focal necrosis (dark stained) of muscle fibers of the heart and reactive proliferation of mesenchymal cells, H.&E., ×221. **c** Fibrinoid damage of an intracerebral arteriole. PAS-stain, ×485. **d** Focal damage in the pancreas with necrosis of acinar epithelia and interstitial edema, which is rich in fibrin. H.&E., ×222

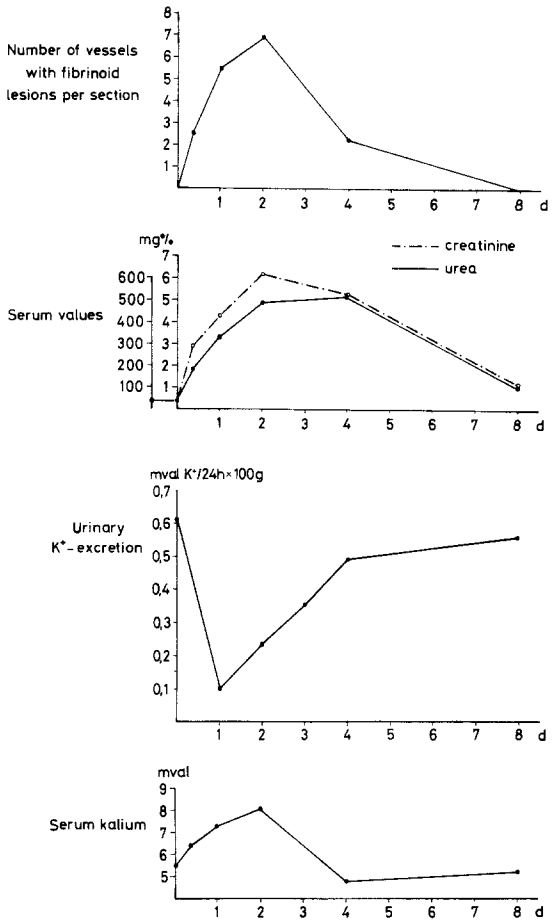

Fig. 11. Correlation of vascular lesions with renal function

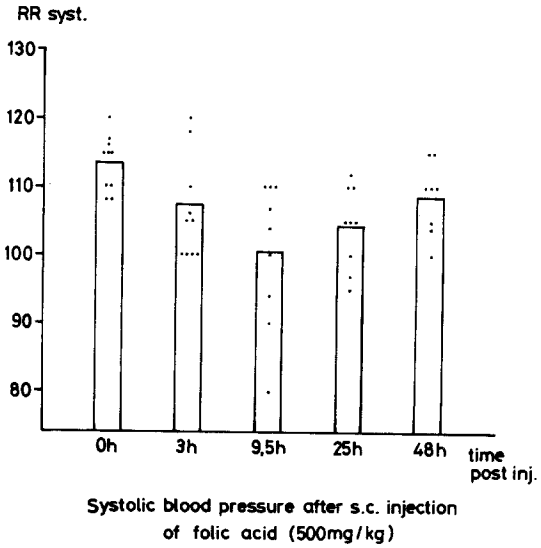

Fig. 12. Systolic blood pressure after s.c. injection of folic acid (500 mg/kg)

of the cytoplasm were seen in the liver of some rats in the early course. In the brain, extensive necrosis of ganglion cells was always present, as was degeneration in the Purkinje cell layer and granular cell layer of the cerebellum.

VII. BLOOD PRESSURE

Mean blood pressure was 109 mm Hg before any treatment. The values for the 10 rats of Group 3 are given in Figure 12. Systemic hypertension was never present as confirmed by sporadic measurements in additional rats of the same or of other groups.

