The Effect of Lithium Carbonate on the Structure of the Rat Kidney [1]

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ABSTRACT Female Sprague-Dawley rats were used to study effects of lithium carbonate on the ultrastructure of the rat kidney. Experimental rats received daily intraperitoneal injections of lithium carbonate in dosages of 10, 30 or 100 mg/kg/day. These animals were killed on day 12 to 60. Control rats were either non-injected or injected with either sodium chloride or sodium carbonate. Kidneys from control rats showed no abnormal changes. The 10 and 30 mg/kg lithium carbonate dosages caused progressive mitochondrial swelling, dilatation of cisternae of rough endoplasmic reticulum and occasional swelling of apical cytoplasm in tubular cells localized in the distal portion of the nephron. The 100 mg/kg lithium dosage produced damage in all portions of the nephron. However, the most severe damage, consisting of mitochondrial swelling, dilatation of the rough endoplasmic reticulum, apical cytoplasmic rarefaction and liquefaction, karyolysis and karyorrhexis was noted in the distal convoluted tubules and collecting ducts. The present study demonstrated that low dosages of lithium carbonate do affect the structure of the rat kidney.

Lithium was introduced into medical practice in 1949 as a salt substitute for patients with cardiac decompensation. The results were tragic since it led to the death of a number of patients (Hanlon et al., '49; Corcoran et al., '49). At about the same time, Cade ('49) noted that lithium salts calmed a variety of hyperactive patients. With this start, lithium salts were gradually proved by clinical studies to be effective in the manic phase of manic-depressive psychosis (Schou et al., '54; Gershon and Yuwiler, '60; Schlagenhauf et al., '66; Whaton and Fieve, '66; Van Der Velde, '70; Platman, '70).

Experimental studies conducted on the dog and rat, indicated that lithium affected primarily the kidney, with some changes occurring in other tissues (Radomski et al., '50; Schou, '58). A light microscopic study of kidneys from lithium-treated dogs showed changes in the distal convoluted tubules, the collecting ducts and the loops of Henle (Radomski et al., '50). Rats treated with lithium presented changes in the proximal convoluted tubules (Schou, '58).

Most experimental studies have used high dosages of lithium administered for short periods of time. Thus it was not known if low dosages of lithium carbonate administered for long periods of time would produce changes in the structure of the kidney. Therefore, the purpose of the present investigation was to study the effect of long-term treatment of lithium carbonate at low dosages on the ultrastructure of the rat kidney.

MATERIALS AND METHODS

Female Sprague-Dawley rats ranging in weight from 200 to 250 gm were used. All animals received up to 230 ml of fresh tap water daily and had free access to Purina Laboratory Chow 56. The experimental animals received daily intraperitoneal injections of aqueous lithium carbonate in dosages of 10, 30 or 100 mg/

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kg/day and were killed on day 12, 20, 30, 40 and 60 after the initial injection. However, the animals that received the 100 mg/kg lithium dosage were not able to survive for 60 days. The control animals received daily intraperitoneal injections of either a sodium chloride solution equivalent in volume and tonicity to one of the three lithium dosages, or a sodium carbonate solution in which the molar concentration of the carbonate ion was equivalent to one of the three lithium carbonate dosages. These animals were killed on the same time sequence used for the lithium-treated animals. Non-injected control rats were also used.

All animals were fixed by total body perfusion through the left ventricle for ten minutes with 1% glutaraldehyde in a modified Tyrode's buffer at pH 7.4 (Maunsbach, '66) and osmolarity of 325 mOsm/kg/H_2O. The fixative also contained 0.1% procaine (Forssman et al., '67). Subsequently, the tissue was post-osmicated for two hours in 1% osmium tetroxide in Tyrode's buffer at pH 7.4, dehydrated and embedded in Epon 812 (Luft, '61). Thin sections were doubly stained with 3% uranyl acetate (Watson, '58) and lead citrate (Venable and Coggeshall, '65), and viewed in a Philips EM-200 and an RCA EMU-3F electron microscope. Thick sections were cut and stained with toluidine blue for light microscopy.

OBSERVATIONS

Complete fixation of the entire kidney was necessary to insure that ultrastructural changes in kidneys from lithium-treated rats could be related correctly to the lithium ion and not to poor fixation. Good preservation of kidney ultrastructure was achieved in all control rats by the perfusion technique and fixative utilized (Maunsbach, '66) (figs. 1–4). No consistent differences were noted when kidney tissue from non-injected controls was compared to that from either of the injected control groups. Exploded mitochondria were rare.

