Cell Proliferation in Rat Kidneys After Prolonged Treatment With Lead

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Effects of chronic lead intoxication on cellular proliferation in rat kidneys were investigated by autoradiography. The rats were given intraperitoneal injections of lead acetate in aqueous solution for 6 months. At the end of this period, the proliferative activity of proximal tubular epithelial cells was about 15 times greater in rats given lead than in untreated controls. In the leaded rats approximately 40% of the proximal tubular cells contained intranuclear inclusions, approximately 0.5% of proximal tubular epithelial cells were labeled and approximately 6% of labeled cells contained intranuclear inclusions. Thus, cells with intranuclear inclusions can replicate DNA. Effects of chronic lead poisoning on the replication of proximal tubular cells in rats subjected to left uninephretomy before inception of treatment with lead were substantially the same. No renal carcinomas were found after 6 months of treatment with lead, but there was epithelial hyperplasia in some proximal tubules, with occasional atypia. The presence of increased synthesis of DNA and epithelial hyperplasia in the kidneys of rats chronically poisoned with lead suggests that the renal carcinogenicity of lead, observed by others, is related to lead-induced stimulation of renal cell proliferation (Am J Pathol 68:359–370, 1972).

IN A VARIETY OF ANIMAL SPECIES, chronic treatment with lead results in the formation of intranuclear inclusion bodies in proximal tubular epithelial cells of the kidneys.[1–4] In rats and mice, administration of lead for more than a year often induces renal neoplasms.[5–8] Little consideration has been given, however, to possible effects of lead on cell replication. Recently, we have demonstrated that a single injection of lead markedly stimulates DNA synthesis in rat kidneys within 2 days.[9]

This paper deals with three questions about the effects of prolonged administration of lead on rat kidneys: What is the state of cell proliferation in the tubular epithelium after lead has been administered for 6 months? Do renal tubular cells that contain intranuclear inclusions display signs of nuclear activity? Does the absence of one kidney influence proliferative activity of the tubular epithelium in the remaining kidney after chronic poisoning with lead?

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We have found, by autoradiography, that the uptake of ^{3}H-thymidine is significantly greater in the proximal tubular epithelium of rats treated with lead for 6 months than it is in the proximal tubular epithelium of controls, and that renal epithelial cells with characteristic intranuclear inclusions can synthesize DNA. We have also noted the development of focal epithelial hyperplasia, sometimes atypical, in segments of proximal tubules.

Materials and Methods

Adult female Sprague-Dawley rats (Chordata Corp, Rochester, NY), weighing 210 to 240 g were used. The rats were housed individually in a room with 12-hour light–dark cycles, and given Purina rat chow and tap water *ad libitum*.

Experimental groups

Group A (Controls). Six control rats were not treated during the experimental period of 6 months.

Group B (Leaded Group). Eighteen rats were injected intraperitoneally once a week with lead acetate in sterile water, at doses of 1 to 7 mg lead per rat for 6 months. Injections were skipped when the rats appeared lethargic.

Group C (Uninephrectomized Group). The left kidney was removed from each of 12 rats at the beginning of the experimental period. The kidney was removed under ether anesthesia, through a dorsolateral subcostal incision. These rats were not treated further.

Group D (Uninephrectomized and Leaded Group). Twelve rats were uninephrectomized as was Group C, and were injected with lead acetate, as was Group B, for 6 months.

Labeling and Autoradiography

The rats in the four experimental groups were sacrificed after 6 months. One hour before sacrifice, all rats were injected intraperitoneally with ^{3}H-thymidine (Specific Activity 15.2 Ci mM, Schwarz Mann, Inc, Orangeburg, NY) in doses of 0.2 μCi g body weight. Kidneys were excised and weighed after their capsules were removed. A central slice of each kidney, obtained by cutting through the long axis, was fixed in Carnoy's fluid, embedded in paraffin and sectioned at 4 μ. Deparaffinized sections mounted on slides were dipped into NTB2 nuclear track emulsion (Eastman Kodak Co. Rochester, NY), exposed for 2 weeks, developed in D-19 Kodak developer, fixed, washed and stained with hematoxylin and eosin.