Discussion

LESIONS OF THE RENAL PARENCHYMA

High-dose folate injection was introduced into experimental renal pathology in 1966 (Taylor et al., 1966; Threlfall et al., 1966). At first, the main interest centered on the question of a "chemically induced hypertrophy", since an increase of renal weight, protein, RNA and DNA synthesis and the mitotic rate were initially the most impressive findings (Taylor et al., 1968; Baserga et al., 1968; Mullin et al., 1976). Together with these changes, however, temporary renal insufficiency was detected and further examined. Several investigators tested high-dose FA injection (250 mg FA/kg b.wt. as originally applied by Taylor et al.) as a new experimental model of ARF. Whereas some authors (Torhorst et al., 1970; Schmidt et al., 1971) stated that the tubular damage after FA injection was rather discrete histologically, others reported more distinct lesions, such as scattered epithelial necrosis, especially in the straight portions of the proximal tubules and in the distal tubules of the outer medulla (Schubert et al., 1971; Schubert, 1976). Byrnes et al. (1972) found the epithelial damage to be more extensive in the distal than in the proximal tubules. Today most authors agree that the intense proliferation of tubular epithelia after FA injection can be regarded as a regenerative phenomenon, at least partly (Schubert et al., 1971; Schubert, 1976; Hsueh and Rostorfer, 1973; Mullin et al., 1976).

In order to obtain a similar enlargement and increase in dry weight of rat kidney as reported by Threlfall et al. (1966) other investigators (Brade et al., 1969, 1970) had to apply twice the dose of FA intravenously (500 mg/kg b.wt.). The same dose was used by Wilimzig (1974) for intraperitoneal injection and in the present study for s.c. and i.v. injection. In accordance with Brade et al. (1969) and as far as we can see also with Wilimzig (1974), but in contrast to the above cited investigations, we found widespread epithelial lesions which were far more extensive in the proximal than in the distal tubules (see below).

Another important finding was that most (if not all) tubules contained crystalline FA-precipitates and/or casts consisting of protein and debris from tubular epithelial cells. Although FA-precipitates were already described by Taylor et al. (1968) their amount and significance was apparently not fully realized at least in some of the earlier investigations.

This was probably due to the fact that the amount of precipitates cannot be recognized if tissues are fixed in water-containing solutions such as formaldehyde or glutaraldehyde. As mentioned in the chapter "Materials and Methods", FA can also be eluted from paraffin sections if these come into contact with water. Thus, for example, the relatively long water contact during PAS-staining might explain why Schmidt et al. (1976) stated that folate casts were "not as numerous as might have been expected" in freeze-dried sections.

From a solely morphologic point of view, the amount of intraluminal FA-crystals and casts might cause a real obstruction in most tubules. This is strongly evidenced by the results of micropuncture studies in which proximal intratubular pressure was found to be elevated to 42.6 mm Hg 10 min and to 20.9 mm Hg 24 h after i.v. FA-injection (250 mg/kg b.wt.) (Huguenin et al., 1974). Evidence is also supplied by the finding that further alkalinization of the injection solution (see above and Oertel and Herken, 1974) prevented, or at least diminished, not only the amount of intratubular FA-precipitates (by the increasing solubility of FA) but also the impairment of renal function and the development of tubular damage and vascular changes. Similarly, it has been reported in clearance studies that the infusion of FA-injected rats with isotonic $NaHCO_3$ prevented the fall of GFR (Birbaumer et al., 1975). The beneficial effect of diuresis induced by furosemide (Birbaumer et al., 1975; Mullin et al., 1976) or mannitol (Hsueh and Rostorfer, 1973) might also be explained through the prevention of intratubular FA-precipitation.

Although there was a relationship between the amount of FA-precipitates and the degree of renal insufficiency, the maximum of tubular and vascular changes and of functional impairment was reached at a time (24–48 h post-injection) when the intratubular FA-concentrations were already reduced. This discrepancy might be explained by (1) a nephrotoxic effect of FA, possibly favored by FA-crystallization (Brade et al., 1969) and/or (2) circulatory disturbances which are severe enough to cause tubular damage. Some authors have pointed out that tubular epithelia located adjacent to folate casts seem to be the preferred sites of damage (Byrnes et al., 1972). In the present study, however, the main site of tubular damage did not correspond to the main site of FA-precipitates. Most of the necrotic epithelia were found in the partes rectae of proximal tubules where larger intraluminal amounts of FA were only detected 9 h post-injection. Cellular damage by FA would thus seem to play only a secondary role. A nephrotoxic effect of FA discussed by Brade et al. (1969) and Schmidt et al. (1976) cannot be excluded, but is hardly decisive in the present experiment since tubular damage could be largely prevented by use of the 1 M bicarconate solution and since no response of the tubular epithelium to FA was found in vitro (Mullin et al., 1976).

