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MERCURIC CHLORIDE-INDUCED TUBULONECROSIS IN THE RAT KIDNEY: THE RECOVERY PHASE

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Summary.—Regeneration of the renal tubules in rats, after death of the cells of the *pars recta* caused by a dose of 1·5 mg $HgCl_2$/kg, was examined by histological and radioautographic methods. The tubules regenerated from surviving squamoid cells at both ends of the necrotic segments, which often appeared to arise from epithelial cells that had cast off superficial "dead cytoplasm" to leave the basal parts containing the nucleus in a rim of cytoplasm. The tubular epithelium was reconstituted between the 2nd and 5th days by proliferation and sliding extension of the squamoid cells along the tubules and predominantly from the *distal end* of the necrotic segments where the cell proliferation was extremely active. Inflammation in reaction to the dead cells was insignificant. Although the majority of tubules regenerated in a regular fashion some degree of anomalous epithelial proliferation occurred in patches, predominantly in the junctional area between the *pars recta* and the loops of Henle and perhaps most frequently in reference to those nephrons with superficial and mid-cortical glomeruli. The exuberant proliferation led to scattered epithelial growths projecting into the lumen of the tubules, and there was evidence that these obstructed the discharge of necrotic debris. Tubular collapse and atrophy leading to the formation of small scars followed, more often affecting the short looped nephrons.

RENAL TUBULONECROSIS and acute renal failure caused by the systemic administration of mercuric chloride ($HgCl_2$) to experimental animals is often referred to as a "model" for experimental work designed to elucidate a pathophysiological basis for the clinical syndrome of acute renal failure in man (Flamenbaum, 1973; Stein, Lifschitz and Barnes, 1978). However, the clinical syndrome is not solely explained by the presence of tubulonecrosis, which may indeed be absent (Finckh, 1960; Finckh, Jeremy and Whyte, 1962). Much work has been done on the biochemical and physiological effects in the 1st 24 h after injection (McDowell *et al.*, 1976; Zalme *et al.*, 1976; Sato *et al.*, 1977; Stein *et al.*, 1978).

$HgCl_2$ leads to necrosis of the proximal convoluted tubules, mainly the *pars recta*, the extent of involvement increasing with the dose (Rodin and Crowson, 1962; Gritzka and Trump, 1968; Haagsma and Pound, 1979). Most workers have used relatively large doses and, since doses in excess of 4 mg/kg in rats are often lethal, long-term effects have not often been pursued. The assumption is usually made that the tubular epithelium is regenerated without permanent damage to the structure of the kidney or to its function. Permanent lesions have been reported (Oliver, MacDowell and Tracy, 1951; Oliver, 1953). The manner of regeneration of the tubular epithelium is controversial (Cuppage, Chiga and Tate, 1972).

We found (Haagsma and Pound, 1979) that rats given a non-lethal dose of 1·5 mg/kg $HgCl_2$ consistently developed necrosis of the distal part of the proximal convoluted tubules and polyuria. A functional disturbance of the kidney persisted for at least 10 days. There was morphological evidence of permanent damage. This paper describes the regenerative phase of the tubular epithelium and the

development and character of the permanent lesions.

MATERIALS AND METHODS

Animals.—Random-bred male Sprague–Dawley rats, 250–350 g in weight, from the Medical School Animal House, University of Queensland, were fed a 20% protein pelleted diet (Bunge, Australia, Ltd) and water, *ad lib.*

Chemicals.—$HgCl_2$: A solution of 1 g/l was made up using $HgCl_2$, L.R., dissolved in sterile 0·9% NaCl solution (Abbott Laboratories Pty Ltd, Sydney). [^{3}H]-Thymidine, 1·5 mCi per mM, was obtained from the Radiochemical Centre, Amersham, U.K.

Experimental

Rats were injected with 1·5 mg $HgCl_2$/kg s.c. into the anterior abdominal wall. Control rats received an equivalent volume of 0·9% saline. This dose of $HgCl_2$ produced necrosis of the outer stripe of the medulla, but no mortality. At 4, 8, 16, 24, 36, 48 h, 3, 5, 7, 9, 15, 22 and 28 days and longer intervals later, rats were prepared to provide sections for light and electron microscopy as previously described (Haagsma and Pound, 1979).

Kidney fixation.—Kidneys were fixed *in vivo* by perfusion through the abdominal aorta with 2·5% glutaraldehyde in 0·05M sodium cacodylate buffer, pH 7·3, under Nembutal anaesthesia, at a perfusion pressure of 130–140 mmHg for 1 min using 45–50 ml fixative solution.

Histological methods.—Paraffin-embedded sections were stained with routine H.E., periodic-acid–Schiff (PAS), and sulphated toluidine blue (STB) to demonstrate brush borders and basement membranes, Mallory stain to demonstrate interstitial fibrosis. Epon-embedded sections, 1 μm thick, were stained with toluidine blue for high-resolution light microscopy.

Autoradiographic methods.—1. Rats from experimental and control groups from 4 h to 28 days after dosing with $HgCl_2$ were given [^{3}H]-thymidine, 0·75 μCi/g body wt i.p., 1 h before perfusion of the kidneys.

2. One experiment was designed to help elucidate the origin of the cells that repopulate the necrotic segments of the tubules: a single dose of [^{3}H]-thymidine, 0·75 μCi/g i.p., was given to 14 rats 35 h after dosing with $HgCl_2$, thus labelling all cells in S phase at that time. Starting at 36 h 2 rats were killed at 6h intervals.