Scoring of Cells

Epithelial cells in proximal tubules were classified as labeled, unlabeled and cells in mitosis. Each class of cells was further divided into cells with and without intranuclear inclusions. At least 10,000 cells were differentially scored for each animal, using an oil immersion objective.

Renal Lead Content

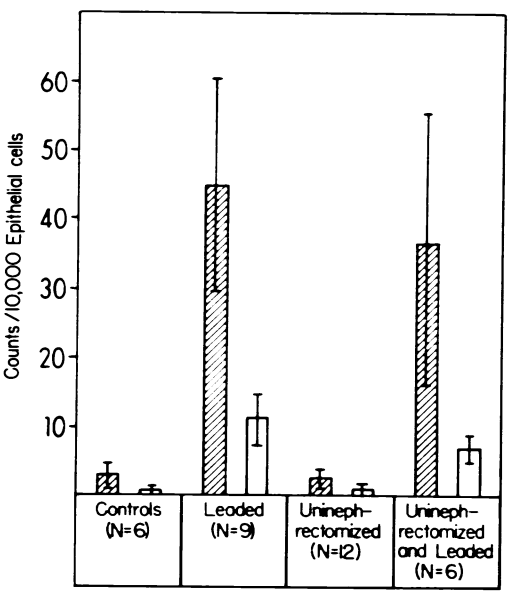
Renal tissue was weighed, rinsed in 0.9% NaCl solution and digested in a 3:2 mixture of concentrated nitric and perchloric acids on a Kjeldahl digestor. After appropriate dilution with a solution of 0.5% $LaCl_3$, lead content was determined in a Jarrel-Ash atomic absorption spectrophotometer.

Results

The results of differential scoring of the proximal tubular epithelial cells for each experimental group are given in Table 1 and Text-figure 1. The amounts of lead in the kidneys are shown in Text-figure 2. In the statistical analysis the 1% level ($P < 0.01$) in Student's t test was taken to indicate significant differences.

Effects of Prolonged Treatment with Lead on Renal Cell Proliferation

Nine of the 18 rats treated with lead survived for 6 months. At the time of sacrifice, blood smears showed basophilic stippling of some erythrocytes in all nine rats. The mean wet weight of kidneys was about 10% higher than that of kidneys from controls. After treatment with lead for 6 months, the mean labeling index of the epithelial cells in the proximal tubules was 45/10,000 cells (Text-figure 1). The uptake of ^{3}H-thymidine was approximately 15 times greater in the leaded rats than in controls ($P < 0.01$). ^{3}H-thymidine labels were found predominantly over cells without intranuclear inclusions. About 6% of the labeled cells also contained intranuclear inclusions (Table 1 and Figure 1A, B). A few cells in mitosis were suspected of containing intranuclear inclusions, but this has yet to be verified.



TEXT-FIG 1—Proliferative activities in epithelia of proximal tubules of kidneys in 4 experimental groups. The counts of labeled cells ±SD (*hatched columns*) and mitoses ±SD (*open columns*) are shown per 10000 epithelial cells.

Histologically, the nuclei of the epithelial cells in the proximal tubules were often enlarged and irregular in shape, but frequently exhibited prominent basophilic nucleoli even when they contained inclusions (Figure 2). About 40% of proximal tubular cells contained recognizable intranuclear inclusion bodies (Table 1) which were characteristically eosinophilic. Sometimes there were two or more inclusions in one nucleus. The epithelial cells of distal tubules contained no intranuclear inclusions but were labeled more frequently in leaded rats than in controls.