On day 12, only the 100 mg/kg lithium dosage produced ultrastructural changes in the nephron. Extensive mitochondrial swelling and dilatation of cisternae of the rough endoplasmic reticulum were found in all portions of the nephron (fig. 5). However, further damage which included karyolysis, karyorrhexis, apical cytoplasmic rarefaction and liquefaction with bulging of the apical portion of the cell was seen in the distal convoluted tubules and collecting ducts (fig. 6).

On day 20 of the experiment, the animals that received the 30 mg/kg lithium dosage showed mottling in the mitochondrial matrices in the loops of Henle and the distal convoluted tubules. No other changes beyond those observed at day 12 could be discerned.

On day 30, the 10 mg/kg lithium dosage produced its first noticeable changes in the nephron. Some mitochondria in the loops of Henle and distal convoluted tubules were slightly mottled and vacuolated (fig. 7). Also the animals that received the 30 mg/kg lithium dosage showed dilatation of the rough endoplasmic reticulum and severe mitochondrial swelling in the same areas (fig. 8). More nephrons appeared to be damaged in the animals that received the 100 mg/kg lithium dosage on day 30 than on day 20.

Animals that received the 100 mg/kg lithium dosage demonstrated severe toxic symptoms by day 40 and were observed to die before day 60. Thus, the remaining 100 mg/kg lithium-treated animals were killed on day 40. In addition to the damage noted in these animals on day 30, the apical portion of the cells of the distal convoluted tubules showed bulging so as to cause apparent blockage of the lumen by day 40. Severe swelling of the mitochondrial matrices in some loops of Henle, distal convoluted tubules and collecting ducts was produced by the 10 mg/kg lithium dosage. The 30 mg/kg lithium dosage demonstrated progressive mitochondrial swelling and dilatation of the rough endoplasmic reticulum.

On day 60, the 30 mg/kg lithium dosage produced moderate rarefaction of the apical cytoplasm in some cells of the distal convoluted tubules and collecting ducts in addition to the previously observed severe swelling and vacuolization of the mitochondria and dilated rough endoplasmic reticulum (fig. 9). The animals that received the 10 mg/kg lithium dosage demonstrated progressive swelling of the

mitochondria and dilatation of rough endoplasmic reticulum (fig. 10).

DISCUSSION

The recommended lithium carbonate dosage for patients in the manic phase of manic-depressive psychosis is 1800 to 3600 mg/day (Gattozzi, '70). This dosage level is reduced to 900 mg/day for prophylactic treatment. The low dosages of 10 and 30 mg/kg/day used in the present study are well within the therapeutic range. However, an excessive (100 mg/kg) dosage was used for purposes of comparing the effects of low and high dosages of lithium on the kidney.

The low dosages that caused mitochondrial swelling and dilatation of rough endoplasmic reticulum in the distal portion of the nephron are strikingly different from the 100 mg dosage that produced damage in all portions of the nephron. However, this high dosage caused uneven damage, the most severe changes being localized in the distal convoluted tubules and collecting ducts. The time of onset also appeared to be dosage-related, occurring later with low dosage. Lithium concentration in kidney tissue rises steadily with chronic administration of lithium (Ho et al., '70). This may explain why our 10 and 30 mg/kg dosages did not cause ultrastructural changes by day 12 but did produce marked changes by day 60.

Noxious conditions such as autolysis initially affect mitochondria, endoplasmic reticulum and cytoplasmic ground substance in cells of the nephron (Suzuki and Motofi, '66; Latta et al., '65). Carbon tetrachloride first affects the endoplasmic reticulum of the liver cell (Trump et al., '65). Nephrotoxins, such as mercuric chloride (Gritzka and Trump, '68), uranium (Stone et al., '61) and glycerin (Suzuki and Mostofi, '66) also produce rarefaction or swelling of mitochondrial matrices. Gritzka and Trump ('68) suggest that swelling in the matrix was caused by increased permeability of mitochondrial membranes resulting in water uptake and some impairment of the phosphorylative capacity of the mitochondria. Isolated mitochondria from kidneys of lithium-treated rats were shown to have a decreased adenosine diphosphate to oxygen (ADP/O) ratio (Evan, '71).