Light microscope autoradiographs were prepared using paraffin sections dipped in Ilford K5 nuclear research emulsion. After 4 weeks' exposure the slides were developed and the autoradiographs counterstained with nuclear red and picric acid to define the cytoplasm and nuclei of cells. Cells were considered to be labelled if they contained at least 5 silver grains over the nucleus. Background counts were seldom over 2 grains per nucleus.

Nomenclature.—The rat kidney can be divided into 4 zones (Peter, 1909; Rodin and Crowson, 1962) *viz*: (a) cortex, which contains the glomeruli, parts of the proximal and distal convoluted tubules, (b) outer stripe of the outer medullary zone, which contains part of the proximal convoluted tubules, including the *pars recta*, and ascending thick limbs of the loops of Henle but no glomeruli. It ascends into the cortex as the medullary rays in relation to the subcapsular glomeruli. (c) Inner stripe of the outer medullary zone, which contains mainly loops of Henle and the first parts of the distal convoluted tubules and (d) inner medullary zone (papilla), which contains mainly collecting ducts and the longer (thin) segments of the loops of Henle.

[^{3}H]-Thymidine and mitotic indices.—[^{3}H]-Thymidine indices were assessed by counting the number of labelled epithelial nuclei in 50 randomly selected high-power fields (each 0·278 $mm^2 \times 400$) or, in some cases, as the areas were small, 50 half fields, in each of the relevant zones (see below). Mitotic indices were determined in the same way. Cells in metaphase, anaphase, telophase and very early interphase were counted, but prophase nuclei were difficult to identify consistently and consequently were not included.

This method of assessment provided the most informative data. Efforts to count indices on a % cell basis or the number of mitoses or labelled cells per tubular profile were inconsistent because of the varying shapes of profiles and the changing patterns of variation of the indices in different segments of the nephrons. The data are supported by the photomicrographs.

RESULTS

Gross findings

Apart from a band of pallor in the outer stripe of the medulla on the cut surface of the kidneys, due to necrotic tissue and seen from the 8th hour to the 7th or 9th day after injection, the kidneys appeared normal. The capsule stripped readily in the early stages but by the 16th day on stripping the capsule small, randomly distributed, irregular depressions about 0·5 mm in diameter remained on the surface of the kidneys. They did not increase in number after this time and therefore their occurrence appeared to be determined by earlier events.

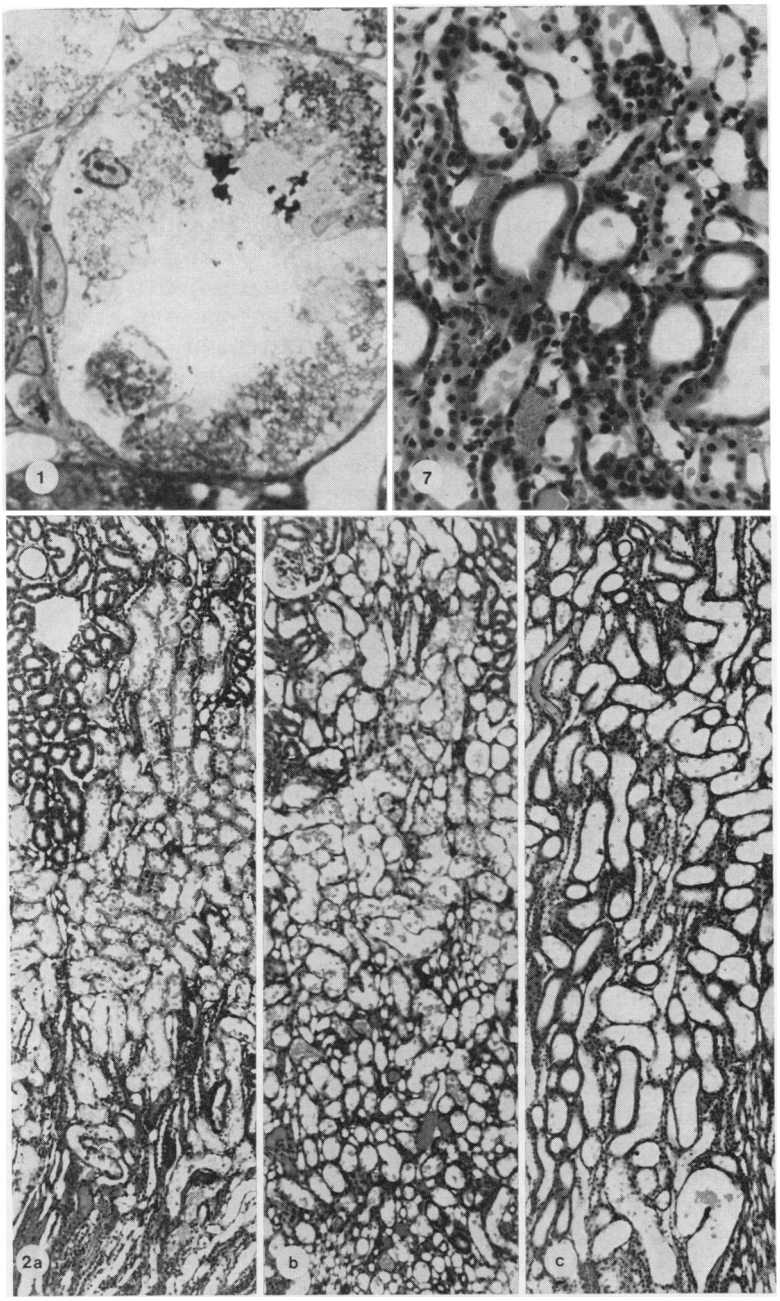

FIG. 1.—Kidney, first part of *pars recta* showing fragmenting and autolysing dead cells and surviving flattened epithelial cell. 36 h after $HgCl_2$. Epon-embedded, toluidine blue. × 1300.