Occasionally, epithelial hyperplasia was found in segments of proximal tubules of rats treated with lead (Figure 3). These foci were easily distinguished from normal surroundings by the hyperbasophilic cytoplasm of the cells and by the increase in number of epithelial cells in the affected tubules. The nucleo-cytoplasmic ratio of the hyperplastic tubular cells was greater than normal. Some tubular cells in areas of hyperplasia were labeled, and some others contained intranuclear inclusions. Here and there, the hyperplasia of proximal tubular epithelial cells was distinctly atypical (Figure 4). Renal carcinomas were not found. In leaded rats, there was no evidence of frank renal necrosis, but desquamated cells were present in the lumen in some of the renal tubules. The renal content of lead was significantly ($P < 0.01$) higher in rats given lead than in controls (Textfigure 2).

Effects of Uninephrectomy on Cell Proliferation in the Remaining Kidney After 6 Months

All uninephrectomized rats survived for 6 months. At the time of sacrifice, the mean wet weight of the remaining kidney was approxi-

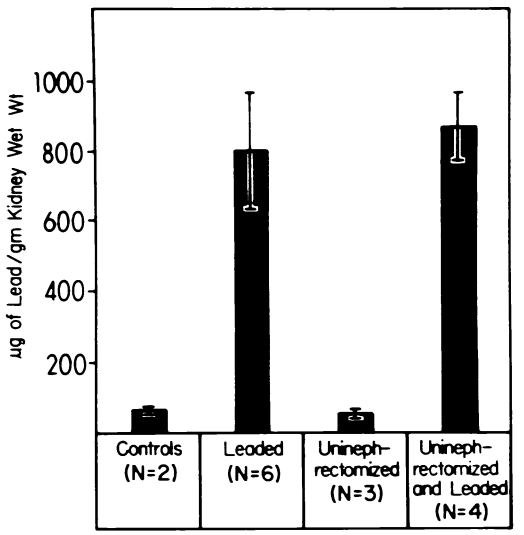
Table 1—Mean Labeling Indices of 4 Experimental Groups*

Experimental group	No. rats	No. kidneys	Labeled cells without inclusions†	SE	Labeled cells with inclusions†	SE	Cells with inclusions†	SE
Controls	6	12	3.0 ± 1.8	0.7	0	—	0	—
Leaded	9	17‡	42.1 ± 13.8	4.6	2.7 ± 2.2	0.7	4047 ± 273	91
Uninephrectomized	12	12	2.6 ± 1.5	0.4	0	—	0	—
Uninephrectomized and leaded	6	6	34.5 ± 20	8.1	2.2 ± 1.3	0.5	4216 ± 370	151

* All animals were labeled with ^{3}H-thymidine for 1 hour

† The counts are per 10,000 epithelial cells of the proximal tubules in the kidney ± SD

‡ Both kidneys were counted in all rats but one



TEXT-FIG 2—Mean lead content of kidneys from 4 experimental groups, determined by atomic absorption analysis of acid digests. Amount of lead is expressed in µg lead/g wet kidney weight ± standard deviation.

mately 30% heavier than that of controls, presumably because of compensatory renal hyperplasia and hypertrophy.[9–11]

Six months after uninephrectomy, the mean labeling index of the proximal tubular cells in the remaining kidneys was not significantly different from that in control kidneys (Text-figure 1). The tubular lumina appeared somewhat enlarged compared with those in controls. The lead content of kidneys from uninephrectomized rats was about the same as that of kidneys from untreated controls (Text-figure 2).

Effects of Prolonged Treatment with Lead on Cell Proliferation in the Remaining Kidneys of Uninephrectomized Rats

Six of the 12 uninephrectomized rats that were treated with lead survived for 6 months. At autopsy the mean wet weight of the remaining kidneys was approximately 50% heavier than that of kidneys from untreated controls, and approximately 20% heavier than that of remaining kidneys from uninephrectomized unleaded rats. The mean labeling index in this group was 37/10,000 proximal tubular cells (Text-figure 1), and the labeling activity and the renal content of lead were significantly greater than in untreated controls and in uninephrectomized unleaded rats ($P < 0.01$). However, the proliferative activity of the proximal tubular epithelium in this group was not significantly different from that in the leaded group without uninephrectomy.