On the other hand, temporary complete renal ischemia is known to cause necrosis of the tubular epithelia (Oliver et al., 1951; Cain and Fazekas, 1963; Kaboth, 1965; Reimer et al., 1972; Arendshorst et al., 1976) and therefore circulatory disturbances might at least contribute to the development of tubular damage after FA-injection. The ultrastructural tubular changes have been reported to be essentially in accordance (apart from some particularities) with the lesions found after temporary renal ischemia (Brade et al., 1970), but renal dry weight, RNA and protein content increased far more after FA-injection.

An obvious topographic relationship between epithelial necrosis and fibrinoid vascular damage was not apparent in the present experiments. On the other hand, a pathogenetic role of the circulatory disturbances might be indicated by our finding that tubular damage together with similar arterial changes occurs also after bilateral ureteral ligation (to be published). However, tubular damage after ureteral ligation is slight and far less extensive than after FA-injection. Thus, several factors might contribute to the tubular damage resulting from the injection of an extremely high dose of FA. Among them, circulatory abnormalities probably play a certain, however ill-defined role.

INTRARENAL VASCULAR CHANGES

Although the FA model of ARF has been used by several investigators (cited above) vascular lesions have never been described.

One important reason might be that most of the investigators applied only 250 mg FA/kg b.wt. instead of the 500 mg dose. In previous experiments (unpublished) we found fibrinoid vascular damage in only 2 out of 10 rats injected with the 250 mg dose. In routine sections, this damage was limited to a single artery in both animals.

The progression of the vascular changes in the present study was quite similar to that found after MC-intoxication: the first stage of smooth muscle cell alteration appeared to be intracellular edema, followed by diffuse imbibition of the cytoplasm by blood plasma material, including fibrinogen. The latter was evidenced by the intracellular precipitation of a material presenting the aspect of fibrin (or fibrinogen). Again, large crystal-like fibrin (or fibrinogen) formations, necrosis of smooth muscle cells and invasion of damaged media segments by red blood cells were observed. The opening of interendothelial junctions, which we observed very occasionally, might possibly provide a way for the entrance of blood plasma material into the vascular wall. However, the leakage might also occur via a transendothelial route which remained unidentified in the present study. Subendothelial fibrin deposits—often reported in arterial hypertension—were not detected.

The influence of harmful factors on the vascular system seemed to be of a temporary nature since most of the damaged muscle cells apparently reached only a prenecrotic stage of degeneration and then recovered. The fibrinoid damage resolved relatively quickly, but distinct residual (and reactive) changes, particularly on the ultrastructural level, were observed as late as 8 days after FA-injection. At least some of the necrotic muscle cells seemed to be substituted by mitosis.

The lack or relatively moderate extent of the arterial lesions after further alkalinization of the injection solution indicates that the arterial damage is not due to a certain chemical property of FA. On the other hand the amount of the vascular lesions seemed to be related to the degree of tubular obstruction in the different experimental groups, so that a causal realtionship can be assumed. The structural changes of the arterial wall were mostly accompanied by distinct irregularities in caliber, in particular by vasodilation. Although it

is difficult to extrapolate from histologic sections to the in vivo state, we therefore suppose that vasomotor changes and circulatory disturbances had developed. They might have preceded and essentially contributed to the development of the fibrinoid media changes.

However, the way in which vasomotor changes might affect the arterial wall is not fully understood as yet. Since the fibrinoid changes do progress with time from the outer third of the media to its inner portions and since the media of these arteries is solely nourished from the luminal side, we speculate that hypoxia of the vascular wall may have preceded the plasmatic imbibition. Hypoxia itself might result from the combination of increased labor of the smooth muscle and of slow blood flow, particularly in dilated vascular segments (see the more detailed discussion in our previous paper). This assumption is in part supported by the occurrence of comparable intrarenal arterial changes after temporary complete renal ischemia. These changes have been discussed to result from hypoxia during the time of circulatory arrest (Cain and Fazekas, 1963) or from vasospasm after restoration of blood flow (Kaboth, 1965; Kashgarian et al., 1976).