Lithium dosages of 10, 30 or 100 mg/kg produce serum lithium levels that are within or below the therapeutic range except for the first two days in the 100 mg/kg lithium-treated animals (Evan, '71). Damage observed with low dosage levels in the present study suggests that lithium may cause subcellular damage in the distal portion of the nephron of the rat kidney at serum lithium levels that have thus far been considered safe (Gattozzi, '70). A correlation between the extent of ultrastructural damage in the nephron and the serum lithium levels cannot be detected at the lower lithium dosages but is present at higher dosages.

Polyuria and polydipsia are common side effects of lithium toxicity (Noyes, '69; Radomski et al., '50; Schou, '58a; Viol and Smith, '71; Lee et al., '71). The lithium dosages used in the present study are known to produce a direct relationship between dosage level of lithium on the one hand and increased water intake and urine output on the other (Evan, '71). Moreover, urine osmolarities decrease as the lithium dosage is increased. Schou ('58) reports that subcutaneous injection of vasopressin does not decrease urine output in a polyuric lithium-treated rat. He concludes that the origin of the polyuria is probably due to direct injury to the cells of distal convoluted tubules and collecting ducts, an injury that causes them to be insensitive to the action of vasopressin. In the present study, electron microscopy of whole tissue presents further evidence of direct kidney injury that might cause polyuria. Lithium is also known to inhibit the action of vasopressin (Harris and Jenner, '69).

Solomon ('67) reports that the kidney medulla is more effective in concentrating lithium than sodium. A possible reason why the portion of the nephron localized in the medulla is affected by lithium is that the kidney medulla has the capacity to concentrate lithium. This would produce a high concentration of lithium in the interstitium around this portion of the nephron, with consequent increase in lithium toxicity. Damage in the distal portion of the nephron might also be traceable to the "normal" function of the nephron in this area. As the filtrate passes down

the nephron, water and sodium are removed. When the filtrate in the treated animals reaches the distal convoluted tubule, it would therefore have a high concentration of lithium. Schou ('58) feels that lithium may gain access to the tubule cell from either the tubule lumen or the capillary side and that the amount of lithium entering may be related by a single factor, the ratio of lithium to sodium.

The present study has shown that low dosages of lithium carbonate administered for long periods of time cause moderate changes in the structure of the kidney.

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PLATES

PLATE 1

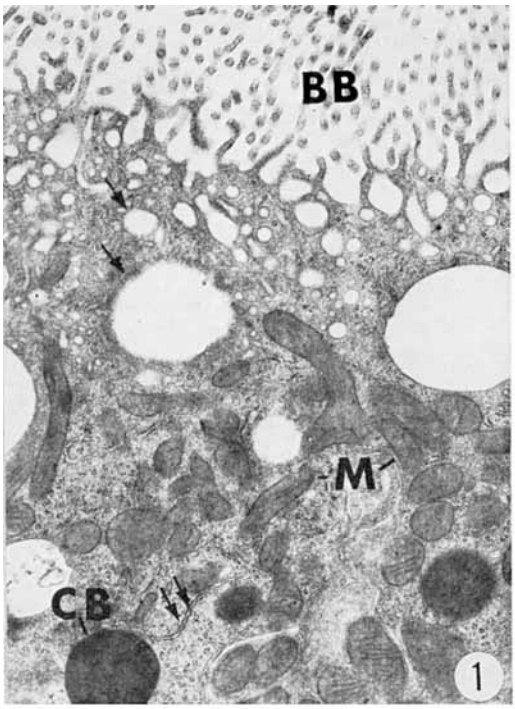
EXPLANATION OF FIGURES

1 Proximal convoluted tubule from a non-injected control rat. The apical portion of the cell presents a brush border (BB), small and large apical vacuoles (arrows), numerous mitochondria (M), and cytoplasmic bodies (CB). Profiles of the rough endoplasmic reticulum are present (double arrows). × 13,500.

2 Loop of Henle from a non-injected control rat. The apical surface demonstrates short microvilli (MV) and tight junctions (T). The mitochondria (M) are ovoid to ellipsoid and the matrix appears uneven in density. A vas rectus (VR) is also present. × 19,500.

3 Distal convoluted tubule from a control rat that received sodium chloride solution adjusted to the 100 mg/kg lithium dosage for 40 days. The mitochondria (M) demonstrate good preservation. G, Golgi body; BI, basal infoldings. × 9,000.