Approximately 6% of the labeled proximal tubular epithelial cells contained intranuclear inclusions (Table 1). Histologic changes in the remaining kidneys of this group were similar to those in kidneys of leaded rats without uninephrectomy. As in the case of leaded rats with both kidneys, epithelial cells of hyperplastic tubules showed hyperchromatic nuclei and hyperbasophilic cytoplasm. There were no renal carcinomas.

Discussion

The present study demonstrates that proliferation of proximal tubular epithelial cells was significantly greater in rats treated with lead for 6 months than in untreated controls. Moreover, the prominence of nucleoli in many cells of the tubular epithelium in the leaded rats may well be an indication of increased RNA metabolism associated with increased DNA replication.[12]

We have reported previously that a single dose of lead (0.04 mg lead/g body weight) stimulates DNA synthesis in rat kidneys within 2 days, without producing morphologically discernible cell damage.[9] This suggests that in chronic, as in acute, lead poisoning, renal tubular cell proliferation may be induced by lead. However, the proliferative activity in the course of chronic lead poisoning could in part be related to regeneration that follows shedding of damaged epithelial cells, although desquamated cells were only infrequently seen after 6 months of treatment with lead. It is unlikely that the increased uptake of ^{3}H-thymidine in the chronically leaded rats was related to repair of damaged DNA, because in the present experiments the mitotic indices of proximal tubular epithelial cells were comparable to the labeling indices (Text-figure 1) if one considers that the number of cells in mitosis is proportional to the duration of the mitotic phase and that in rat kidneys the mitotic phase is about a quarter of the synthetic phase.[13] Therefore, the increase in ^{3}H-thymidine uptake in the leaded rats appears to be a manifestation of genuine DNA replication involving cell division. The labeling activity in proximal tubular epithelium did not differ significantly between Group B (leaded) and Group D (uninephrectomized and leaded). This indicates that uninephrectomy had little or no effect on renal cell proliferation after 6 months. The mean labeling index of proximal tubular epithelial cells in control kidneys was low compared to the labeling index we have previously reported for 4-month-old rats, presumably because of the significantly greater age (approximately 10 months) of the controls in the experiments here reported.

An interesting finding of the present study was that in leaded rats a small number of proximal tubular cells with intranuclear inclusions was distinctly labeled by ^{3}H-thymidine (Table 1). This is evidence that cells with intranuclear inclusions can synthesize DNA. Yet the presence of intranuclear inclusions appears to be unfavorable for cell replication. In leaded rats approximately 40% of all proximal tubular epithelial cells contained intranuclear inclusions, whereas only 6% of labeled cells contained inclusion bodies. It should be noted, however, that small intranuclear inclusions may not be recognized by histologic examination. The intranuclear inclusion bodies have been reported to contain protein as well as lead.[3,14]

Zollinger [5] showed that lead is carcinogenic in rat kidneys. Others [6–8] have reported that ingestion of lead in the diet for a year or more induces renal neoplasm in rats and mice. Some have proposed that this carcinogenic effect of lead results from inhibition of mitosis in the kidney.[7] The findings presented here suggest strongly that it is not the inhibitory but the stimulatory potential of lead on renal cell replication which is related to renal carcinogenesis.

Chemical carcinogens, such as 2-acetylaminofluorene (2-AAF) and ethionine have been shown to stimulate proliferation of hepatocytes in rats and to produce hyperplastic nodules in the liver.[15–16] These reports, as well as other evidence,[17–19] suggest that increased cell proliferation in the target tissue during the latent period is an essential biologic feature of carcinogenesis. Therefore, the significant increase in proliferative activity of renal tubular epithelial cells after chronic treatment with lead is consistent with experimental data on the effects of other chemical carcinogens. Since a single injection of lead stimulates proliferation of renal tubular epithelial cells in rats,[9] it is likely that the increased proliferative activity observed in the chronically leaded rats lasted for the entire period of 6 months.