FIG. 2.—Kidney, proximal convoluted tubules after 1·5 mg $HgCl_2$/kg. *A*. At 48 h, few flat epithelial cells remain near the viable cortex and at the terminal part of the *pars recta*, the intervening zone being free of epithelial cells. *B*. At 3 days, flat cells have now extended along the tubules from either end but mainly from the distal end; the intervening zone is narrowed but still free of cells. *C*. At 5 days, flat epithelial cells now completely reline the tubules, which are now dilated. The epithelial cells are beginning to develop their normal structure. A few casts are present in the distal part of the *pars recta* between the vascular bundles on the 3rd day (*B*). H. E. × 80.

FIG. 7.—Kidney, distal part of *pars recta* showing papillary-like proliferations of the tubular epithelium associated with some degree of obstruction to the passage of cell debris. 9 days after $HgCl_2$. H.E. × 200.

Microscopic findings

The tubulonecrotic lesion after an s.c. dose of 1·5 mg $HgCl_2$/kg and the onset of regenerative changes in these rats has been recorded (Haagsma and Pound, 1979). The maximum extent of involvement was attained between 8 and 16 h after injection. The very terminal part of the *pars recta* immediately before the descending limb of the loop of Henle was occasionally spared but the cells became flattened and basophilic by 36 h. By 24 h the dead cells were in an advanced stage of autolysis, fragmentation and dissolution, and numerous dead cells, fragments of cells, sometimes membrane-bound, and debris were shed into the lumen of the tubules. Many fragments of dead cells, adherent to the basement membrane, persisted in decreasing amounts for up to 9 days. No disruption of the basement membranes was detected.

In the affected parts of the tubules a few very flattened epithelial cells survived lying against the basement membrane, often underlying remnants of dead cells. These cells, at 36 h, had basophilic cytoplasm containing few organelles and a round or oval nucleus with a prominent nucleolus or sometimes 2 nucleoli (Fig. 1). The more cuboidal cells occasionally retained a small tuft of sparse microvilli. Occasional cells contained phagocytosed debris. They were seen in two areas: (1) a narrow zone immediately distal to the proximal surviving segment of the proximal convoluted tubules, and (2) a broader zone at the terminal end of the *pars recta*. Between the two zones they rapidly became less frequent and up to 36 h most profiles of the *pars recta* were devoid of them. Cells, with vacuolated cytoplasm but with more or less normal nuclei that possibly were still viable were occasionally present lying free in the lumen of the tubules. The squamoid cells appeared to arise from epithelial cells which had cast off superficial necrotic parts of the cytoplasm to leave the nuclei with a small rim of cytoplasm attached to the basement membrane (Haagsma and Pound, 1979; Fig. 7).

Tubular regeneration

The necrotic segments were repopulated with flat deeply basophilic epithelial cells which extended from the two areas, particularly from the distal area, in such a way that the cell-free area in between became smaller, and the tubules were completely lined between the 3rd and 5th day (Fig. 2*a–c*). The proliferative activity occurred at different times and intensities in different parts of the nephrons so that it is convenient, for the purposes of this description, in the outer stripe of the medulla, to speak of a narrow *juxta-cortical viable zone* in which no cells were dead, a very narrow *juxta-cortical necrotic zone* in which there were a few surviving squamoid cells, and the *necrotic zone* with no surviving cells, an *inner necrotic zone* where there were a few surviving cells and a broad *junctional zone* which comprised the very terminal parts of the *pars recta* in which more surviving cells were present, sometimes apparently little affected in the first 24 h but which became squamoid by 36 h. In these zones the counts refer to proximal convoluted tubules. [^{3}H]-Thymidine and mitotic indices are set out in Table I and Figs 3*a–c* and 4*a* and *b*. Thymidine and mitotic indices in the normal kidney are very low and lowest in the papilla.

During the 1st 16 h the thymidine indices of the tubular cells in the cortex and outer stripe of the medulla were decreased and in the necrotic zones no labelled cells were seen in the first 36 h. The mitotic indices in these zones showed no significant change. No mitoses were seen up to 48 h in the *necrotic zone*

An increase in the thymidine index was first seen in the cortex and *juxta-cortical viable zone* 24 h after injection. In these zones the mitotic index was increased at 36 h, reached a maximum after 2–3 days and later decreased, although it was still elevated 7 days later. The increase was a little greater in the narrow *juxta-cortical viable zone*, although the cells retained their normal morphology. Although proximal and distal convoluted tubules in the