Our conception that vasomotor changes occur in FA-induced ARF is in contrast to the results of experiments in which the 250 mg dose of FA was applied and in which renal hemodynamics were found unaltered over a 24-h period post-injection (Ayer et al., 1972; Schmidt et al., 1976).

There is no evidence of elevation of systemic arterial pressure (see also Brade et al., 1969) so that systemic hypertension as a possible causative factor of arterial damage can be excluded. However, electrolyte disturbances and azotemia might synergistically contribute to arterial damage. The question then arises whether a release of vasoactive substances of renal origin occurs which might induce or favor the development of vascular changes. Helmchen et al. (1972) found no elevation of plasma renin activity after the injection of 250 mg FA/kg b.wt. The authors concluded that there is no evidence for the participation of renin in the pathogenesis of this model of ARF. However, renin or angiotensin might exert a solely local action within the kidney (Mason and Thurau, 1975). Yamamoto et al. (1972) observed increased renin activity and angiotensin I concentrations in the plasma 48 and 96 h after FA-injection while renal renin content was not changed. However, the very low dose of 250 μg FA/kg was used by these authors. So far, the structure of the JGA has not been systematically studied in FA-induced ARF. From our preliminary studies we have gained the impression that the renin-producing cells of the juxtaglomerular arterioles, and even more pronounced the interstitial cells of the renal medulla, are stimulated in the later course of ARF (after the second day). This might reflect a secondary response. Further work has to be done, however, to clarify the role of renin-angiotensin and medullary prostaglandins in this experimental model of ARF. In any case until now there is little to support the view that the changes in vascular tone after tubular obstruction by FA and casts are initially mediated by renin or angiotensin.

Furthermore an excessive release of catecholamines which are involved in the regulation of renal blood flow and intrarenal hemodynamics (Carrière, 1975; Tyssebotn and Kirkebø, 1975; Rentsch et al., 1976) might contribute to the generation of the vascular changes, and the question also arises whether a first or rapid phase of vasomotor changes might be of a primarily neurogenic

nature. Both, catecholamines as well as renal nerve stimulation, have been reported to cause renal vasoconstriction. According to Gotshall and Itskovitz (1977) this is more marked in the inner than in the outer cortex. The results of angiographic studies (Brady and Fischer, 1964) indicated that nerve stimulation causes constriction of both, the large and small arterial segments whereas the catecholamines constrict only the smaller segments while the large segments are passively dilated. Interestingly, Källskog and Wolgast (1975) have suggested that the tension of the tubular wall could be a factor which might effect the preglomerular vascular tone.

THE QUESTION OF RENAL AUTOREGULATION

The rat kidney maintains a relatively constant blood flow and GFR at a mean perfusion pressure between 105 and 145 mm Hg (Arendshorst et al., 1975). The long known autoregulative response is based on the intrinsic ability of the kidney to modify intrarenal vascular resistance. Autoregulation therefore depends on the functional integrity of the intrarenal arterial vessels, particularly of the vascular smooth muscle (Thurau, 1966). This is also evidenced by the fact that autoregulation can be abolished in the dog if the vascular smooth muscle is paralyzed by potassium cyanide, papaverine, acetylcholine or large doses of novocain (Miles et al., 1954; Ochwadt, 1956; Thurau and Kramer, 1959; Gross et al., 1976). Hypoxia was found to cause a moderate impairment (Waugh and Shanks, 1960). From the vascular changes demonstrated in the present study we conclude that the functional integrity of vascular smooth muscle is temporarily abolished (at least focally) and that renal autoregulation might be temporarily impaired. This might also be true for the MC-induced ARF and for the renal insufficiency following ligation of both ureters where we found similar vascular changes. The problem has also been discussed in the case of temporary renal ischemia (Cain and Fazekas, 1963; Thoenes, 1964). Since the autoregulative vascular reactions seem to be only a part in a more complex sodium conserving mechanism (Thurau, 1966), the vascular lesions might well be accompanied by a complex disturbance of intrarenal regulatory mechanisms.