We did not find neoplasms in the kidneys of rats treated with lead for 6 months, presumably because the experimental period was not long enough for the production of neoplasia.[6–7] However, the spotty presence of epithelial hyperplasia in the proximal tubules, sometimes atypical, may be indicative of the development of a preneoplastic state. The concept of preneoplasia is supported by studies on hepatocarcinogenesis. Hyperplastic nodules in rat liver, induced by 2-AAF or by aflatoxin B, appear to be sites in which hepatomas originate.[15]

Perhaps sustained stimulation of mitosis in renal tubular cells by lead was the cause of the observed hyperplasia. If so, persistent inter-

action of lead with tubular epithelial cells may on occasion produce an enduring alteration in the regulation of cell replication that gives rise to neoplastic transformation.

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Acknowledgments

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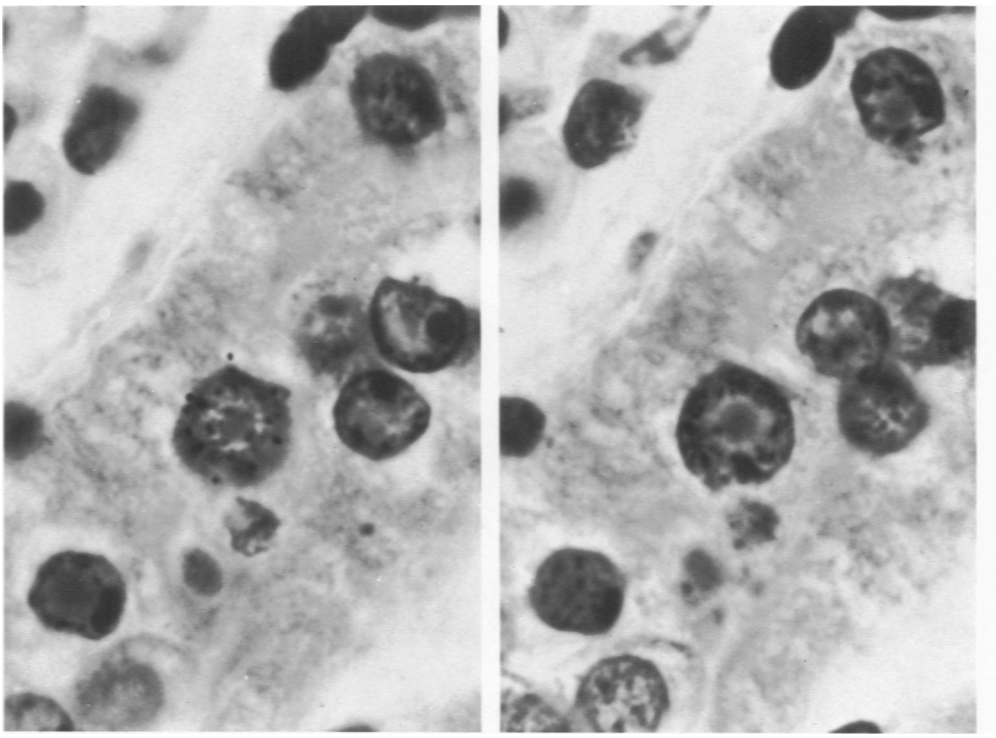
[*Illustrations follow*]