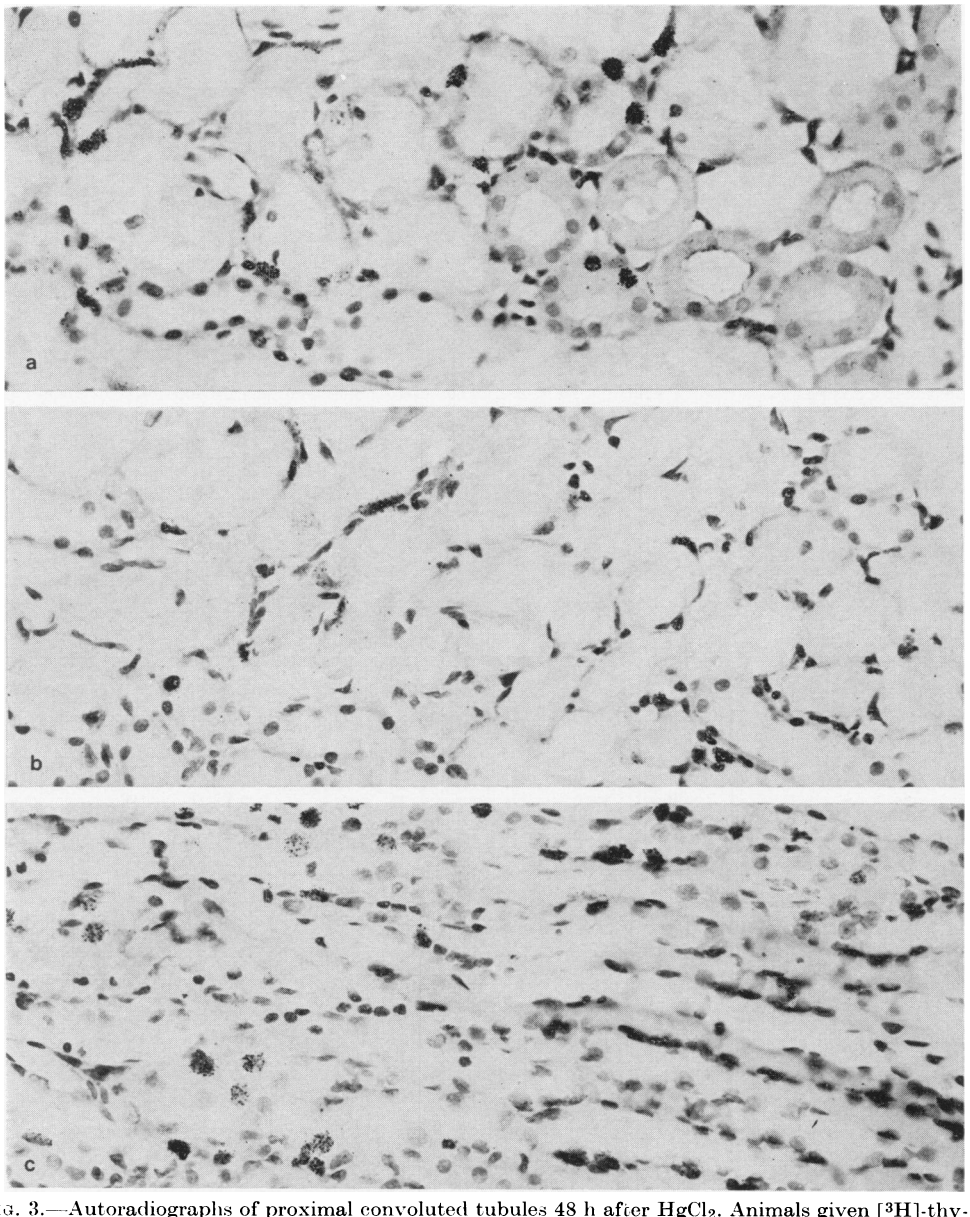

FIG. 3.—Autoradiographs of proximal convoluted tubules 48 h after $HgCl_2$. Animals given [^{3}H]-thymidine 1 h before death. *A*. Labelled cells are present in small numbers in the surviving tubules of the cortex near the necrotic zone and in a few cells of the adjacent juxta-viable necrotic zone. *B*. Very few cells are labelled in the necrotic zone and these are probably interstitial cells. *C*. Numerous cells are labelled in the terminal part of the *pars recta* (junctional zone). × 300.

cortex were not counted individually the increases involved both.

The thymidine index of the surviving squamoid cells was increased at 36 h in the *juxta-cortical necrotic zone*. Only at 48 h were labelled cells seen in the *necrotic zone* and the thymidine indices increased in the *inner necrotic zone*, but the indices increased abruptly to very high levels, reaching a maximum on the 3rd day. A similar abrupt increase in the thymidine index, starting at 36 h, occurred in the broad

TABLE I.—*[^3H]-Thymidine and mitotic indices in rat kidneys at different times after injection of $HgCl_2$*

Zone	Index	Control	Time after injection of $HgCl_2$ (hours) 4	6	8	16	24	36	48	72	5×24	7×24
Cortex	[^{3}H]	17±3	21	19	6	9	59	73	280	185	139	63
	M	2±1	2	2	2	1	3	14	22	15	17	7
Outer stripe of outer zone of medulla												
Juxta-cortical viable zone	[^{3}H]	19	17	9	9	9	39	900	650	1250	704	—
	M	1	1	0	0	1	1	67	120	196	53	—
Juxta-cortical necrotic zone	[^{3}H]	19	7	9	3	0	0	50	1250	3200	1350	550
Necrotic zone	[^{3}H]	16	0	0	0	0	0	0 (50)	500	2270	1150	370
	M	2	0	0	0	0	0	0 (13)	4 (14)	630	136	49
Inner necrotic zone	[^{3}H]	16	7	0	0	0	0	0 (30)	1800	4500	1400	350
Junctional zone	[^{3}H]	18	17	17	11	5	8	300	3500	3900	1300	410
	M	2	0	1	0	0	0	14	295	450	45	23
Inner stripe of outer zone of medulla	[^{3}H]	19	18	20	17	16	11	80	1400	1280	740	285
	M	1	0	2	1	1	1	3	55	155	55	22
Inner zone of medulla	[^{3}H]	1	2	3	3	2	1	19	120	314	103	50
	M	0	0	0	0	0	1	3	22	30	17	3

Figures in brackets refer to ascending loops.

junctional zone. Mitotic indices of the squamoid cells in these zones increased at about the same times and followed a similar course although in general the maximum values occurred about a day later. After 7 days the thymidine and mitotic indices were still elevated, and remained higher than normal, particularly in the necrotic zones, for up to 28 days.