4 Collecting duct from a non-injected control rat. Both a dark cell (DC) and a light cell (LC) can be seen. In both cell types mitochondria (M) were mostly ovoid in shape and contained a matrix of uneven density. The luminal surface of the cells was convex in shape with short, irregular microvilli (arrows). A nucleus (N) and profiles of the rough endoplasmic reticulum are present (double arrows). × 8,900.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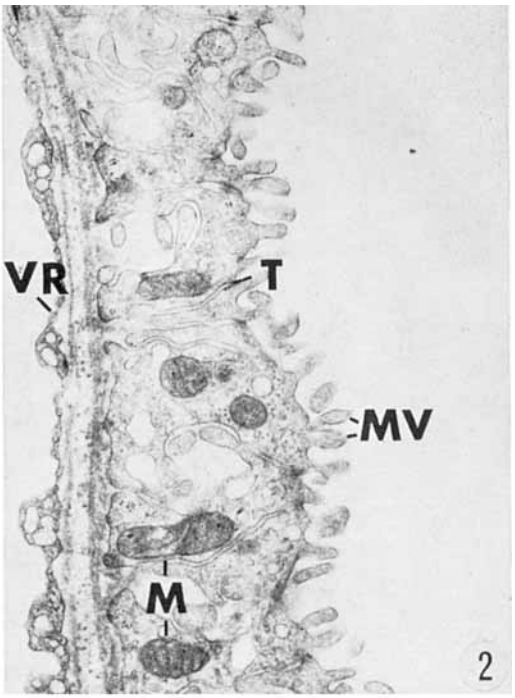
M
CB
1


VR
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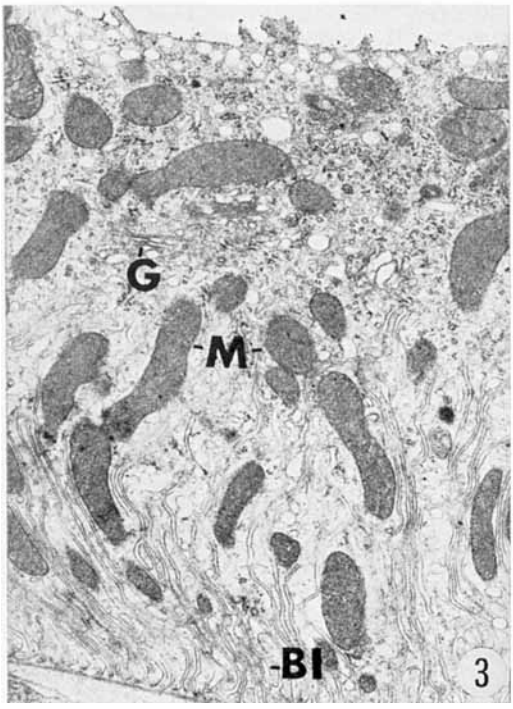
2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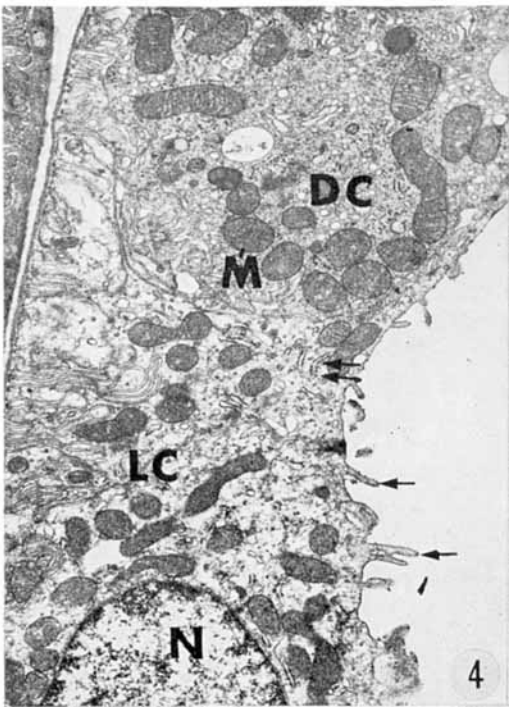
DC
M
LC
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4