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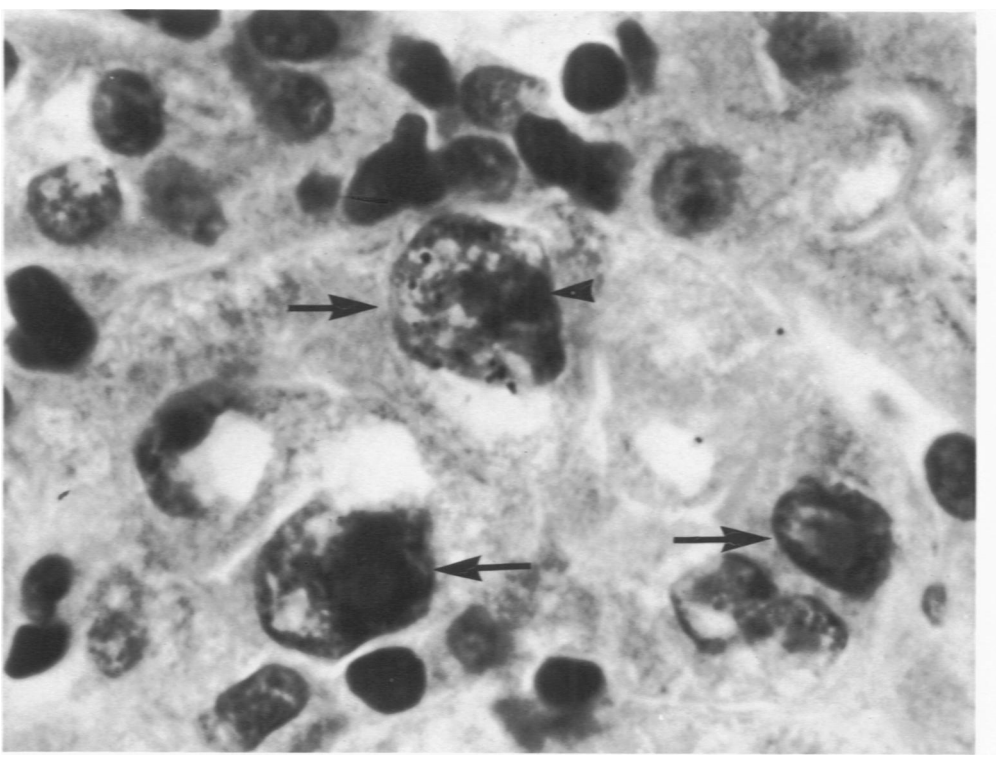

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Fig 1—Proximal convoluted tubule of rat given lead during 6 months. Labeled nucleus of tubular epithelial cell with inclusion body, focused in **A** to show exposed silver grains of photographic emulsion, and in **B** to show intranuclear inclusion body (H&E, × 1700). **Fig 2**—From rat given lead for 6 months. Three proximal tubular epithelial cells (*arrows*) with intranuclear inclusion bodies. One of the nuclei is also labeled by ^{3}H-thymidine (*grains*). Note nucleolus (*arrowhead*) in the labeled nucleus (H&E, × 1700).

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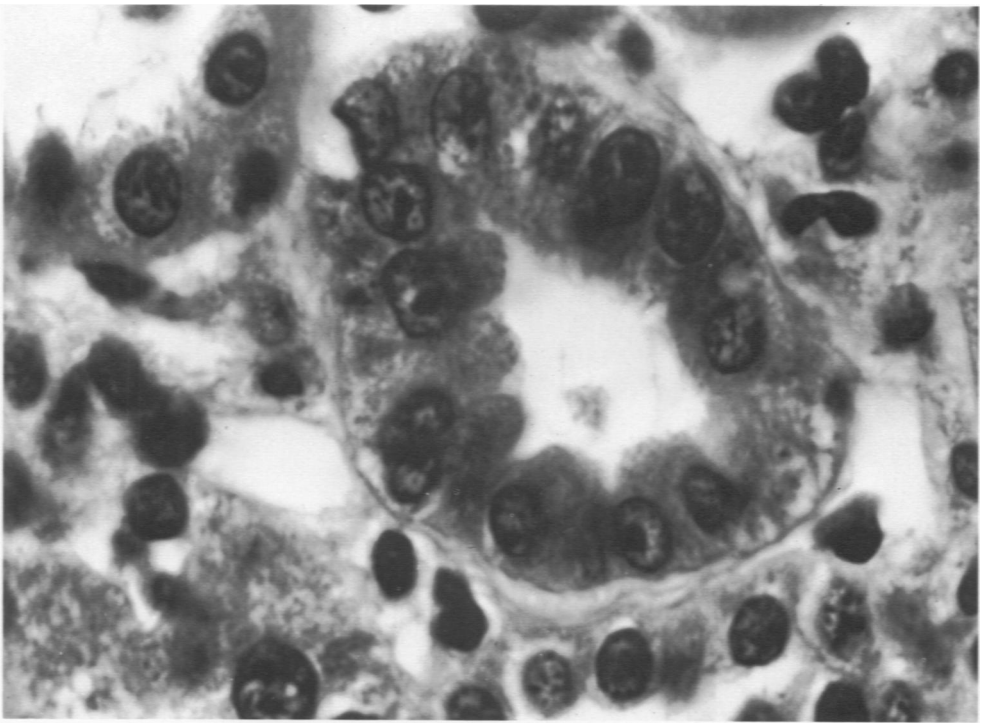