The thymidine and mitotic indices also increased in the cells of the inner stripe of the outer medulla and in the papilla from 36 h, reaching a maximum on the 2nd or 3rd day. These patterns of increase follow those of the cells of the cortex, rather than of the necrotic zones. The increases involved all types of tubules, which were not counted individually, but in the papilla few labelled cells could be recognized in collecting ducts. It is also of note that no change in the very scanty number of cells that take up thymidine in the glomerular tufts was found, nor were any mitoses seen. An occasional cell of Bowman's capsule was observed to be labelled.

In rats of Experiment 2 given a single dose of [^{3}H]-thymidine at 35 h, labelled cells were practically confined to cells of the *juxta-cortical viable zone*, a few cells in the *juxta-cortical necrotic zone* and cells in the *junctional zone* (Table I); none were found in the intervening segment. From the 48–54h stage labelled cells were found in all the regenerating segments, although not all cells were labelled. These cells had most probably been derived from the adjacent labelled segments. The occasional large granular, apparently viable, cells previously noted lying free in the lumen of the necrotic tubules had also sometimes taken up thymidine, suggesting that they might play a part in colonizing the necrotic tubules.

Characteristics of the repopulating cells

The proliferative stimulus, obviously, is very intense on the 2nd and 3rd days and it is clear that the source of the repopulating cells is mainly from surviving squamoid cells at the distal end of the *pars recta* and to a lesser extent from cells at the proximal end. Sections from the edge of the proliferating zone when active extension along the tubules is occurring on the 3rd day show that the density of nuclei in cross-sections of the regenerating tubules is greater than the density of nuclei in longitudinal sections suggesting

that the cells slide along the basement membrane of the tubules lengthwise resembling the manner of peripheral extension of cells in a tissue culture.

The newly proliferated epithelial cells were without microvilli, *i.e.* at 3 days. Over the next 11 days the cells gradually became taller, developed a round nucleus, cytoplasmic organelles and brush borders characteristic of normal tubular cells, while the cytoplasmic basophilia decreased as the cells matured. Considerable variation was present between different tubules, along the length of tubules, and between cells in the same tubular profile; *e.g.* almost normal tubular cells with brush borders were seen occasionally adjacent to flat basophilic cells in the same profile even after 11 days. The "maturation" was always more advanced at both ends but particularly the distal end of the regenerated segment. The cytoplasmic basophilia decreased gradually but was evident up to 28 days.

The mean diameters of tubules in the *pars recta* of control rats, measured in H.E.-stained sections of perfusion-fixed material between basement membranes, was $42{\cdot}7 \pm 3{\cdot}7$ μm. At 24, 36, 48 and 72 h after $HgCl_2$ the diameters were not significantly changed (Table II), although the lumens were enlarged because of loss of cells and the flat regenerated epithelium. The diameters were significantly enlarged from the 5th to 11th day and the lumens were enlarged because of this and the flat character of the cells in most tubules. Even after 28 days the tubules had not returned to normal size. On the other hand no change in the diameter of the surviving cortical parts of the proximal tubules, apart from the atrophy dealt with below, was found at any stage. The number of nuclei per profile in the regenerating tubules was greater than normal from the 5th to the 11th day (Table II) and was still increased at 28 days, that is, the increase appeared to be in proportion to the diameter of the tubules. At later stages when most tubules had returned to normal occasional dilated profiles persisted, or became more dilated and lined by attenuated epithelial cells.

TABLE II.—*Diameters of profiles of* pars recta *and number of nuclei per profile at different times after* $HgCl_2$

	Diameter μm	No. of nuclei
Controls	$42{\cdot}7 \pm 3{\cdot}7$	$7{\cdot}3 \pm 1{\cdot}0$
24 h	$42{\cdot}4 \pm 2{\cdot}7$	—
36 h	$41{\cdot}7 \pm 4{\cdot}3$	—
48 h	$44{\cdot}6 \pm 6{\cdot}3$	—
72 h	$43{\cdot}0 \pm 5{\cdot}8$	n.r.
5 days	$53{\cdot}9 \pm 5{\cdot}1$	$11{\cdot}7 \pm 1{\cdot}4$
7 days	$56{\cdot}7 \pm 4{\cdot}1$	$13{\cdot}3 \pm 1{\cdot}3$
9 days	$51{\cdot}1 \pm 5{\cdot}3$	$17{\cdot}0 \pm 2{\cdot}3$
11 days	$51{\cdot}0 \pm 4{\cdot}9$	$15{\cdot}3 \pm 2{\cdot}6$
28 days	$47{\cdot}0 \pm 4{\cdot}1$	$8{\cdot}7 \pm 1{\cdot}4$

— None to count.
n.r. = Too irregular to count at this stage.
25 profiles at random were measured at each stage, usually from 2 rats. Nuclei were counted only in more or less circular profiles.