EXTRARENAL VASCULAR CHANGES

As far as we know, vascular changes outside the kidney have only been described in MC-induced ARF (Zimmermann et al., 1977) and after bilateral ureteral ligation (Toussaint, 1953; personal unpublished observations in the rat). It became clearly evident in the present study that the amount of extrarenal arterial lesions, especially in the splanchnic area, parallels the amount of intrarenal arterial alterations.

In comparison to MC-intoxication, the damage to the arterial vessels of the heart was much heavier. Lesions of brain and liver vessels were not detectable after MC-injection. Extrarenal parenchymatous changes were also far more distinct following FA-injection, especially in the heart,

but also in the pancreas and in the liver, whereas an extensive necrosis of the ganglion cells of the brain was found in MC-injected rats, too.

The detection of extrarenal vascular changes should lead to concentrate not only on intrarenal events in ARF. However, further experiments are needed to explain the findings. The distribution of vascular damage throughout the body is similar to that found in systemic hypertension which was not observed in the present study. For the time being, we assume that vasomotor changes may also occur outside the kidney and that an excess of catecholamines as well as neural factors, in addition to others, might participate in the generation of the structural changes. The autoregulation of blood flow which is not limited to the kidneys (Johnson, 1964), might therefore also be temporary disturbed in certain extrarenal circulatory districts.

FINAL REMARKS

As in other experimental models of ARF, the impairment of renal function after high-dose FA-injection cannot be explained by a single pathophysiologic abnormality. Several factors seem to be involved among which tubular obstruction and vasomotor changes might play a dominant role from the morphological point of view. We suppose that the intratubular crystallization of FA causes functional tubular obstruction which induces severe vasomotor changes leading to structural damage of the vascular wall and to intrarenal circulatory disturbances. The latter might be of special importance in the *maintenance* of ARF. The histologic findings indicate that the imbalance of the vascular tone persists into the recovery phase of ARF. This is in accordance with clearance studies indicating that GFR is still low 10 days after FA-injection (Brade et al., 1969; Brade and Propping, 1970). The present study does not answer the question whether resp. to what degree tubular leakage might be an important pathophysiologic event.

As already mentioned, vascular lesions are also found after temporary complete renal ischemia. It is therefore of special interest that intratubular obstruction has recently been emphasized as the major factor in both the generation and maintenance of the oliguria following temporary unilateral renal arterial occlusion in the rat. There seemed to be a delayed development of a predominant increase in preglomerular vascular resistance as a response to tubular obstruction, and oliguria 24 h after removal of the renal arterial clamp was thought to be the result of a combination of persisting luminal obstruction, intrarenal vasomotor changes and passive tubular backflow (Arendshorst et al., 1975, 1976). Similar observations have been made after obstruction of individual nephrons by viscous oil or by complete unilateral ureteral ligation both for a period of 24 h (Arendshorst et al., 1974). Tanner et al. (1973), Tanner and Sophasan (1976) and Tanner and Steinhausen (1976) have also stressed the role of tubular obstruction, mainly caused by intraluminal casts consisting of protein and debris from tubular epithelial cells, in the pathogenesis of ARF after temporary complete renal ischemia. The previous reports about structural vascular alterations have not been mentioned in these recent publications. Since

we found evidence of tubular obstruction in the present study and since, on the other hand, autoregulation of the GFR apparently also depends on an uninterrupted delivery of fluid to the distal tubule (Ploth and Schnermann, 1975) we doubt that the vasomotor changes which we assume to occur *after* tubular obstruction are primary mediated via the macula densa on an individual nephron basis. However, this does not exclude the possibility that in the very early stage of ARF, when tubular obstruction is not yet manifest, a tubuloglomerular feedback system as propagated by Thurau and coworkers is stimulated, thereby reducing GFR and favoring intratubular FA-precipitation. After tubular obstruction has become manifest, a tubulovascular relationship might exist which is not governed by the JGA. Further investigations are necessary to support or refute these reflections.

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