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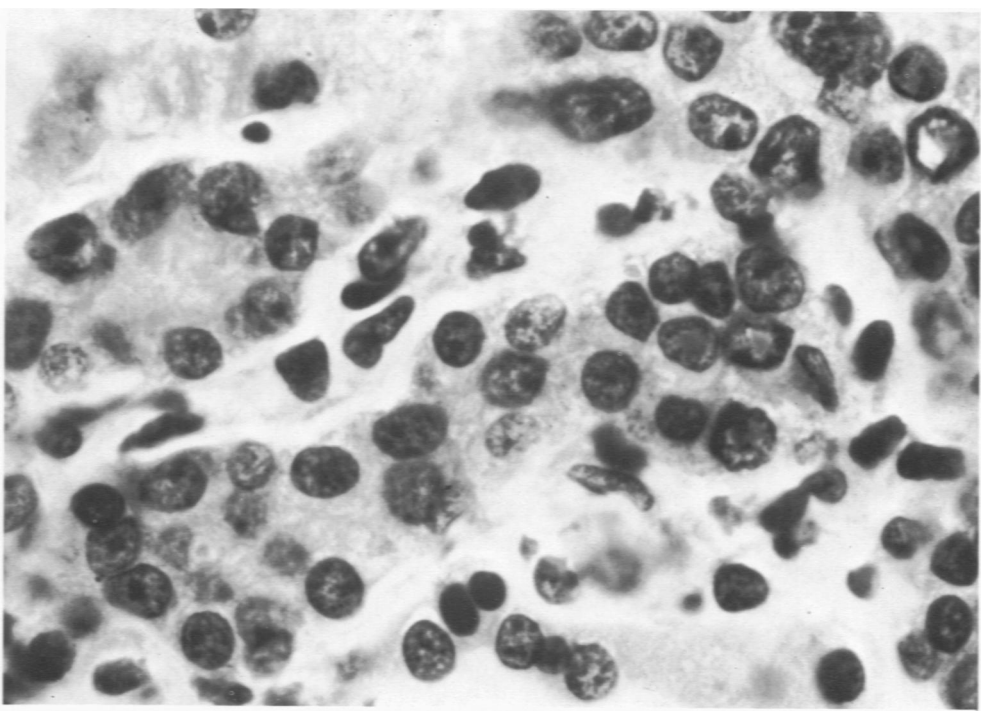

Fig 3—From rat given lead for 6 months. Hyperplasia of epithelial cells seen in cross section of proximal convoluted tubule. Several nuclei have abnormal, irregular shapes, appear to be larger than normal. Cells are more crowded than normally. Compare with cells near upper left corner, which line another segment of the proximal tubule (H&E, × 1700).
Fig 4—From rat given lead for 6 months. Irregular hyperplasia of proximal tubular epithelium. Note inclusion bodies in several nuclei (H&E, × 1200).