Tubular collapse

On the 5th day small groups of collapsed proximal tubular profiles surrounded by a small collection of lymphocytes were seen at all levels in the cortex but predominantly in the subcapsular zone (Figs 5 and 6). These lesions became more prominent with time. It became obvious that many of the profiles were actually sections through the convolutions of the same collapsed proximal tubule as it descended towards the medulla. By the 7th day a few collapsed glomeruli were seen usually closely associated with or lying within foci of proximal tubular collapse and lymphocytic infiltration. These glomeruli appeared hypercellular, had few patent capillaries which usually contained a few retained red cells, and a partly collapsed or even absent Bowman's space. The proportion of affected glomeruli did not change with time. After the 11th day collapsed ascending thick limbs of the loops of Henle were also seen traversing the outer zone of the medulla and the cortex.

It seems obvious from their frequent association that these lesions all involve the same nephrons. The collapsed tubules later atrophied and became surrounded by

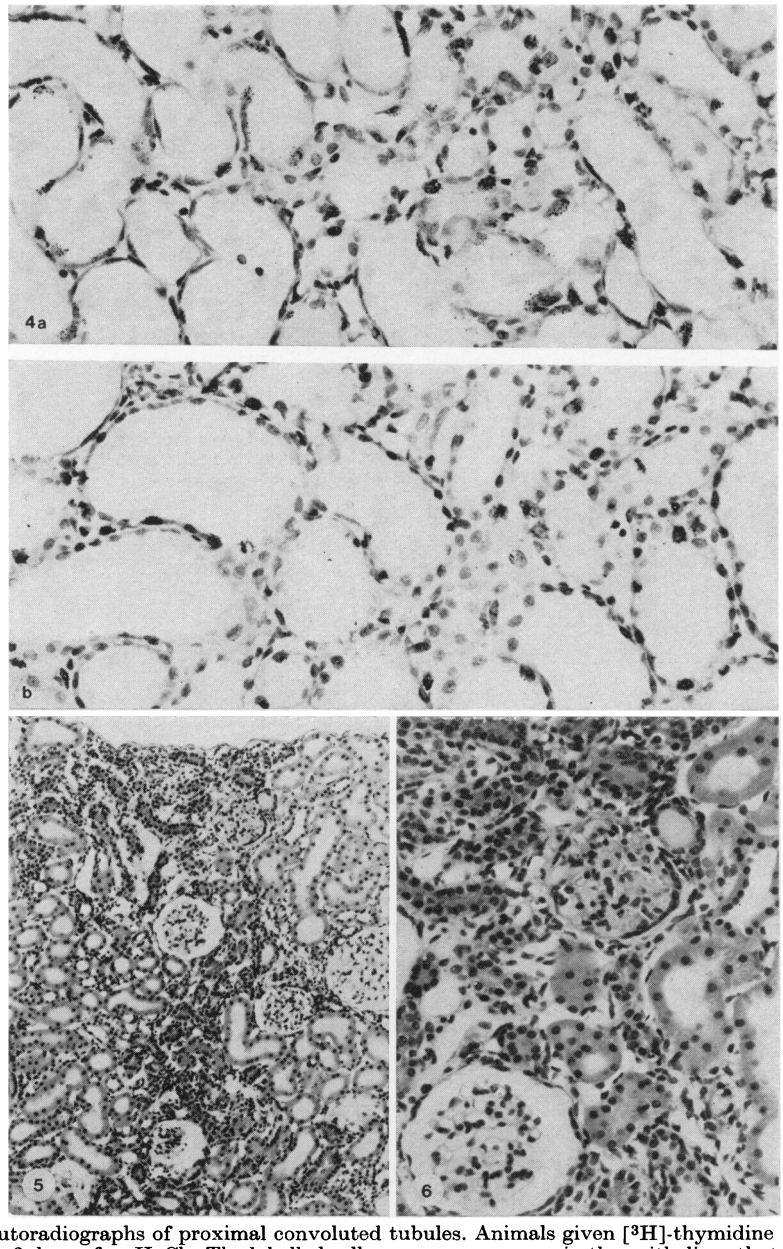

FIG. 4.—Autoradiographs of proximal convoluted tubules. Animals given [^{3}H]-thymidine 1 h before death. *A*. 3 days after $HgCl_2$. The labelled cells are now numerous in the epithelium that is repopulating the necrotic zone. *B*. 5 days after $HgCl_2$. Numerous labelled cells are still present in the epithelium that completely lines the now dilated tubules. × 240.

FIGS. 5, 6.—Kidney, focus of collapsed proximal tubules with early interstitial fibroblastic proliferation and persistent mild lymphocytic infiltration, with corresponding focal depression on the surface. An altered glomerulus with few patent capillaries and loss of Bowman's space is present and can be compared with an adjacent normal glomerulus. Collapsed tubules are present at various levels and probably represented convolutions of the same tubule as it passes through the cortex. 13 days after $HgCl_2$. H.E. Fig. 5 × 120. Fig. 6 × 200.

connective tissue with some lymphocytic infiltration, producing the small scars on the surface of the kidneys noted on gross examination. By the 112th day some of the collapsed tubules had broken up into nests or small strands of cells in fibrous connective tissue and the glomeruli were reduced to shrunken eosinophilic masses with a few hyperchromic nuclei, no visible capillaries and no Bowman's capsule. Very occasionally, segments of tubules became dilated and lined by attenuated epithelium.