PLATE 2

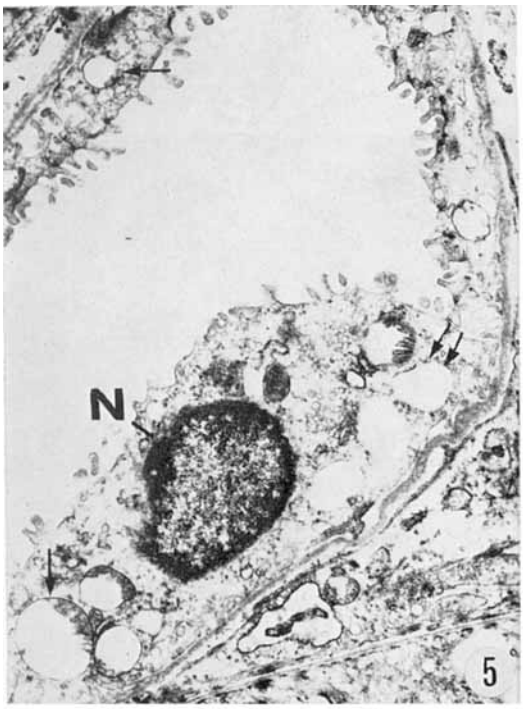
EXPLANATION OF FIGURES

5 Loop of Henle from an animal that received 100 mg/kg lithium dosage for 12 days. Extensive mitochondrial swelling (arrows) and dilatation of cisternae of the rough endoplasmic reticulum (double arrows) can be noted. N, nucleus. × 9,800.

6 Distal convoluted tubule from an animal that received the 100 mg/kg lithium carbonate dosage for 12 days. Karyolysis (N) is obvious. Mitochondrial swelling (arrows), cytoplasmic rarefaction and dilatation of cisternae of the rough endoplasmic reticulum (double arrows) can also be noted. × 5,200.

7 Distal convoluted tubule from an animal that received the 10 mg/kg/day lithium carbonate dosage for 30 days. The mitochondria have mottling to partial vacuolation (arrows). BM, basement membrane. × 27,000.

8 Distal convoluted tubule from an animal that received the 30 mg/kg lithium carbonate dosage for 30 days. Mitochondria (M) appear severely swollen. × 30,000.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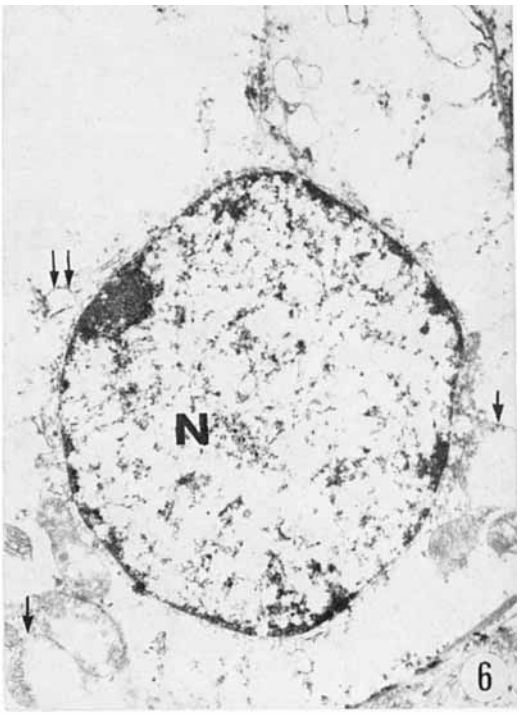
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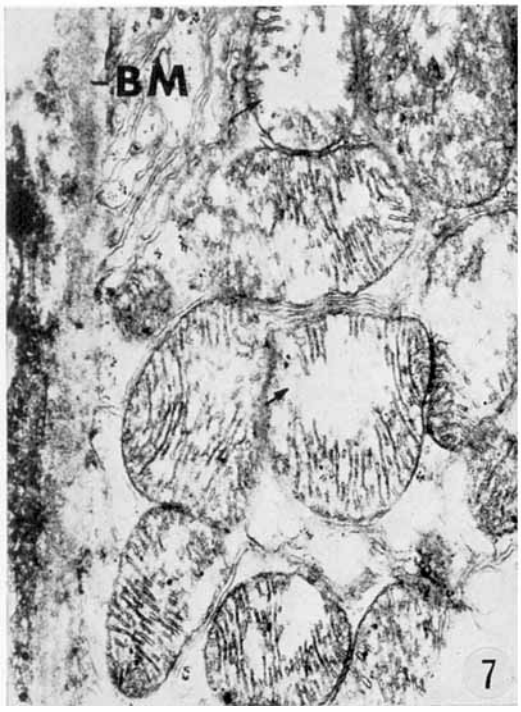
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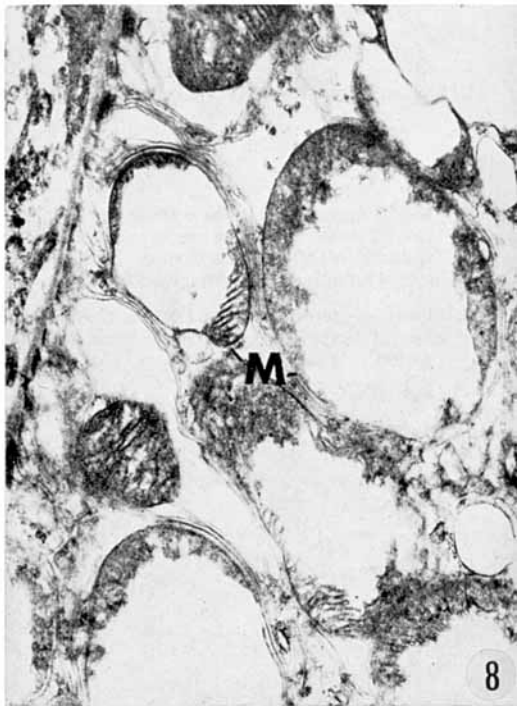
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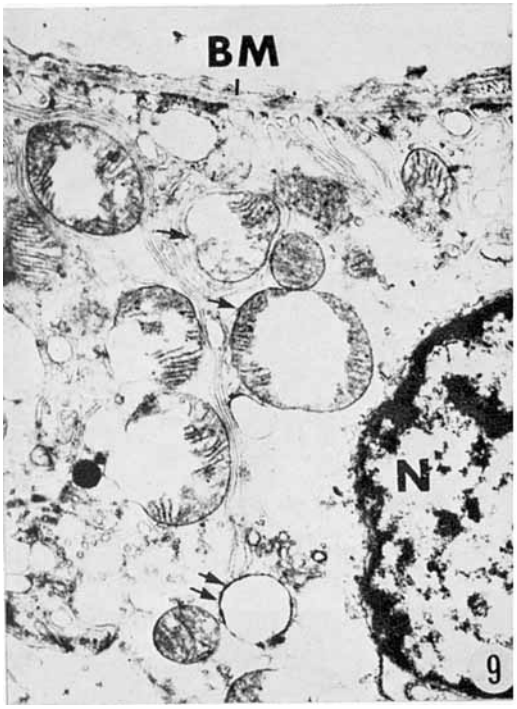