Irregular cell proliferations

The majority of the tubules were reconstituted to normal. Epithelial cell proliferation was particularly active in the junctional zone from 3 to 5 days (Table I), especially in those nephrons between the vascular bundles where the epithelium piled up in the lumen even, in isolated tubules, to the extent of actual occlusion. These proliferations tended to regress by the 7th day and were no longer present after 9 days.

In a small number of nephrons the proliferating epithelium formed irregular masses that projected into the lumen of the tubules. These projections varied in size from a few cells (Fig. 7) to larger papillary masses (Fig. 7). These lesions occurred most frequently in the juxtamedullary zone, where epithelial proliferation was most active, and were most prominent between the 5th and 9th days and in some cases caused a banking up of necrotic debris (Fig. 7). By the 18th day they were seldom seen. In other situations solid cords of epithelial cells appeared to project into the interstitial tissue at about the same time, *i.e.* from the 3rd to the 5th days, and then started to atrophy.

Although fragmentation of the basement membranes, as described by Cuppage and Tate using a larger dose of $HgCl_2$ (1968), was not seen at any stage, in the areas of active proliferation the basement membranes became thinned and duplicated or even triplicated, as though split; but this is more likely to be associated with active epithelial proliferation and laying down of new basement membranes rather than being an initial effect of the action of $HgCl_2$.

Casts

The appearance of casts in the urine in small numbers at 24 h, rising to a peak at 2–5 days and then declining, has been recorded (Haagsma and Pound, 1979). In kidney sections, a number of loose granular casts were seen at 24 h, mainly in distal convoluted tubules and collecting ducts. At 36 h casts were present in all tubules distal to the *pars recta* and large amounts of debris were present in the *pars recta*, which banked up by 48 h to form dense aggregates in the junctional zone. By the 3rd day less debris was present in the *pars recta* and the casts were much denser in the junctional zone particularly between the vascular bundles (Fig. 2). By the 5th day the number of tubular casts was reduced.

Inflammatory responses

A minor degree of margination of leucocytes and lymphocytes in arterioles, peritubular capillaries and *vasa recta* with migration of very small numbers of these cells into the interstitial tissue in the necrotic zone was seen from 24 to 36 h but regressed in 5 days to be replaced by a few monocytes and lymphocytes.

A few small collections of lymphocytes remained around collapsed segments of proximal tubules at all levels of the cortex. By the 7th day young vascular connective tissue with some lymphocytes began to form at these sites and also about the irregular proliferations growing into the tubular lumen. Such foci were seen at all levels of the cortex and persisted to give rise to foci of connective tissue.

DISCUSSION

Twenty-four hours after a dose of 1·5 mg $HgCl_2$/kg the major part of the *pars recta* of the proximal convoluted tubules was necrotic (Haagsma and Pound, 1979).

The dead cells underwent autolytic dissociation and dissolution but many cells, especially at either end of the necrotic zone, appeared to break up into membrane-bound fragments. The debris of the dead cells passed into the lumen and was passed in the urine.

A few very flat epithelial cells survived at both ends of the necrotic segment adjacent to relatively non-affected segments of the nephrons but were seldom identified in the intervening area. These cells appeared to arise from tubular epithelial cells that had cast off dead fragments of cytoplasm into the lumen leaving surviving basal parts of the cell, containing the nucleus, adherent to the basement membrane, where they rapidly became extended lengthwise along the tubules. The characteristics of these cells, also described by others (Cuppage and Tate, 1967; Cuppage *et al.*, 1972; Siegel and Bulger, 1975) make it clear that they are functionally active in protein synthesis. A very occasional squamoid cell retained a small tuft of microvilli. The proximal zone of these cells was narrow but the distal zone was quite broad.

The tubular epithelium regenerated rapidly from these surviving squamoid cells. DNA synthesis followed by mitosis was seen first in the surviving squamoid cells in the zones at each end of the necrotic segment at 36 h and only 12 h later in cells in the intermediate zones where cells were not seen until 48 h. The increase in number of cells in DNA synthesis and in mitosis was quite abrupt in the necrotic zones, and reached very high levels that remained high for up to 5 days. The sequence of development of DNA synthesis and of subsequent mitosis in the various zones, the extension of labelled cells along the tubules from the 36th to 48th h, the lengthwise orientation of the cells and the greater density of nuclei in cross-section than in longitudinal section of tubules indicate that the regenerative process is one of proliferation and sliding of the cells along the tubules. The presence of an undamaged basement membrane is probably conducive to such a rapid process by providing a structural framework as was suggested (Oliver, 1953; Cuppage and Tate, 1967, 1968) and as is thought to be the case in the regeneration of other necrotic tissues such as muscle (Vracko, 1972), lung (Vracko and Benditt, 1972) and liver. The fact that the proliferating zone is much broader and more active at the distal end of the necrotic segment, and observations of the extension zones show that the tubular epithelium is regenerated mainly from cells in this zone, that is to say the growth proceeds mainly *up* the tubules. We have likened this to the peripheral extension of cells in a tissue culture, but similar sliding movement of epithelial cells is seen at the edges of healing wounds in various epithelia. The little affected cells in the tubules immediately adjacent to the necrotic area also had increased rates of DNA synthesis and mitosis greater than elsewhere in the unaffected kidney (see below), but there was no evidence that these cells contributed to the regeneration process except, perhaps, by conversion to squamoid cells.

The rapid regeneration and the high number of cells synthesizing DNA and in mitosis conform to previous accounts of the regeneration of the tubules (Cuppage and Tate, 1967; Cuppage, Cunningham and Tate, 1969; Cuppage *et al.*, 1972), but our findings differ in detail of the radioautographic observations and mitotic counts in the different zones, and in the suggested mode of origin of the surviving cells from which regeneration occurs. These authors imply the presence of surviving cells along the necrotic segments, which we have seldom found after this dose of $HgCl_2$. Only later were a few, apparently isolated, replicating cells found in this zone. We have indicated some observations to suggest the possibility that the tubules may be colonized by a few cells carried down from above in the glomerular filtrate, although we were not able to record observations to confirm this. Morphological and radioautographic

studies have led to other authors (Noltenius, Schellhas and Oehlert, 1963, 1964) to postulate that the tubular epithelium is regenerated from surviving cells at the end of the *pars recta*. On the other hand, after a small dose of $HgCl_2$ (0·6 mg/kg) a proportion of tubules are spared and regeneration of the affected tubules is more rapid, from cells along much of the tubule (Pound, unpublished results). Clearly the intensity of the initial injury determines the distribution of surviving cells.

The tubular epithelial lining was complete in 4–5 days and the squamoid cells gradually developed into cells of the normal size and characteristics of the area. Considerable variations in this development at any one time were seen in different parts of the tubules, zones nearest the viable tissue developing faster, but even in single profiles the presence of cells with brush borders adjacent to flat cells suggests that development may proceed stepwise. The persistence of deep basophilia for at least 10 days and in lesser degree for as long as 28 days, and the persistence of the elevated [^{3}H]-thymidine and mitotic indices indicate that, although the epithelial lining is complete within a very short time, epithelial cell turnover persists for a considerable time.

Probably associated with this is the dilatation of the regenerating segments of the tubules from the 3rd day. The dilatation thus started at the time when large casts accumulated at the beginning of the descending loops, which are narrow, and perhaps offered an obstructive element. However, it persisted long after most casts had been discharged. Moreover, it seems unlikely that the dilatation is a simple passive effect of an increased tubular pressure consequent on normal glomerular filtration into an obstructed tubule (Oliver, 1953; Siegel and Bulger, 1975) because the first parts of the proximal convoluted tubules, which were not involved in the necrosis, were not dilated. It seems likely that the dilatation is associated with the active proliferative activity, that is, it is a growth effect. Oliver (1953) considered that the obstructive effect of a cast might be disproportionately greater than suggested by its apparent size. Some casts persisted for a considerable time mainly in the loops of Henle and occasionally became clothed in an envelope of epithelial cells as has been noted previously (Sheehan and Davis, 1959) or appeared to become organized. It seems possible that such persistent casts could be related to collapse and atrophy of occasional nephrons.

While most tubules were reepithelialized to normal structures in orderly fashion, focal proliferations of epithelium, to form intratubular papillary lesions or in some cases appearing to form extensions into the interstitium as though tubules were budding, were seen. Such lesions have been recorded by others using $HgCl_2$, other nephrotoxic chemicals or ischaemia to induce renal tubular necrosis (Oliver, MacDowell and Tracy, 1951; Oliver, 1953; Cuppage and Tate, 1967, 1968; Siegel and Bulger, 1975) and have been ascribed to the influence of focal areas of disruption of basement membranes. We have not been able to demonstrate such disruption of basement membranes in our material up to 3 days. The focal proliferations occurred in the zones where epithelial proliferation was most exuberant, *i.e.* mainly in the junctional zones, and only very occasionally elsewhere. In these zones reduplication of basement membranes, or splitting, was seen but is more likely to be related to the proliferation, since it occurred with and not before this. It was not otherwise related to the focal proliferative lesions. First seen on the 5th or 7th day, soon after the tubular epithelial proliferation was most intense, they were seldom seen after 17 days and therefore must have regressed or become incorporated into the epithelium of collapsed tubules.

The relationship of retained casts and epithelial focal proliferations to collapse and atrophy of nephrons is not certain. Since nephrons appear to be affected as a

whole there must be a focal lesion, implying a mechanism whereby the glomerular blood supply is reduced. It has not been possible to identify such a lesion, but it is significant that the process involved predominantly tubules with subcortical glomeruli, that is, nephrons with short loops of Henle, and it is perhaps relevant that the densest and most persistent casts were seen in the junctional zone between the vascular bundles, that is, in tubules related to the short looped nephrons (Kriz, Schnermann and Koepsell, 1972), suggesting that perhaps an anatomical peculiarity contributes to the localization of the lesion. It may be commented that after a larger dose of $HgCl_2$ (2·5 mg/kg), dense often calcified casts are frequent up to 13 days mainly in proximal tubules of the junctional zone. These are associated with greater numbers of collapsed and atrophic tubules, and persistently dilated ducts with flat epithelia (Pound, unpublished data).

The rapidity of tubular epithelial regeneration has some general implications for any pathological process involving death of tubular cells, but there is evidence that the time sequence of events may be influenced by the toxic effect of Hg. Thus after 45 min of ischaemia to one kidney, proliferation of renal epithelium throughout the kidney reached a high maximum after 24 h (Haagsma, unpublished) and, following the tubular necrosis induced by glycerol (Finckh, 1960), in our experience the epithelial proliferation in the necrotic tubules started at 24 h and is similarly intense.

It is of interest that after local destruction of the *pars recta*, some proliferative activity occurs throughout the kidney. It is possible that the toxic agent does in fact injure cells in the kidney generally. Alternatively products of the dead tissue may stimulate other cells to proliferate (Cain, Egner and Redenbacker, 1976).

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