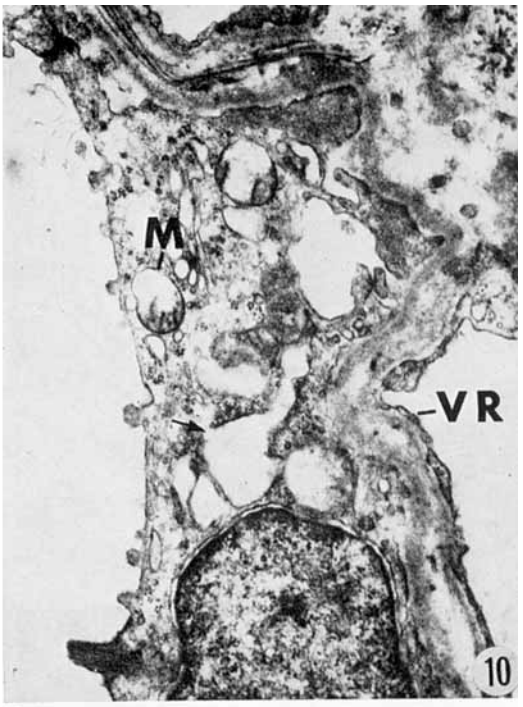

EXPLANATION OF FIGURES

9 Distal convoluted tubule from an animal that received the 30 mg/kg/day lithium dosage for 60 days. The damage at this dosage has progressed to include karyolysis (N), cytoplasmic rarefaction, mitochondrial swelling (arrows), and dilatation of cisternae of rough endoplasmic reticulum (double arrows). BM, basement membrane. × 16,700.

10 Loop of Henle from an animal that received the 10 mg/kg/day lithium carbonate dosage for 60 days. Extensive mitochondrial swelling (M) and dilatation of rough endoplasmic reticulum (arrow) is evident. VR, vas rectus. × 9,000.